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BIOCONTROL EFFICACY OF NATIVE *Metarhizium rileyi* TO VARIOUS LIFE STAGES OF *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae)

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ABSTRACT

The efficacy of an entomopathogenic fungus (EPF) was investigated as a potential biological control agent against onion armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), which is an important lepidopterous insect pest infesting onion crop in the Philippines. The pathogenic effect of a native isolate of *Metarhizium rileyi* was assessed against the pre-imaginal stages of *S. exigua* such as eggs, various larval instars, prepupa, and pupa by exposure to conidial concentrations of *M. rileyi* in laboratory bioassays. The EPF showed no significant effect on the hatchability of *S. exigua* eggs, but later caused mortality to the neonates from hatched treated eggs. Various instars of *S. exigua* larvae were all susceptible to *M. rileyi* with infection usually initiated at 4 days after treatment (DAT) and peaked at 6 to 7 DAT. Higher conidial concentrations caused higher and faster larval mortality as compared to lower concentrations with mean time to mortality of 6.2 to 7.1 days. There was no trend observed on the lethal concentration (LC_{50}) values that ranged from 5.59×10^5 to 5.95×10^6 conidia/ml relative to various instars. Adult emergence of prepupae and pupae was not significantly affected but abnormalities were observed in adults. Our findings revealed the infectivity of native *M. rileyi* against *S. exigua* and suggest its potential as biological control agent against this major economic insect pest of onion.

Key words: biological control, entomopathogenic fungus, bioassays, mummification, onion armyworm

INTRODUCTION

Spodoptera exigua (Hübner), locally known in the Philippines as *harabas* or onion armyworm, is a prolific, destructive, and polyphagous insect pest. A female adult lays 829-1,675 eggs in its lifetime while the hatched neonates are known to be gregarious feeders (Navasero et al. 2019). *S. exigua* infests more than 90 plant species in Asia including ornamental crops, weeds, and agricultural crops (Fu et al. 2017). In the Philippines, an outbreak of *S. exigua* was first reported in 2016 affecting onion fields in the provinces of Tarlac, Nueva Ecija, and Pangasinan. In Nueva Ecija, the infestation in 5,000 ha of onion field resulted to an estimated crop loss of PhP 1.6 billion. Chemical pesticides were extensively used in attempting to salvage the fields (Navasero et al. 2017). Aside from causing harm to the environment and imposing health risks, reliance to and misuse of chemical pesticide lead to its inefficacy and may induce resistance to target pests. Several studies noted resistance development of *S. exigua* against various synthetic pesticides (Che et al. 2013). In addition, chemical pesticides commonly used against *S. exigua* were found to be toxic to its known predators and parasitoids (CABI 2019; de

Castro et al. 2018; Liu et al. 2016). Hence, a safer, sustainable, and more environment-friendly means to control this insect pest must be explored.

The use of entomopathogens such as virus and fungi are being explored to control this insect pest (Navasero et al. 2017). These microorganisms are safe to use as these are naturally found in the environment (Dara 2017). *S. exigua* multiple nucleopolyhedrovirus (SeMNPV) was found infective to five larval instars of *S. exigua*, inflicting high mortality and reduced growth and development of the insect pest (Montecalvo and Navasero 2019). Likewise, a previous research reported successful isolation of entomopathogenic fungus (EPF) *Metarhizium rileyi* from field collected *S. exigua* that significantly caused mortality and affected development of the 3rd larval instar of the insect pest (Montecalvo and Navasero 2020). In this research, this *M. rileyi* isolate was further studied against *S. exigua* considering its biocontrol potential and being a native isolate from this insect pest.

The EPFs are beneficial fungi that cause disease and mortality to its host insect characterized by stiffening of the insect body with subsequent growth of mycelia and production of conidia. The most studied EPFs are *Beauveria bassiana* and *M. anisopliae*, which are both generalists infecting approximately 700 and 300 arthropod species, respectively (Rohrlich et al. 2018; Sbaraini et al. 2016). *M. rileyi*, on the other hand, is known to infect only around 60 species of insects, which are mainly lepidopterous insects that belong to the Noctuidae family (Fronza et al. 2017). These EPFs pose less threat to the natural enemies in the field while maintaining their virulence to their host.

M. rileyi is a dimorphic hyphomycete that causes epizootic mortality to various lepidopterous pests (Sinha et al. 2016). This EPF infects its host by adhesion to the cuticle, tissue invasion, enzymatic activity, and toxicosis (Fronza et al. 2017; Mengzhao et al. 1992). Some lepidopterous pests infected by *M. rileyi* are *Helicoverpa armigera* (da Costa et al. 2015), *Anticarsia gemmatalis* and *Chrysodeixis includens* (Lopes et al. 2020), *S. litura* (Liu et al. 2019; Namasivayam and Bharani 2015), and *S. frugiperda* (Cruz-Avalos et al. 2019; Montecalvo and Navasero 2021b; Ramanujam et al. 2020).

Higher virulence of *M. rileyi* is expected against *S. exigua* than other EPFs such as *M. anisopliae* and *B. bassiana* since *M. rileyi* was isolated from *S. exigua*. Considering the virulence of *M. rileyi* to *S. exigua*, this study aimed to further assess the potential of this EPF as a biological control agent against *S. exigua*. Bioassays were conducted to elucidate the pathogenicity of this isolate to the different life stages of *S. exigua* particularly eggs, 1st to 5th larval instars, prepupa, and pupa. Likewise, its biocontrol efficacy was determined based on calculation of lethal concentration (LC) and time.

MATERIALS AND METHODS

Laboratory rearing. *S. exigua* was obtained from the existing culture at the Biological Control Laboratory of the National Crop Protection Center, College of Agriculture and Food Science, University of the Philippines Los Baños, Laguna, Philippines. The parental stock was originally collected from an infested onion field in Nueva Ecija, Philippines. Adults were mated in Mylar cages and fertilized egg masses were harvested. Homogenous larvae were obtained from eggs hatched on the same day. Newly hatched larvae were fed daily with fresh castor oil leaves in plastic pans until appropriate larval instar was reached. Consequently, homogenous prepupae and pupae were obtained from larvae that reached prepupal stage and pupated on the same day, respectively.

Preparation of conidial suspensions. The native *M. rileyi* was originally isolated from a naturally infected larvae of *S. exigua* collected from an infested onion field in Nueva Ecija, Philippines (Montecalvo and Navasero 2020). The fungus was revived and subcultured in potato dextrose agar (PDA). This isolate was previously characterized based on cultural and morphological characteristics. Molecular identification through comparison of the DNA sequence of the fungal isolate to known sequences by BLASTn program confirmed the identity of the isolate.

Sporulated cultures of *M. rileyi* were used to reinfect larvae. *M. rileyi* was reisolated in PDA with yeast extract (PDAY) from mummified larvae to enhance virulence. Conidia were harvested from the cultures by scraping fungal growth and were suspended in 0.1% Tween 80 solution. Conidia in the suspension were quantified using a Neubauer improved hemocytometer (Blaubrand 717805, GMBH + CO KG, Germany). Conidial suspensions with various concentrations (1×10^5 to 1×10^9 conidia/ml) were prepared by diluting the conidial stock suspension in 0.1% Tween 80 solution.

Bioassay of *M. rileyi* against various life stages of *S. exigua*

Hatchability of eggs and mortality of hatched larvae. Freshly laid egg masses of *S. exigua* were counted under a dissecting microscope. Egg masses with nearly similar counts were randomly distributed in UV-sterilized Petri plates with damp sterile cotton. Egg masses were mist-sprayed with 1×10^9 conidia/ml of *M. rileyi*, while 0.1% Tween 80 solution was sprayed in the control set-up. Each treated and control set-up was replicated four times, with approximately 97 to 112 eggs per egg mass.

Fresh castor oil leaves were surface-sterilized by washing in 0.05% sodium hypochlorite for 10 min followed by washing twice in sterile distilled water for 1 min each. These castor oil leaves were placed inside the Petri plates, which were sealed using Parafilm to prevent the escape of neonates. Neonates and unhatched eggs were counted daily. Ten (10) newly hatched larvae per replicate were transferred in sterile Petri plates and were fed daily until 3rd larval instar was reached. Surviving 3rd instar larvae were single cultured by transferring and feeding one larva per Petri plate until the adult stage was reached. Mortality and mycosis were recorded daily. The experiment was done in four replicates.

Dose-mortality of various larval instars. Leaf discs of fresh castor oil leaves were prepared and surface-sterilized as previously described. Various suspensions of *M. rileyi* (1×10^5 to 1×10^9 conidia/ml) were mist-sprayed on both surfaces of the leaves and were air dried. Tween 80 (0.1% v/v) solution was sprayed for control. Two inoculated leaves, one on top of the other, were placed in a sterile Petri plate containing a sterile damp cotton ball. For the 1st and 2nd larval instars, 10 larvae were placed in a Petri plate and single cultured upon reaching 3rd larval instar. The 3rd, 4th, and 5th larval instars were cultured singly in a Petri plate. Fresh surface-sterilized castor oil leaves were fed to the larvae daily. Each treatment was done in triplicates with 10 individuals per replicate. Mortality was noted daily and mycosis of the larvae was documented. The percentage of mortality was corrected (Abbott 1925) and mean time to mortality was calculated (El-Hawary and Abd El-Salam 2009).

Adult emergence of prepupa and pupa. Prepupa and pupa were surface-sterilized by washing in 1% sodium hypochlorite for 1 min then washing twice in sterile distilled water for 30 sec then air dried. These prepupae and pupae were treated by submerging in 1×10^9 conidia/ml of *M. rileyi* for 1 min. For the control, prepupae and pupae were submerged in 0.1% Tween 80 solution. After airdrying, treated prepupae and pupae were distributed singly to sterile specimen cups. Adult emergence was counted daily. The control and treated set-ups were replicated thrice with 10 prepupae or pupae per replicate.

Statistical design and analysis. All experiments were arranged in CRD. T-test was conducted to compare the results of fungal treated versus the control set-up in the bioassays of *S. exigua* eggs, prepupa, and pupa. Treatment means in bioassays conducted in larval instars were compared by analysis of variance using Tukey's honest significant difference test. Lethal concentration (LC) values were calculated using PriProbit software ver. 1.63.

RESULTS AND DISCUSSION

Hatchability of eggs and mortality of neonates. The native isolate of *M. rileyi* had no significant ovicidal effect on *S. exigua* (Fig. 1). Interestingly, 93.75% of the larvae that hatched from the treated egg masses succumbed to fungal infection, while the remaining 6.25% reached pupal stage, but no

longer emerged into adult (Fig. 2.). Some pupae were observed to exhibit incomplete pupation. On the contrary, *S. exigua* in the control set-up pupated successfully and emerged into adults.

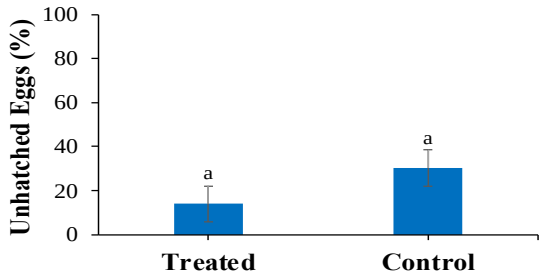

Fig. 1. Unhatched eggs of *Spodoptera exigua* treated with *Metarhizium rileyi*. Bars represent the standard error of the means. Those with same letters are not significantly different by t-test (P< 0.05).

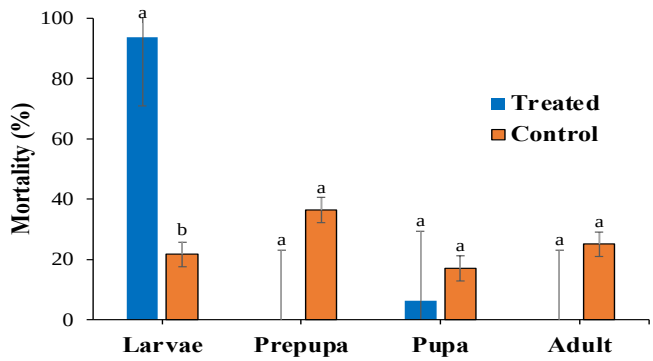

Fig. 2. Mortality in different life stages of *Spodoptera exigua* which hatched from egg masses treated with *Metarhizium rileyi*. Bars represent the standard error of the means. Pairs of bars with the same letters are not significantly different by t-test (P< 0.05).

Our observations suggest that the ovicidal activity of *M. rileyi* was not apparent based on its effect on hatchability. This result agrees with previous studies wherein eggs of *Spodoptera* spp. were exposed to *M. rileyi* but did not show significant effect in terms of hatchability (Montecalvo et al. 2022). Both studies by Cruz-Avalos et al. (2019) and Ramanujam et al. (2020) reported that *M. rileyi* was one of the strains which did not infect the eggs of fall armyworm (*S. frugiperda*) after treatment with various strains of EPF. In contrast, *B. bassiana* and *M. anisopliae* reduced hatchability of *S. frugiperda* eggs (Montecalvo and Navasero 2021a). *M. anisopliae*, *Isaria fumosorosea*, and *B. bassiana* also infected freshly laid *S. litura* eggs (Asi et al. 2013).

Although the hatchability of eggs was not affected, this research also presented the lethal effect on the larvae hatched from the egg masses exposed to *M. rileyi*. These neonates may have acquired *M. rileyi* from the surface of the treated egg masses upon emergence, hence, these larvae succumbed to fungal infection. A similar study in *S. littoralis* showed that *M. rileyi* caused larval mortality after contamination of first instar larva from treated egg mass, which might be infected by the germinating fungus in the egg integument or the larva acquired the conidia from the egg cuticle which fed upon chorions (Rodriguez-Rueda and Fargues 1980).

Dose-mortality of various larval instars. Based on laboratory bioassays against various larval instars of *S. exigua*, the mortality across time points revealed that all larval instars of *S. exigua* were susceptible

to *M. rileyi* with infection initiating at 4 days after treatment (DAT) and significantly increased at 6 to 7 DAT (Fig. 3).

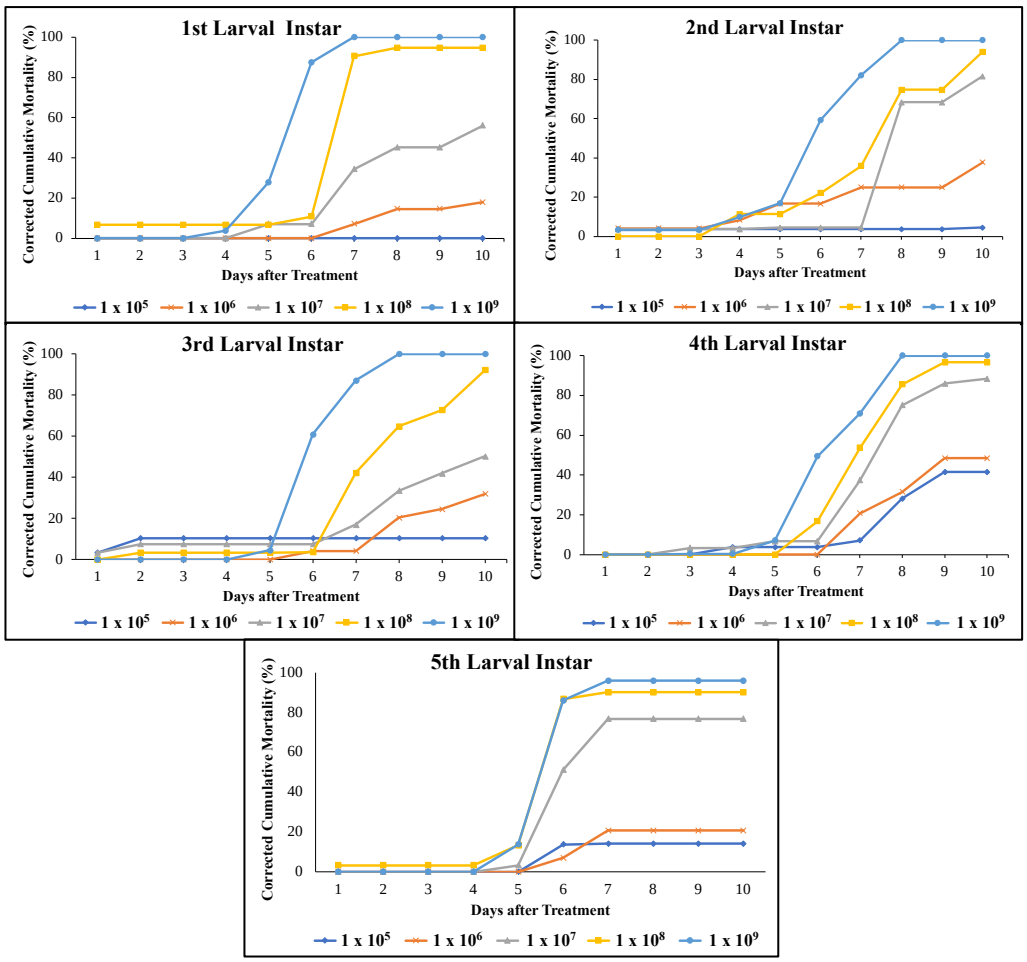

Fig. 3. Corrected cumulative mortality of larval instars of *Spodoptera exigua* exposed to conidial concentrations of *Metarhizium rileyi*: 1 x 10^5 conidia/ml (A); 1 x 10^6 conidia/ml (B); 1 x 10^7 conidia/ml (C); 1 x 10^8 conidia/ml (D); and 1 x 10^9 conidia/ml (E).

Mycosis was confirmed in *S. exigua* cadavers having stiff bodies with white fungal growth and olive green sporulation (Fig. 4). This finding is consistent with the initial results where 3rd instar larvae of *S. exigua* were treated with various conidial concentrations (1 x 10^4 to 1 x 10^8 conidia/ml) of the same *M. rileyi* isolate (Montecalvo and Navasero 2020).

Fig. 4. Larval cadaver of *Spodoptera exigua* mycosed by *Metarhizium rileyi.*

Increasing conidial concentration resulted in higher mortality that rose significantly days after exposure to *M. rileyi* (Fig. 5). Conidial concentrations of 1 x 10^8 and 1 x 10^9 conidia/ml induced the highest lethal infection up to 100% mortality in all larval instars of *S. exigua*, while lower conidial concentrations induced lower and slower mortalities at 10 DAT (up to 41.48% in 1 x 10^5 conidia/ml; 45.19% in 1 x 10^6 conidia/ml; and 88.33% in 1 x 10^7 conidia/ml).

Increasing trend in fungal infection with increasing conidial concentrations was also observed in *S. exigua* treated with various conidial concentrations (1 x 10^4 to 1 x 10^9 conidia/ml) of *M. anisopliae* and *Paecilomyces fumosoroseus* (Han et al. 2014) and 3rd instar larvae of *S. exigua* treated with various conidial concentrations (1 x 10^4 to 1 x 10^8 conidia/ml) of *M. rileyi* (Lee et al. 2012). Likewise, significant number of early larval instars of *S. frugiperda* succumbed to a native isolate of *M. rileyi* (Montecalvo et al. 2022).

Larval mortalities are influenced by molting, pupation, and conidial concentration. Inoculum may be lost during molting (Meekes 2001). Kim and Roberts (2012) observed low fungal infection of early nymphal stages of cotton aphids due to low number of conidia attached to the insect cuticle, low levels of conidial germination, and rapid ecdysis that may result in removal of conidia before the germ tubes penetrated the host hemolymph.

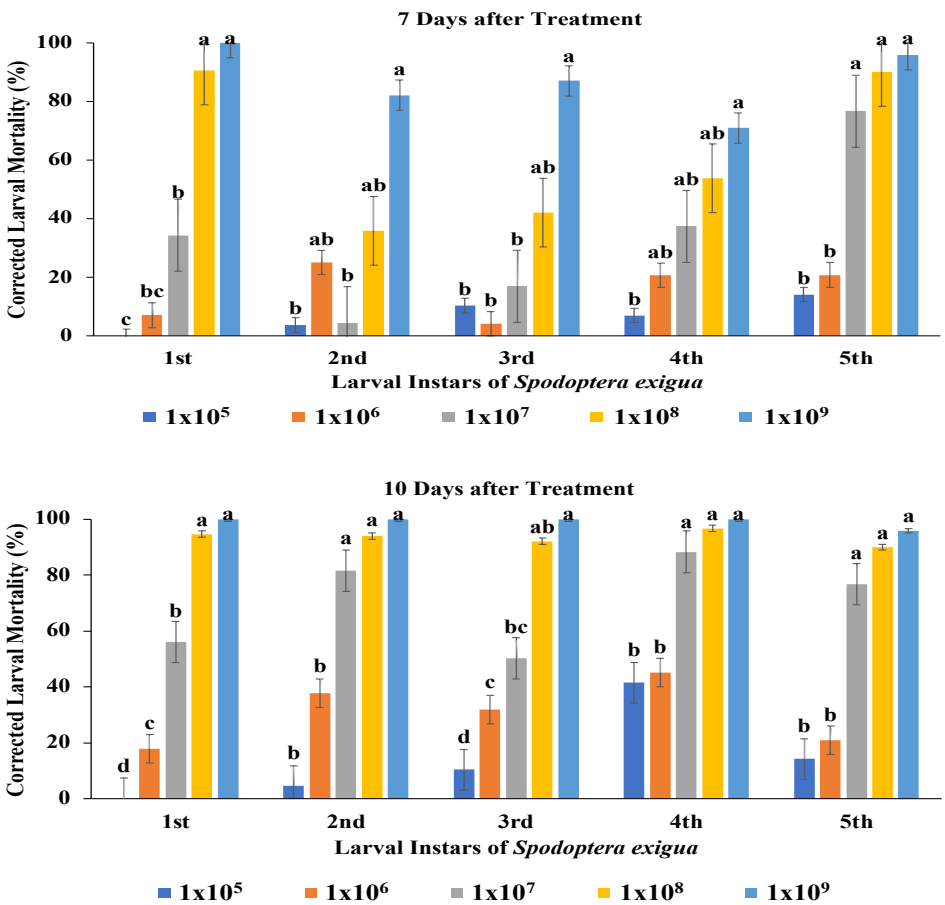

Fig. 5. Corrected cumulative mortality of larval instars of *Spodoptera exigua* exposed to conidial concentrations of *Metarhizium rileyi*. Bars represent the standard error of the means. Pairs of columns with the same letters are not significantly different in HSD (P< 0.05).

This study also presented the calculated LC_{50} of each larval instar using probit analysis at 95% fiducial limit (Table 1). The LC_{50} values revealed that different conidial concentrations of *M. rileyi* were required for 50% of each larval instar to be infected with no distinct trend. Calculated LC_{50} at 10 DAT ranged from 5.59 x 10^5 to 5.95 x 10^6 conidia/ml. This is contrary to the findings of Kaur et al. (2011) wherein LC_{50} values increased with the larval stage when *S. litura* was treated with *B. bassiana*. Likewise, increasing LC_{50} conidial concentrations of this *M. rileyi* isolate was observed when it cross-infected *S. frugiperda* with increasing LC_{50} values ranging from 1.44 x 10^5 to 9.36 x 10^8 conidia/ml for young to old larval instars (Montecalvo and Navasero 2021b). Larval size may also have contributed to its susceptibility to EPF with larger larvae requiring more fungal inoculum.

Table 1. Lethal concentration of *Metarhizium rileyi* against *Spodoptera exigua* larvae at 95% fiducial limit.

Larval instar	**LC_{50}** (conidia/ml)	**95% Fiducial Limit** (Lower-Upper)
1st	5.95 x 10^6	3.08 x 10^6 - 1.09 x 10^7
2nd	2.01 x 10^6	7.49 x 10^5 - 4.02 x 10^6
3rd	5.08 x 10^6	1.95 x 10^6 - 1.07 x 10^7
4th	5.59 x 10^5	1.63 x 10^5 - 1.32 x 10^6
5th	3.12 x 10^6	1.25 x 10^6 - 6.71 x 10^6

Mean time to larval mortality suggests that increasing conidial concentrations of *M. rileyi* resulted in earlier larval mortality (Fig. 6). The earliest larval mortality was observed with conidial concentrations of 1 x 10^8 conidia/ml (7.1 days) and 1 x 10^9 conidia/ml (6.2 days) which were significantly faster than lower conidial concentrations. Our findings conform with our earlier observations that increasing conidial concentrations of *M. rileyi* resulted in earlier larval mortality. There was no significant difference on the mean time to larval mortality among different larval instars (6.9 to 8.0 days). In a previous study, this isolate caused mean time to mortality of 4.5 to 8.9 days during cross infection to larval instars of *S. frugiperda* (Montecalvo and Navasero 2021b). Shorter lethal time due to higher conidial concentrations were observed in earlier studies (El-Hawary and Abd El-Salam 2009; Han et al. 2014).

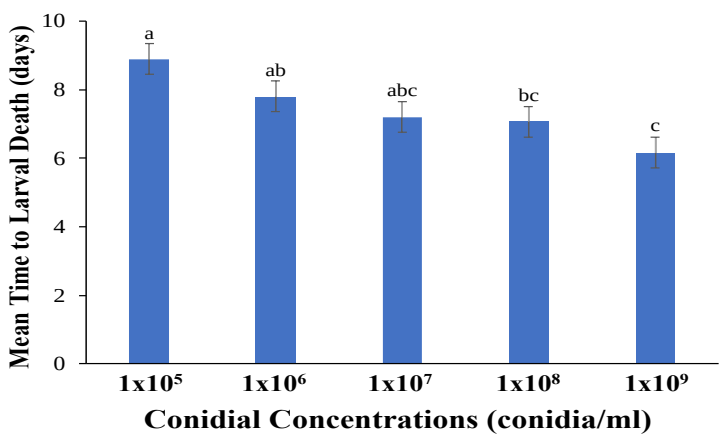

Fig. 6. Mean time to larval mortality of *Spodoptera exigua* as affected by conidial concentrations of *Metarhizium rileyi*. Bars represent the standard error of the means. Bars with the same letters are not significantly different in HSD (P< 0.05).

Adult emergence of prepupa and pupa. Prepupa and pupa of *S. exigua* were not susceptible to *M. rileyi* (data not shown). *M. rileyi* had no effect on the emergence of adults from treated prepupae and pupae. This is different from the results obtained by Garrido-Jurado et al. (2020) where high mortalities were observed when *S. littoralis* prepupae and pupae were treated with various isolates of *B. bassiana* and *M. brunneum*. On the contrary, prepupa of *S. frugiperda* was affected by *M. rileyi* but pupa was not susceptible to fungal infection (Montecalvo et al. 2022). On the other hand, *B. bassiana* and *M. anisopliae* caused low mortalities in *S. frugiperda* prepupae but these EPF did not affect adult emergence of treated pupae (Montecalvo and Navasero 2021a). Nevertheless, the emergence of deformed adults such as reduced size of wings from treated prepupae and pupae in their study was also observed in this experiment implying the possible impact on the mating ability of the adults. Pupae may not be susceptible to fungal infection due to their thick and sclerotized cuticle that serves as barrier to fungal infection (Hajek and St. Leger 1994).

This paper presented the virulence of the *M. rileyi* isolate which also caused epizootics to several insect pests. This *M. rileyi* isolate cross infected two armyworm species including *S. frugiperda* (Montecalvo and Navasero 2021b) and true armyworm, *Mythimna separata* (Montecalvo et al. 2021). Likewise, a native isolate of *M. rileyi* naturally infected *S. frugiperda* collected in Quezon province in the Philippines (Montecalvo et al. 2022) suggesting that *M. rileyi* is a potent EPF against invasive insect pests such as *S. exigua* and *S. frugiperda*. The use of this EPF in the integrated pest management of *S. exigua* is promising since the fungus can establish endophytically in its host.

CONCLUSION AND RECOMMENDATION

The native isolate *M. rileyi* caused lethal infection to its original insect host, *S. exigua*. Different life stages of *S. exigua* were exposed to local isolate of *M. rileyi*. This EPF did not affect hatchability of eggs, however, larvae hatched from the treated eggs succumbed to fungal infection. Larval instars succumbed to fungal infection, however, prepupa and pupa were not susceptible to *M. rileyi*. This EPF is a potent biocontrol agent against *S. exigua* larvae. Its biocontrol efficacy can be further assessed by conducting screenhouse and field trials implying the need to optimize mass production methods and to formulate this EPF.

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INTENTION TO PURCHASE LOCAL FOOD OF VIETNAMESE CONSUMERS IN THE COVID-19 PANDEMIC CONTEXT: AN APPLICATION OF THE THEORY OF PLANNED BEHAVIOR

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ABSTRACT

The COVID-19 pandemic has changed consumers' habits and behavior for food shopping and consumption in favor of local food. This study sought to determine the intention of Vietnamese consumers to purchase local food in the COVID-19 pandemic context. Data came from the interviews of 286 consumers using standard questionnaire. Five provinces from the North to the South: Hanoi, Quang Ninh, Dong Thap, Ninh Thuan and Dak Lak, which represent typical areas of Vietnam such as urban, rural areas; coastal, plain and mountainous areas, respectively were considered in 2021. Based on an extended theory of planned behavior (TPB), descriptive statistics, exploratory factor analysis, and regression analysis were used to establish the relationship between behavioral intention and its determinants. Four determinants affected the intention to purchase local food among Vietnamese consumers in the COVID-19 pandemic context: subjective norm, trust in local food, perceived behavioral control, and attitude of consumers to local food. The impact of the COVID-19 pandemic on consumers was found not significant. Policy implications are discussed to promote sustainable local food development in Vietnam in the future.

Key words: consumer behavior, local food, TPB, Vietnamese consumer

INTRODUCTION

The COVID-19 pandemic created many challenges when the numbers of infection cases increased rapidly in all countries and territories. National and regional governments all around the world imposed policies to control the situation, including social distancing, safety mandates, and lockdown restrictions. This in turn slowed down the economy, affecting many business sectors, caused an increase in unemployment rates, and reduced consumers' income and spending (Jo et al. 2021). These policies and situations cause changes in the daily lives of people, from how they work to how they shop and how they entertain themselves. Consumers also changed their behavior to the new orientation and habits of food consuming, food shopping, food delivery and dining out (Jo et al. 2021; Sheth 2020). Consumers have increased home cooking and baking (Gerritsen et al. 2020), spent more time on new recipes and wasted less food (Huntepr 2020), and eaten out less during the pandemic (Flanagan et al. 2021). They prefer online shopping with direct delivery to their door (Jo et al. 2021). Also, local food has become more important during the COVID-19 pandemic (Bui et al. 2021).

There is no consensus in defining local food (Feldmann and Hamm 2015). Local food is understood differently, based on the social and spatial context, as consumers and scholars are very flexible in understanding the term (Carroll and Fahy 2015). It is mainly defined as food that is produced, processed, and retailed within a defined geographical area (Adams and Adams 2011; CFIA 2019; Kumpulainen et al. 2018; Moya et al. 2013). The distance between the location of production and consumption ranged from 10 miles, 30 to 50 miles (Thilmany et al., 2010) to 100 miles (Adams and Adams, 2011; Durham et al. 2009; Feldmann and Hamm 2015; Thilmany et al. 2010). In some other studies, food can be considered local when it is consumed in the same county, town, state, or country from which it originated (Brown 2003; Darby et al. 2008; Moya et al. 2013).

The previous literature showed that local food promotes certain dimensions of sustainability (Mäkiniemi and Vainio 2014) that brought benefits to the consumers: local foods are of higher quality (Feldmann and Hamm 2015), are fresher (Lim and Hu 2016), more nutritious (Bianchi and Mortimer 2015), and tastier than other products (Jensen et al. 2019) thanks to the shortened transportation time from production to consumption, and less use of preservatives (Bui et al. 2021; Motta and Sharma 2016; Skallerud and Wien 2019). Local food is also considered to protect the ecological environment (Zhang et al. 2020) by using less chemicals during production (Lim and Hu 2016), reducing the use of transportation fuels, thus minimizes the emission of greenhouse gases (Bianchi and Mortimer 2015; Moya et al. 2013). It is believed that local food consumption supports local producers (Jensen et al. 2019) and contributes to the local economic development because the money the consumers spend remains within their community (Jensen et al. 2019; Memery et al. 2015). From these benefits, local food has become a new trend in many countries such as in the US (Hedberg and Zimmerer, 2020), Europe (Kumpulainen et al. 2018; Skallerud and Wien 2019), and others (Lim and Hu, 2016) with the total sale of around $20 billion in 2020 (Zhang et al. 2020).

Like all countries and territories in the world, Vietnam is not immune to the COVID-19 pandemic and its accompanying economic headwinds. Although it has first achieved some successes in containment and reduced the impacts of COVID-19, thanks to the compliant population that mostly adhered to safe distancing requirements, COVID-19 posed a negative impact on people's lives, especially the poor people. Many have become unemployed, resulting in no or low incomes (Bui et al., 2021). Similar to most consumers around the world, Vietnamese consumers have changed their behavior and habits. They adjust many aspects of their lives, including their way of shopping by avoiding the long queues in front of shops and supermarkets; more thoughtful spending, reducing shopping frequency through larger baskets; shifting to stores closer to their home, with more focus on local food; and increasing e-commerce (Tien 2020). Consumers also tend to order and shop online via mobile applications with more direct delivery to their doors than traditional shopping. Consumers also buy more local origin products cheaper and easier to buy than higher priced imported ones. This seems to show that the COVID-19 crisis encourages consumer preferences for local foods (Phuong et al. 2021).

Many studies have investigated the intention to purchase local food (Holt et al. 2018; Kumar et al. 2021; Minh et al. 2021) and effects of COVID-19 in terms of food businesses, market conditions, changes in consumer behaviors, revenues, and mobilities in the world (Bucak and Yiğit, 2021; Djekic et al. 2021; Jo et al. 2021; Nakat and Bou-Mitri 2021; Rizouet al. 2020; Wang et al. 2020). However, the intention to purchase local food of Vietnamese consumers in the context of COVID-19 is currently under-explored. No empirical evidence is available to explore the motivation of Vietnamese consumers' motivation to purchase local food. These gaps in the literature need to be addressed. Beyond that, this study aims to determine the intention to purchase local food of Vietnamese consumers in the COVID-19 pandemic context and suggest some policy recommendations to promote sustainable local food development in Vietnam in the future.

MODEL AND METHODOLOGY

Theory of Planned Behavior (TPB). Recently, the TPB has been an influential theoretical approach in the psychology domain (Shalender and Sharma 2021). It has been frequently used to investigate the behavioral predispositions of consumers and their buying behavior (Sabah 2016). Moreover, it is argued that buying decision is complex and need an intentional cognitive process (Giampietri et al. 2015). The TPB can be applied to predict the behavior of individuals by their intentions (Aggestam et al. 2017). However, Sniehotta et al. (2014) highlighted the limitations of the TPB, and in particular, the idea that individuals have an intention but may not act upon it (Ajzen 2006; Sniehotta et al. 2014). In other words, factors that go beyond an individual's control influence their intentions and predictions of behavior (Ajzen 2006; Kor and Mullan 2011).

The TPB argues that the intention of a person relies on personal attitudes, subjective norms (SN), and perceived behavioral control (PBC) and is used to determine and understand human behavior (Ajzen 1991). In general, the intention may increase with a positive attitude towards the behavior, SN, and PBC (Giampietri et al. 2018; Lim and An 2021). In this study, attitudes refer to the feelings or emotions related to performing a behavior (Ajzen 1991). Attitude can have positive effect to intention of the people (Lim and An 2021). SN represents the perception of significant others about a given behavior. The main assumption for adding this factor to the model is the argument that human's buying behavior is adopted according to other people's attitude towards given behavior (Sabah 2016); SN is social in nature because a person performs an action on the basis of the opinions of her/his acquaintances, which is also influenced by perceptions of social pressure to make a decision about the action for him/her (Park 2000); PBC is related to the perceived ease or difficulty of performing the behavior (Aggestam et al. 2017).

The concept of the TPB was extended in other studies (Giampietri et al. 2018; Mazzocchi et al. 2008), by noting that trust is a behavioral determinant whose nature is jointly relevant to the TPB concepts of attitudes, SN, and

PBC. In addition, the studies have found that the direct interaction between local food producers and consumers and repeated exchanges can provide consumers with a sense of trust built on shared know-how and a mutual understanding with local food producers (Hartmann et al. 2015; Hunt 2007; Meyer et al. 2012; Tregear 2011). Moreover, trust can drive loyalty and new solid relationships between producers and consumers (Hartmann et al. 2015). In Vietnam, trust is an important factor that affects consumer behavior (Nam and Huan 2018). So in this study, trust was added to extend the TPB of Ajzen (1991).

The study was conducted in the complex COVID-19 pandemic context in Vietnam. Many recent studies have been conducted around the world that indicates the impact of COVID-19 on food production and business (Bucak and Yiğit 2021; Nakat and Bou-Mitri 2021; Soon et al. 2021; Wang et al. 2020). Bui et al. (2021) studied short food supply chains in Vietnam during COVID-19 and argued that COVID-19 affected Vietnamese consumer behavior. A more in-depth study on local food purchases during COVID-19 is necessary to asscess food consumer behavior for implementing policy to promote local food selling.

Factors affecting the intention of Vietnamese consumers to purchase local food in the COVID-19 pandemic context were tested based on the TPB model (Fig. 1). In particular, the following hypotheses were tested:

H1. Consumer's subjective SN towards purchasing local food increases their buying intention.
H2. Consumer's attitude towards purchasing local food increases their buying intention.
H3. Consumer's trust towards purchasing local food increases their buying intention.
H4. Consumer's PBC towards purchasing local food increases their buying intention.
H5. COVID-19 pandemic increases their buying intention.

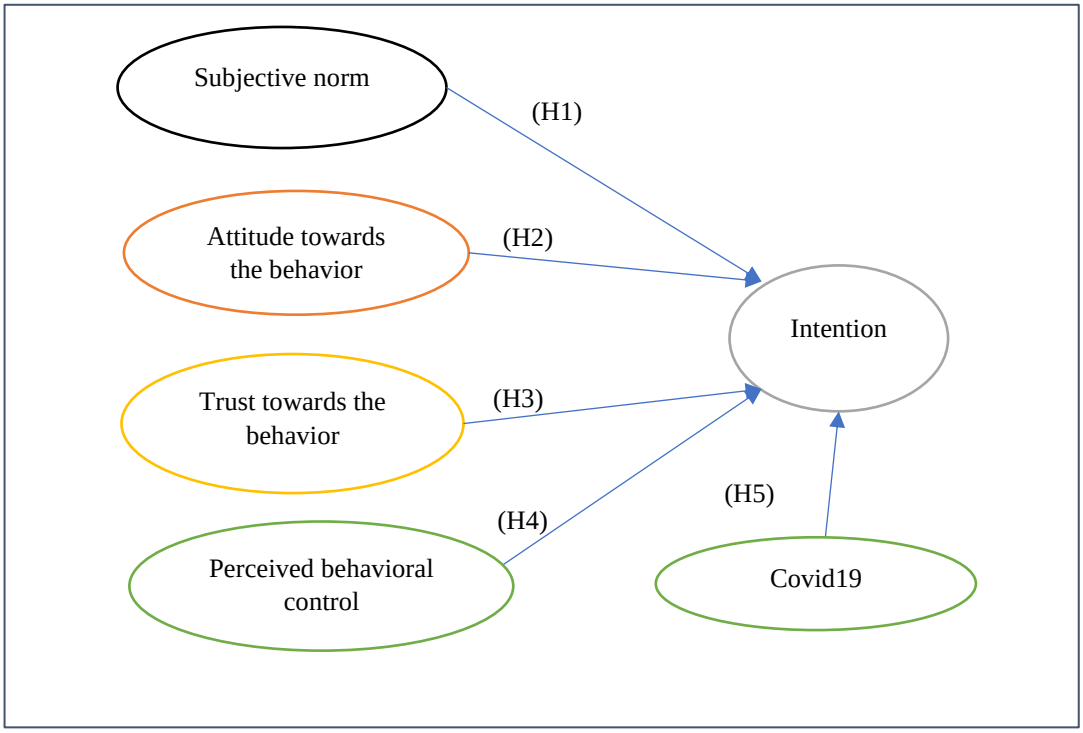


Fig. 1. The extended Theory of Planned Behavior model related to Vietnamese consumer intention to buy local food

Questionnaire development. All items used in this study are measured by five-point scales that ranged from 1 (strongly disagree) to 5 (strongly agree) in relation to local food purchase behaviors of the respondents. The scales used were adapted from previous studies. The questions were revised by discussing and testing within two group discussions (7 people each group) with consumers in Hanoi to clarify the meaning of the questions in Vietnamese. The variable COVID-19 was also developed via discussions in the groups. The original sources of the questions used in the survey are described in Table 1.

Table 1. The items used in this study and sources

Variables	Items		Reference
INT	INT1	I intend to purchase more local food during COVID-19	(Ajzen, 2006; Giampietri et al., 2018; Lam and Hsu, 2006; Zeithaml et al., 1996)
	INT2	I plan to purchase more and more local food from local producers during COVID-19	
	INT3	I am willing to buy more local food during COVID-19	
	INT4	I will make an effort to purchase more local food during COVID-19	
SNO	SNO1	My acquaintances understand me buy more local food as a wellbeing food	(Ajzen, 2006; Lam and Hsu, 2006; Lim and An, 2021)
	SNO2	My acquaintances think that I should eat more local food	
	SNO3	My acquaintances approve me eating more local food	
ATT	ATT1	Purchasing more local food is pleasurable	(Ajzen, 2006; Lam and Hsu, 2006)
	ATT2	Purchasing more local food is favorable	
	ATT3	Purchasing more local food is enjoyable	
TRUST	TRUST1	I perceive purchasing more local food to be reliable	(Giampietri et al., 2018; Mazzocchi et al., 2008)
	TRUST2	Purchasing more local food appears trustable to me	
	TRUST3	I trust in purchasing more local food.	
PBC	PBC1	I can easily eat more local food whenever I want	(Ajzen, 2006; Giampietri et al., 2018; Lam and Hsu, 2006; Lim and An, 2021)
	PBC2	If I wanted to, I could easily purchase more local food	
	PBC3	Purchasing more local food is up to me.	
COVID-19	COVID1	COVID-19 pandemic had made my life change	(Meixner and Katt, 2020; Qi and Ploeger, 2021)
	COVID2	COVID-19 pandemic has change society	
	COVID3	COVID-19 pandemic will shift my consumption behavior	

Data collection. Five provinces were chosen to conduct the survey that include Hanoi, Quang Ninh, Dong Thap, Ninh Thuan, and Dak Lak, which represent areas and regions of Vietnam as urban, rural areas, coastal, plains, and mountain areas of the country (Table 2). The number of 286 respondents was chosen randomly by available resources to interview "face to face". Six trained interviewers conducted the interviews from December 2020 to March 2021 in local markets and supermarkets in each province. The interview length was 40 minutes on the average.

Table 2. Distribution of the sample

			Sample	
Provinces	**Areas**	**Region**	**Frequency**	**Percent (%)**
Dong Thap	Rural, Plain areas	South	73	25.5
Dak Lak	Rural, Mountainous area	Middle	52	18.2
Ninh Thuan	Plain, rural, coastal areas	Middle	51	17.8
Quang Ninh	Coastal, plain and mountain	North	60	21.0
Hanoi	Urban, plain	North	50	17.5
Total			**286**	**100**

Source: Survey data, 2021

Data analysis tools. Descriptive statistics were used to describe the demographic characteristics of the respondents. An exploratory factor analysis was conducted, and Cronbach's alpha was used to check the internal consistency of the variables. However, the total sample must be more than 5 times Items for Exploratory Factor Analysis (Hair et al, 1998). In this analysis, we have 18 items; the total sample is more than 90 observations, so 286 respondents is enough for Exploratory Factor Analysis. After that, a regression analysis was conducted within a TPB framework to estimate the relationship between behavioral intention and its determinants (Mazzocchi et al. 2008). A multiple regression

model was utilized to analyze the relationship between the intention to purchase local food by Vietnamese consumers and its determinants.

The multiple regression model can be specified as follows:

$$INT = \beta_1 * SNO + \beta_2 * ATT + \beta_3 * TRUST + \beta_4 * PBC + \beta_5 * COVID\text{-}19 + error$$

Where: INT: intention to buy local food
SNO: subjective norm
ATT: attitude towards local food
TRUST: trust in local food
PBC: perceived behavioral control
COVID-19: impact of COVID-19
β_i: estimated coefficients

RESULTS AND DISCUSSION

Profile of the respondents. The majority of the respondents were aged from 25 to 54 years. Around 56% of them were female, the rest were male. Regarding the educational level, the proportion of the respondents with the high school level was 36.7%, the number with college and vocational education level was 34.5%. The highest percentages of jobs were farmers (25.2%) and workers (17.1%). Most family incomes were under 200 million Vietnam Dong[1] per year, with an average household size of 4.2 persons. Vietnamese ethnicity occupied 95.8%, the rest were minority ethnicities (Table 3).

Table 3. Demographic characteristics of the respondents (N=286)

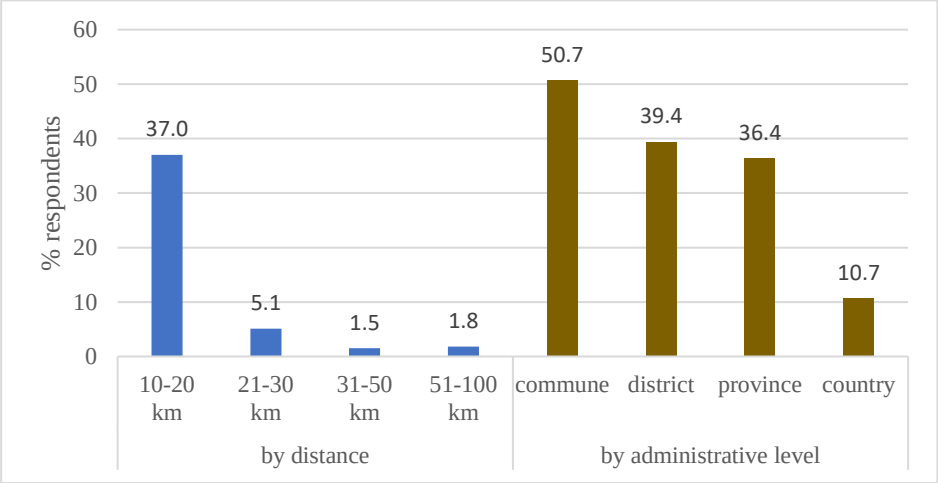
Variable	Category	Frequency	Percent (%)
Age (years)	< 24	47	16.4
	25 to 34	97	33.9
	35 to 54	66	23.1
	45 to 54	46	16.1
	55 to 64	19	6.7
	> 64	11	3.8
Gender	Male	126	44.1
	Female	160	55.9
Education	Under high school	35	12.2
	High school	105	36.7
	College and vocational	98	34.3
	University and higher	48	16.8
Occupation	Worker	49	17.1
	Farmer	72	25.2
	Officer and staff	32	11.2
	Businessman/woman	37	12.9
	Others	96	33.6
Family income (million Dong)	< 100	101	35.3
	100 to 199	125	43.7
	200 to 299	42	14.7
	300 to 399	10	3.5
	≥400	8	2.8

[1] Dong is the currency of Vietnam. 01 USD=23,095 Vietnam Dong. https://portal.vietcombank.com.vn/en-Us/Personal/TG/Pages/exchange-rate.aspx?devicechannel=default. Consulted on 28/07/2021.

Variable	Category	Frequency	Percent (%)
Ethnic	Vietnamese (Kinh)	274	95.8
	Minority ethnic	12	4.2
Total		**286**	**100**

Source: Survey data, 2021

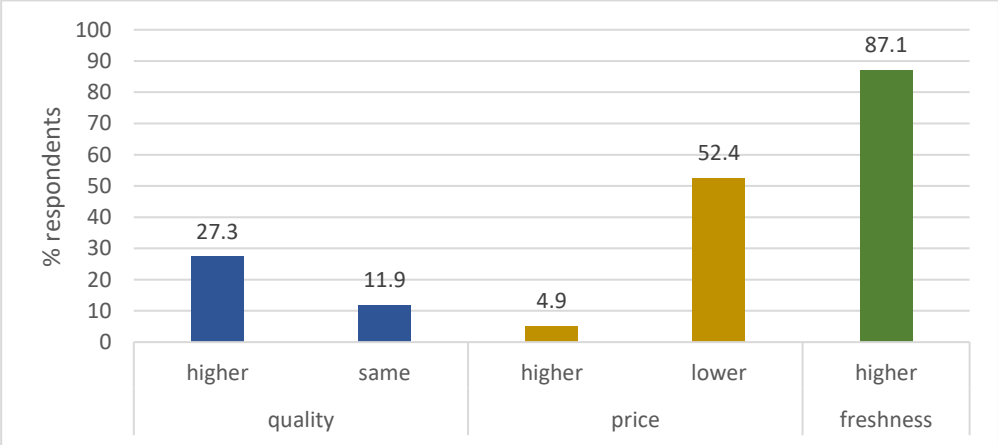
Local food is a product that is produced within their commune (50.7%), or district (39.4%), or within their province (36.4%). However, there were some people who thought that local food could be extended to the food with a national brand name, such as Vinacafe (a Vietnamese company that produces and distributes roast and instant coffee). There were 37% respondents who thought that local food is produced within 20 km from their place and 8.4% that gave higher numbers for the distance in kilometers (Fig. 2).



Source: Survey data, 2021

Fig. 2. Opinion of respondents about local food (N=286)

Most of the respondents answered that the local food was fresher (87.1%) and cheaper than other foods. Some others thought that the quality of the local food was higher (27.3%). These results can be explained by the fact that local food is produced and sold near the producer's areas. The farmers do not spend much time and cost on storage and transportation. So, the local food is normally fresher and cheaper than imported food (Fig. 3).

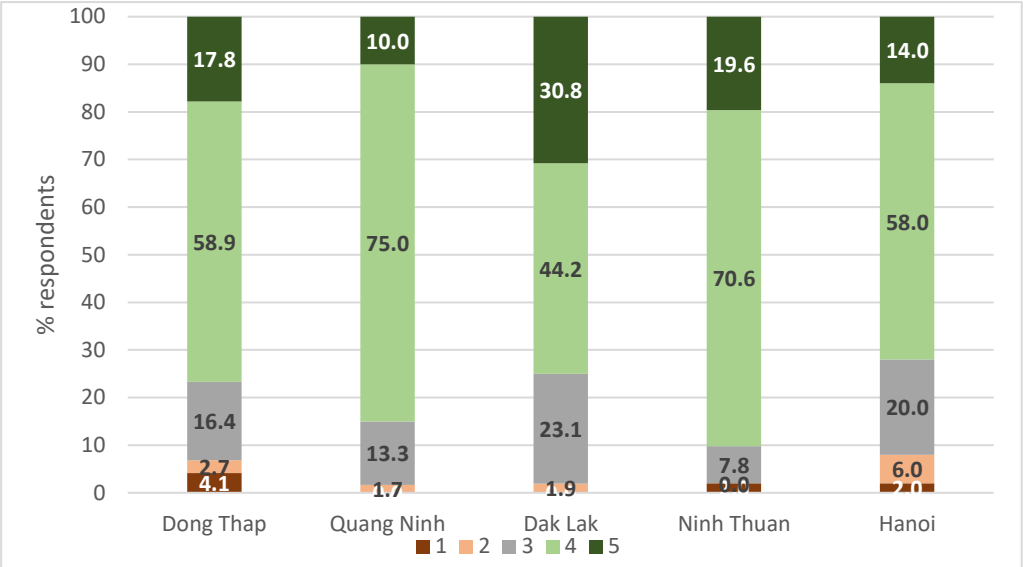


Source: Survey data, 2021

Fig. 3. Evaluation of respondents about local food (N=286)

COVID-19 pandemic has influenced the food consumption of the Vietnamese (Nga et al., 2021). Figure 4 shows the impact of the COVID-19 pandemic to the respondents by provinces. Almost all the respondents agreed or strongly agreed that the COVID-19 affected their life, with varying shares per province: Dong Thap (76.7%), Quang Ninh (85.0%), Dak Lak (75.0%), Ninh Thuan (90.2%), and Hanoi (72.0%). This has created a change in their activities

and purchasing behavior. The respondents' opinion differed among provinces due to the different impacts of the COVID-19 pandemic on different provinces before survey time.



Note: 1 strongly disagree - 5 Strongly agree
Source: Survey data, 2021

Fig. 4. Impact of COVID-19 pandemic to respondents by provinces

Descriptive statistics of items are shown in Table 4. All of the items' means are more than 3.0. Most of respondents also agreed that Covid-19 had variable effects on consumer behavior.

Table 4. Descriptive statistics of the factors

Variables		Items	Mean	Std. Deviation
INT	INT1	I intend to purchase more local food during COVID-19	3.83	.432
	INT2	I plan to purchase more and more local food from local producers during COVID-19	3.88	.366
	INT3	I am willing to buy more local food during COVID-19	3.84	.402
	INT4	I will make an effort to purchase more local food during COVID-19	4.24	.201
SNO	SNO1	My acquaintances understand me buy more local food as a wellbeing food	3.02	1.036
	SNO2	My acquaintances think that I should eat more local food	4.46	.693
	SNO3	My acquaintances approve me eating more local food	3.84	1.159
ATT	ATT1	Purchasing more local food is pleasurable	3.40	.764
	ATT2	Purchasing more local food is favorable	3.37	.796
	ATT3	Purchasing more local food is enjoyable	3.33	.805
TRUST	TRUST1	I perceive purchasing more local food to be reliable	4.02	.586
	TRUST2	Purchasing more local food appears trustable to me	3.97	.609
	TRUST3	I trust in purchasing more local food.	4.07	.554
PBC	PBC1	I can easily eat more local food whenever I want	4.61	.615
	PBC2	If I wanted to, I could easily purchase more local food	4.27	.841
	PBC3	Purchasing more local food is up to me.	3.98	.844
COVID-19	COVID1	COVID-19 pandemic had made my life change	3.38	.969
	COVID2	COVID-19 pandemic has changed society	3.20	.925
	COVID3	COVID-19 pandemic will shift my consumption behavior	3.40	.930

Source: Survey data, 2021

Exploratory factor analysis (EFA) of intention to local food. The reliability of the scale was determined by computing the alpha coefficient. The items which have a corrected item-total correlation less than 0.3 were excluded. The items with a credibility alpha of more than 0.6 was deemed acceptable as they imply that the research concept is new to the respondents (Peterson 1994; Slater 1995). Thus, those with Cronbach Alpha of more than 0.6 was used in this research in the context of Vietnam' consumers during COVID-19. After dropping INT4 because the corrected item-total correlation was less than 0.3, the results of Cronbach's Alpha reliability are shown in Table 5.

Table 5. Explorative Factor Analysis

Factors	Code	Corrected Item-Total Correlation	Cronbach's Alpha if Item Deleted	Cronbach's Alpha
Subjective norm	SNO1	.560	.572	.707
	SNO2	.570	.626	
	SNO3	.520	.653	
Attitude	ATT1	.711	.710	.818
	ATT2	.684	.736	
	ATT3	.620	.802	
Trust	CON1	.693	.755	.828
	CON2	.683	.766	
	CON3	.684	.766	
	CON4	.693	.755	
Perceived behavioral control	PBC1	.709	.782	.833
	PBC2	.724	.740	
	PBC3	.690	.778	
COVID-19	COVID1	,658	,758	.816
	COVID2	,650	,764	
	COVID3	,696	,718	
Intention	INT1	.431	.506	.614
	INT2	.355	.603	
	INT3	.491	.411	

Source: Survey data, 2021

Exploratory factor analyses (EFA) were conducted to elicit if items measuring consumer's intention to purchase local food as well as items measuring TPB constructs allow for computing the respective scales. The extraction method used was Principal Component Analysis, and the rotation method used was Varimax. The EFA obtained Kaiser-Meyer-Olkin (KMO) value of 0.702. The KMO value reflects the sampling adequacy and should exceed the minimum value of 0.6 (Hoque 2018).

The eigen value for each component should be greater than 1.0 and the cumulative variance explained should be greater than 60% (Shkeer and Awang 2019). The eigen values obtained ranged between 2.082 and 2.710; the variance explained for component 1 is 22.580%, component 2 is 19.830%, component 3 is 17.351%, component 4 is 13.038%. The total variance explained for measuring this construct is 72.799%. The results showed the four components that emerged with the respective items under each component.

The EFA procedure for exploring the measuring items for intention to purchase local food in Vietnam has resulted in four components with three items each. The total items retained were 12. The EFA of intention to purchase value for the dataset was then determined using principal axis factoring and promax rotation. Items with factor loadings below 0.45 and communalities of less than 0.5 were considered for removal Comrey and Lee (1992). The result showed that 12 items with factor loading greater than 0.7 are selected (Table 6). This result showed that measuring items could explore purchase intention to local food in Vietnam.

Table 6. Exploratory factor analyses of independent variables

Items	Component 1	2	3	4	5
Perceived Behavioral Control 1	.882				
Perceived Behavioral Control 2	.872				
Perceived Behavioral Control 3	.860				
Trust towards the behavior 2		.869			
Trust towards the behavior 1		.868			
Trust towards the behavior 3		.850			
Attitude towards the behavior 1			.871		
Attitude towards the behavior 2			.848		
Attitude towards the behavior 3			.846		
COVID2				.867	
COVID3				.862	
COVID1				.831	
Subjective Norm 2					.849
Subjective Norm 1					.813
Subjective Norm 3					.753

Source: Survey data, 2021

Factors influencing the intention to purchase local food. The extracted factors from the explanatory factor analysis were incorporated into the linear regression analysis in order to examine the impact of independent variables on the dependent variable, purchase intention (Table 7).

Table 7. Regression model – Factor influencing the intention to buy local food

Variables	Coefficients	t	p-value	Collinearity Statistics	
				Tolerance	VIF
SNO	.375	8.583	< .001	.922	1.085
PBC	.356	8.331	< .001	.961	1.040
TRUST	.404	9.408	< .001	.955	1.047
ATT	.253	5.812	< .001	.928	1.078
COVID-19	.051	1.178	.240	.929	1.077

Source: Survey data, 2021

Results showed that the model adjusted R2 = 0.508, indicating that 50.8% of the variance in purchase intention on local food is explained by the five factors SNO, PBC, TRUST, ATT, and COVID-19. With p-value< 0.001, the result confirmed that the regression model is consistent with the data collected. With p-value > 0.05, COVID-19 pandemic had no significant impact on intention to purchase local food, but the rest of the variables are statistically significant at 1% significance level. Thus, the regression model is written as:

$$INT= 0.375SNO + 0.356PBC + 0.404TRUST + 0.253ATT$$

With the regression coefficient of 0.375 for SNO and $p < 0.001$, H1 is accepted, suggesting a positive relationship between subjective norm and behavioral intention identified in this study, which is consistent with previous studies (Giampietri et al. 2018; Lam and Hsu 2006; Lim and An 2021). This result shows that sample consumers behave in such a way that the more the people around them approve of them buying local food, the more willing they are to purchase them. So behavioral intention to purchase local food is related to perceived social pressure from important people around them.

The regression coefficient 0.356 for PBC at $p<0.001$ means that the more positive the perceived behavioral control is, the higher the intention to purchase local food. This result is similar to studies of Lam and Hsu (2006), Giampietria et al. (2018), Lim and An (2020) that indicated perceived behavioral control is a significant predictor in positively influencing consumers' behavioral intention to purchase a product. A similar study conducted during COVID-19 in Indonesia and Malaysia to determine food safety and evaluated intention to practice safe eating out measures showed perceived behavioral control had a significant influence on Vietnamese behavioral intention to purchase local food in a COVID-19 pandemic context (Soon et al. 2021).

Similarly, for TRUST with coefficeint of 0.404 and p<0.001, H3 is accepted, indicating that consumer's trust is the most important factor that affects positively the intention to purchase. This positive relation is consistent with the findings of Giampietria et al. (2018) and Mazzocchi et al. (2008). The stronger the trust in the product and its seller, the higher is the intention to purchase a product. In this study, when consumers have more trust on local food and local sellers, it is easier for them to consume said local food.

With regression coefficient of 0.253 forATT and p < 0.001, H2 is accepted which means that attitude towards purchase of local food was positively related to behavioral intention. That is, if sample consumers have a more positive attitude toward local food, they will have a stronger intention to purchase local food. This finding is not surprising since Lam and Hsu (2006) and Giampietria et al. (2018) had similar results.

Finally, with a regression coefficient for COVID-19 of 0.051 with p>0.2, H5 is rejected, suggestive of the fact that perceptions of the pandemic did not alter the behavioral intention of the consumers. This result is not consistent with previous studies of Minh et al. (2021) and Kumar et al. (2021). This result may be due to earlier survey time (2021) wherein Vietnam controlled well COVID-19 pandemic, thus it did not clearly show the change in behavioral intention of the consumers.

CONCLUSIONS AND IMPLICATIONS

This study confirmed four identified determinants affecting the intention to purchase local food of consumers in the COVID-19 pandemic context. These factors are subjective norm, trust in the sellers and local food, perceived behavioral control and attitude of consumers to local food. The COVID-19 pandemic did not affect significantly behavioral intention. This may be due to the behavioral intention for local food has been present even before the COVID-19 pandemic. However, it might also be the case that the COVID-19 variable is endogenously influenced by the other factors so that the effect of the COVID-19 pandemic is also captured by the other variables.

It is recommended that policymakers, businesses, and producers promote the consumption of local food in order to better satisfy consumers' needs and get the highest business efficiency:

- Since the intention to purchase local is affected by the subjective norm, it is necessary to strengthen communication, promotion, and raising people's awareness about local food through many communication channels such as local media channels, leaflets, social networks, websites, etc., especially through the well-known people in the society.
- Producers should increase the trust of the consumers by applying good practice in the production process, providing enough and clear information on the production and processing through traceability, labels, and brand name.
- The local government should better support local producers by organizing events, festivals, tourist markets, night markets to create the chance for local consumers to join, try and buy local products.
- The national government should create a good environment and policies to promote and ensure the quality of food supply to improve the trust and change the attitude of consumers toward food supply in general and local food in particular.

Because the survey was implemented at the first period of the COVID-19 pandemic when the situation was not serious, the results would differ in the later period when COVID-19 became severe and the government applied social distancing, thus changing consumer behavior. Future research will involve in-depth qualitative interviews to get more information about the real motivation and intention to buy local food of the consumers in the post-COVID-19 period.

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EFFECTS OF TEMPERATURE ON POLLEN GERMINATION OF 'SABARA' JABOTICABA (*Plinia cauliflora* (Mart.) Kausel) *IN VITRO*

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ABSTRACT

Jaboticaba (*Plinia cauliflora* (Mart.) Kausel) is a sub-tropical fruit native to Brazil. It is cultivated in South America and Southeast Asia. 'Sabara' jaboticaba can produce fruit all year round under favorable conditions. However, sometimes fruits drop under high temperatures. This study was carried out in Tokyo University of Agriculture from 2018 to 2021. Sucrose concentration and thermal response on pollen germination *in vitro* were investigated. The favorable sucrose concentration for pollen germination was 10% while, pollen germination percentage and pollen tube length were highest at 25°C. The favorable temperatures for pollen germination were 20-30 °C. In addition, low (10-15°C) temperatures exposure time did not affect pollen germination. However, in the high temperature treatments, pollen tube length decreased at 35°C for 2 h and over, or 40°C for 1 h and over. Fruit drop in hot summer might be due to the inhibition of pollen germination under high temperatures. It is desirable to use devices such as an electric fan under high temperatures to manage temperature under greenhouse conditions and in the field when the trees are in bloom.

Key words: artificial medium, jabuticaba, pollen germination percentage, pollen tube elongation, thermal response

INTRODUCTION

Jaboticaba (*Plinia cauliflora* (Mart.) Kausel) belonging to the family Myrtaceae is native to Brazil (Wu et al. 2013; Shinohara et al. 2021; TPL 2020) and cultivated in tropical, subtropical, and temperate climates, such as South America, the USA, China, and Southeast Asia (Marica et al. 2018; Salomão et al. 2018; Mitra 2010). The fruit is consumed mainly fresh (Teixeira et al. 2011). Several processed products such as jams, jelly, juice, and wine are also known (Montes et al. 2005). 'Sabara'jaboticaba strain are the most cultivated in Brazil (Freitas et al. 2020). The fruit is dark purple, with a thin and smooth skin and a very sweet flavor. 'Sabara' can be produced throughout the year under favorable conditions. However, it has been observed that fruit dropped in hot summer in Japan. Thermal responses on reproductive physiology in many plants have been reported (Thakur et al. 2010; Zinn et al. 2010; Matsuda et al. 2015). Some studies reported fruit drop under low or high temperatures due to the inhibition of pollen germination, such as peach (Kozai 2014), loquat (Yahata and Nakai 1994), mango (Sukhvibul et al. 2000), and cherry (Hagihara et al. 2018). Previous studies on pollen

germination have been conducted on an artificial medium and in pistils in various fruits, such as persimmon (Fukui et al. 1990), cherimoya (Lora et al. 2006), salak palm (Matsuda et al. 2020), peach and durian (Kozai 2014). Pollen germination test in pistils to determine whether the pistil or pollen causes the reaction is not possible. In addition, pollen germination of each cultivar might be a factor in determining the cultivation area of cultivars in lychee (Matsuda and Higuchi 2013).

There have been no reports on the thermal response of pollen germination in jaboticaba. Jaboticaba flowers bloom early in the morning, at which time pollen are attached to the stigma. After that, pollen germinate in the pistil. Therefore, pollen were exposed to high temperatures on the stigma during the daytime in summer. Thus, temperature treatments were conducted after placing the pollen onto the medium. In this study, the optimal temperature and tolerance of low and high temperatures on pollen germination were investigated in jaboticaba.

MATERIALS AND METHODS

Plant material. 'Sabara' strain of jaboticaba (*Plinia cauliflora* (Mart.) Kausel.) were cultivated under greenhouse conditions (winter minimum temperature > 10°C) in Tokyo University of Agriculture (35.6° N, 129.6° E) in Japan. Although 'Sabara' was propagated from seed, its characteristics are stable due to polyembryonic seed.

Sample collection. Pollen were collected from at least 30 full bloom flowers early in the morning because jaboticaba flowers open early in the morning. The flowers were collected from at least three trees, ranging from 15 to 35 years of age.

Pollen germination and pollen tube length. The number of germinated pollen grains was counted from 200 pollen grains and was replicated 4 times. In all experiments, pollen germination percentage was measured using this method. Pollen possessing pollen tube longer than the pollen grain were recorded as germinated. Pollen germination was observed with a fluorescence microscope (BX53, Olympus, Tokyo, Japan). The images were obtained with an attached camera (DP70-SET-A, Olympus, Tokyo, Japan) and software (CellSens, Olympus, Tokyo, Japan). After collection of images, pollen tube length was calculated using ImageJ (National Institutes of Health, Bethesda, MD, USA).

Time-course of pollen germination. Pollen of 'Sabara' were placed onto the medium, consisting of 1 % agar and 10 % sucrose. The set-up was then incubated at 25°C under dark conditions. Pollen germination percentage and pollen tube length were measured at 3, 6, 9, 12, and 24 h after incubation. Pollen tube length was measured from 90 pollen grains.

Effects of sucrose concentration. Pollen of 'Sabara' were placed onto the medium, consisting of 1% agar with 0, 1, 10, 20, or 30% sucrose. The set-ups were then incubated at 25°C under dark conditions. Pollen germination percentage and pollen tube length were measured at 24 h after incubation. These treatments were conducted on the same day. Pollen tube length was measured from 90 pollen grains in all treatments.

Effects of temperature. Pollen of 'Sabara' were placed onto the medium, consisting of 1 % agar and 10 % sucrose. The set-up was incubated at 10, 15, 20, 25, 30, 35, or 40°C under dark conditions. Pollen germination percentage and pollen tube length were measured at 24 h after incubation. Pollen tube length was measured from 30 pollen grains at 10°C, 10 pollen grains at 40°C, and 90 pollen grains for the other treatments. Each temperature treatment was conducted on the same day.

Low and high temperature exposure time. Pollen of 'Sabara' were placed onto the medium, consisting of 1 % agar and 10 % sucrose. In the low temperature treatments (10 and 15°C), the set-up

was incubated for 0.5, 1.0, 2.0, or 3.0 h. In the high temperature treatments (35 and 40°C), the set-up was incubated for 1.5 h at 25°C after placement. These were then incubated for 0.5, 1.0, 2.0, or 3.0 h at 35 or 40°C. These were cultivated for 24 h at 25°C after low and high temperature treatments. Pollen germination percentage and pollen tube length were measured. As a control, pollen were incubated for 24 h at 25°C after placement without treatment. Pollen tube length was measured from 90 pollen grains in all treatments. These experiments were conducted because 'Sabara' flowers open early in the morning. The low temperature treatments (10 and 15°C) sought to simulate low temperatures in the early morning, while the high temperature treatments (35 and 40°C) simulated high temperatures during daytime. The low and high temperature treatments with control were conducted on the same day.

Statistical analysis. One-way ANOVA was conducted for statistical analysis of pollen germination and pollen tube length followed by Tukey test ($P < 0.01$) in the open-source statistical language R environment (Version 4.0.0., R Development Core Team 2020). Pollen germination percentage was analyzed with language R after arcsin transformation. For the effects of low and high temperatures exposure time, we conducted statistical analysis compared each temperature with control.

RESULTS AND DISCUSSION

Time-course of pollen germination. Pollen germination rate and pollen tube elongation determines the incubation time of pollen in 'Sabara' jaboticaba. The pollen germination rate reached a maximum at 6 h incubation (Fig.1). On the other hand, pollen tube elongation continued to be observed until 9 h. There were no significant changes observed in pollen germination and pollen tube elongation until 24 h. Incubation for more than 9 h therefore was sufficient for pollen germination in 'Sabara' jaboticaba.

Compared to other fruits, the time required for pollen germination in 'Sabara' jaboticaba was showed no noticeable difference. Maximum pollen germination rate and pollen tube elongation were reached at 2 h and 6 h in cherry (Beppu and Kataoka 1999) and 9 h and 18 h in lychee (Matsuda and Higuchi 2013). However, the favorable sucrose concentration to germinate pollen is differs depending on the kind of fruits (Iwanami 1980). Therefore, the favorable sucrose concentration in 'Sabara' jaboticaba was investigated.

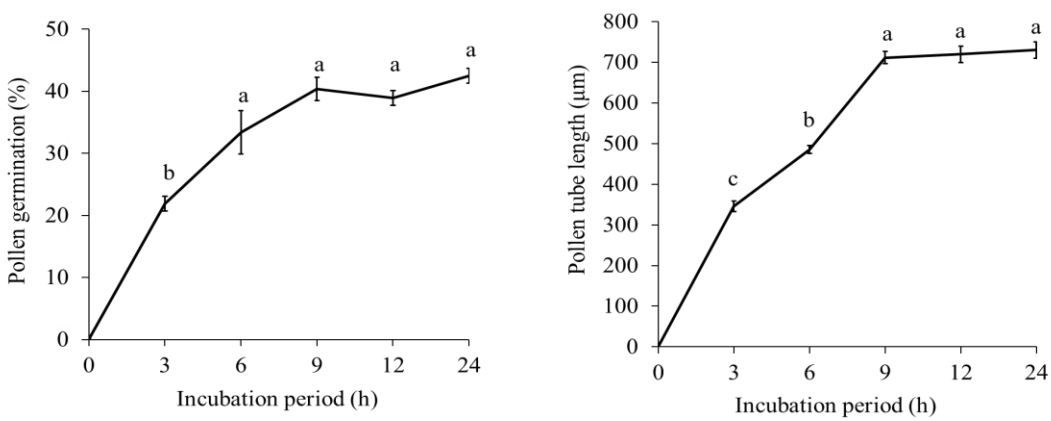

Fig. 1. Time-course of pollen germination percentage (left) and pollen tube elongation (right) in 'Sabara' jaboticaba. Pollen were incubated at 25 °C for 3, 6, 9, 12 or 24 h on the medium, consisted of 1% agar and 10% sucrose under dark condition. Vertical bars indicate S.E. Different letters indicate significant difference using Tukey test at $P < 0.01$.

Sucrose concentration effects. Sucrose concentration affected pollen germination percentage and pollen tube elongation (Fig. 2). These parameters were highest at 10 % sucrose, followed by 1 % and

20 % sucrose. Pollen germination percentage and pollen tube length were 48.1% and 656 μm, respectively for 10 % sucrose. In 30 % sucrose, these were 2.9 % and 106.1μm, respectively, while in 0 % sucrose, these were 9.1 % and 188.7μm, respectively.

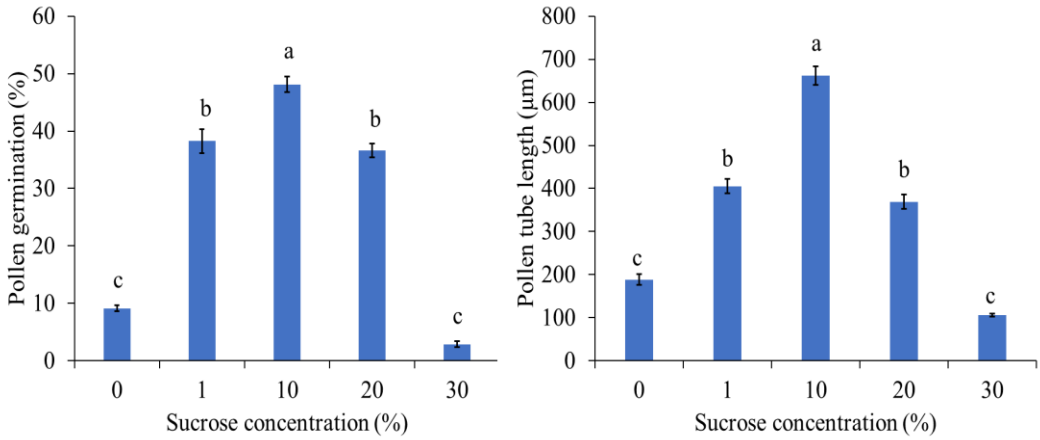

Fig. 2. Effects of sucrose concentration on pollen germination percentage (left) and pollen tube elongation (right) in 'Sabara' jaboticaba. Pollens were incubated at 25 °C for 24 h in medium supplemented with 1 % agar and each sucrose concentration under dark conditions. Different letters indicate significant difference using Tukey test at $P < 0.01$.

Temperature effects. Pollen germination percentage and pollen tube length were evaluated under different temperatures (Fig. 3). Pollen germination percentage and pollen tube length were linked in each treatment. These were highest at 25 °C. Furthermore, these were decreased to less than half at below 15 °C or above 35 °C, compared to 25 °C. In 10 °C, these were 1.75 % and 79.2μm, respectively. In 40 °C, these were 0.5 % and 88.0μm, respectively. The favorable temperatures of pollen germination in jaboticaba were 20-30 °C. Therefore, we considered that jaboticaba can produce fruit throughout the year in tropical and sub-tropical regions. Favorable temperatures for pollen germination have been reported in other tropical and sub-tropical fruits, *i.e.*, 25-27°C in avocado (Loupassaki et al. 1997), 22-25 °C in cherimoya (Yonemoto et al. 1999), 25-30 °C in lychee (Matsuda and Higuchi 2013), 20-30°C in white sapote (Yonemoto et al. 2000), 30 °C in longan (Pham et al. 2015), and 30-40°C in pitaya (Macha et al. 2006). However, pollen germination percentage and pollen tube length decreased below 15 °C or above 35 °C in 'Sabara' jaboticaba. Temperatures can be below 15 °C or above 35 °C in temperate, tropical, and subtropical regions such as South America and South Asia countries. Recently, it is over 40 °C, event in temperate regions.

Based on the results, it was suggested that jaboticaba production should be careful about low temperatures in winter and high temperatures in summer at the daytime in temperate, tropical, and subtropical regions. From practical point of view, it is desirable to use black cheesecloth or use an electric fan in cultivated areas under high temperature. Under low temperature, it is advisable to turn a heater. However, it is not known how long pollen can withstand temperatures below 15°C or above 35°C. Therefore, we investigated the effects of exposure time on pollen germination under low and high temperature conditions.

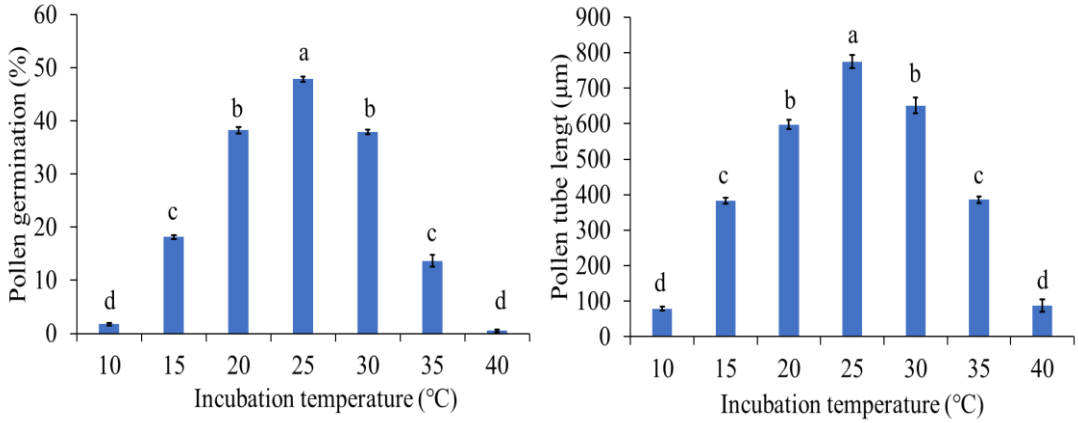

Fig. 3. Effects of temperatures on pollen germination percentage (right) and pollen tube elongation (left) in 'Sabara' jaboticaba. Pollen were incubated at each temperature for 24 h in the media supplemented with 1 % agar and 10 % sucrose under dark condition. Different letters indicate significant difference using Tukey test at $P < 0.01$.

Low and high temperatures exposure time. Although collected pollen was treated on agar medium at 10°C or 15°C for 3 h before incubation at 25°C, pollen germinated as well as those incubated at 25°C immediately after placement on the medium (Table 1). In addition to germination percentage, pollen tube length was not affected by the low temperature treatments. These results indicate that pollen of 'Sabara' can tolerate low temperatures of 10°C and 15°C for 3 h.

Table 1. Effects of exposure time in low temperatures on pollen germination and pollen tube in 'Sabara' jaboticaba.

Incubation temperature	Exposure time (h)	Pollen germination (%)	Pollen tube length (µm)
25°C	0	50.3 ± 0.5 a	802.7 ± 25.6 a
10°C	0.5	51.1 ± 2.9 a	782.9 ± 17.5 a
	1.0	53.3 ± 1.8 a	805.0 ± 16.4 a
	2.0	54.9 ± 0.7 a	802.1 ± 17.1 a
	3.0	55.9 ± 0.7 a	789.4 ± 16.8 a
15°C	0.5	51.0 ± 1.8 a	751.0 ± 13.0 a
	1.0	55.6 ± 0.9 a	770.1 ± 15.2 a
	2.0	51.7 ± 1.7 a	754.9 ± 20.0 a
	3.0	50.8 ± 2.0 a	760.0 ± 13.3 a

Different letters within a column indicate significant difference using Tukey test at $P < 0.01$.

In the high temperature treatments, pollen germination percentage was not affected by the high temperature treatments at 35 or 40 °C for 3 h (Table 2). However, pollen tube length decreased for treatments at 35°C for 2 and 3 h or at 40 °C for 1, 2 and 3 h incubation compared to control.

Table 2. Effects of exposure time at high temperatures on pollen germination and pollen tube in 'Sabara' jaboticaba.

Incubation temperature	Exposure time (h)	Pollen germination (%)	Pollen tube length (µm)
25°C	0	50.3 ± 0.5 a	802.7 ± 25.6 a
35°C	0.5	50.4 ± 1.1 a	782.8 ± 20.3 a
	1.0	53.3 ± 1.4 a	695.4 ± 21.4 a
	2.0	50.9 ± 1.0 a	647.7 ± 26.8 b
	3.0	50.3 ± 1.9 a	632.5 ± 26.3 b
40°C	0.5	52.1 ± 1.5 a	826.9 ± 19.7 a
	1.0	50.1 ± 1.2 a	606.6 ± 24.5 b
	2.0	57.0 ± 2.9 a	451.8 ± 24.1 c
	3.0	54.8 ± 2.7 a	319.2 ± 11.0 d

Different letters within a column indicate significant difference using Tukey test at $P < 0.01$. The control is the same as Table 1.

Pollen exine is composed of sporopollenin, an organic biopolymer of extremely high stability (Wiermann and Gubatz 1992). Therefore, pollen is extremely durable. Pollen from tens of thousands of years ago has been found deposited in lakes or land (Berthou and Leereveld 1990; Karrow and Anderson 1975; Nakagawa et al. 1996). It was considered that pollen germination percentage not affected in the treatments because pollen is protected by sporopollenin. On the other hand, pollen tubes are composed of cell walls containing pectin, callose, and cellulose (Aloisi et al. 2017; Mascarenhas 1993). Therefore, pollen tubes might have been more sensitive to thermal changes than pollen grains. It is therefore necessary to proactively manage temperature by using black cheesecloth roof or an electric fan, when the temperature in the cultivated area is at 35°C for less than 2 h or at 40°C for 1 h in summer at daytime.

CONCLUSION

Pollen germination percentage and pollen tube elongation *in vitro* were affected by sucrose concentration and temperature. The sucrose concentration (10%) was favorable, and pollen germination decreased at lower (0–1 %) and higher (20-30 %) sucrose concentrations. The favorable temperatures for pollen germination ranged from 20-30°C and decreased at temperatures below 15°C and above 35°C. Pollen germination was not affected by low temperatures (10-15°C). Pollen tube elongation was inferior to that at 25°C as pollen was exposed to higher temperatures (35-40°C) for a long time. Jaboticaba fruit drop in hot summer days might be due to the inhibition of pollen germination under high temperatures. It is desirable to use black cheesecloth or an electric fan in the cultivated area under high temperature and a heater under low temperature. It is not clear how pollen reacts to temperatures before anther dehiscence. Future studies on the thermal responses on pollen germination during anther dehiscence and in the pistil may reveal more details about the thermal response mechanism and improved productivity.

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ELUCIDATION OF PHYSICOCHEMICAL CHANGES IN FRUIT DEVELOPMENT OF 'SABARA' JABOTICABA (*Plinia cauliflora* (Mart.) Kausel)

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ABSTRACT

Jaboticaba is a sub-tropical fruit native to Brazil, that is cultivated in South America and Southeast Asia etc. One of the barriers for commercializing this fruit is the difficulty in distinguishing the fruit maturation stages and the fruit cannot be kept on the tree for a long time due to rapid maturation. This study aimed to understand the comprehensive maturation mechanism and make use of maturation indicators to determine the harvesting time. The primary metabolites were analyzed at three different maturation stages using high performance liquid chromatography (HPLC) and gas chromatography - mass spectrometry (GC-MS) as a data driven research. Metabolite analysis revealed the amount and the changes in individual metabolites and metabolic pathways such as the tricarboxylic acid cycle (TCA cycle), anaerobic respiration, shikimate pathway, and γ- aminobutyric acid (GABA) shunt in the process of fruit maturation. Based on the metabolome analysis, acetaldehyde and ethanol might be effective maturation indicators because these compounds changed more rapidly than sugars or organic acids. Thereafter, the physicochemical changes, including acetaldehyde and ethanol, were investigated during fruit development. The results showed that acetaldehyde and ethanol increased sharply from the ripe to overripening fruit, which could be used as the maturation indicators.

Key words: fruit firmness, fruit size, maturation indicators, metabolites composition, primary metabolism

INTRODUCTION

Jaboticaba (*Plinia cauliflora* (Mart.) Kausel), belonging to the family Myrtaceae, is native to Brazil (Shinohara et al. 2021; Wu et al. 2013b), and cultivated in tropical, subtropical, and temperate climates, such as South America, the USA, China, and Southeast Asia (Marica et al. 2018; Salomão et al. 2018). The fruit is consumed fresh (Teixeira et al. 2011b) while several processed products such as sweets, jams, juice, jelly, and wine are also known (Montes et al. 2005). It has been reported that feeding jaboticaba peel to rats prevented liver steatosis and improved insulin resistance (Lenquiste et al. 2012; Lenquiste et al. 2019). Jaboticaba fruit has the potential to contribute to human health (Wu et al. 2013a). 'Sabara' jaboticaba is cultivated mostly in Brazil (Freitas et al. 2020). The fruit is dark purple, with a thin and smooth skin, and a very sweet taste.

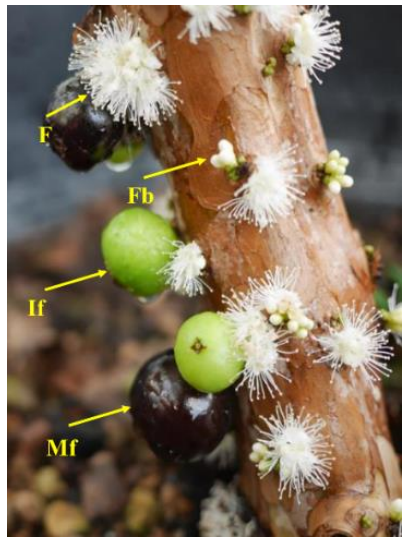

Fig 1. Jaboticaba 'Sabara' was grown in pot. Abbreviations are as follows: F, Flower; Fb, Fb, Flower bud, If, Immature fruit, Mf, Mature fruit.

However, farmers have to check each fruit visually when harvesting because the maturation stages in jaboticaba are varied. Furthermore, jaboticaba fruits mature so rapidly that the freshness of the fruit deteriorates within a few days. Therefore, it is difficult to judge the fruit maturation stages based solely on its sugars and acid content, softening, and coloration, as opposed to temperate fruit such as apple, peach, and pear, which have a long maturation period. Thus, the establishment of new maturation indicators is required.

Previous studies on fruit maturation of jaboticaba reported changes of structural and non-structural carbohydrate during fruit development (Barros et al. 1996; Magallhães et al. 1996). Some reports have studied changes of sugars, organic acids, anthocyanin, polyphenol, and ellagitannin at different maturation stages (Becker et al. 2015; Pereira et al. 2017). In addition, some reports studied comparison of organic acid, phenolic compositions, or volatile compounds among varieties (Roquim et al. 2013; Freitas et al. 2020; Jham et al. 2007). However, these studies did not propose maturation indicators. Further information such as metabolic and physicochemical changes following the fruit maturation is needed.

Metabolome analysis investigated changes of metabolites during fruit maturation for understanding the comprehensive fruit maturation, i.e., strawberry (Zhang et al. 2010), raspberry (Dincheva et al. 2013), guava (Lee et al. 2010), pitaya (Wu et al. 2019), mangosteen (Parijadi et al. 2018), peach (Lombardo et al. 2011), and pear (Oikawa et al. 2015). Data-driven research was adopted in many metabolome analyses. In other words, the theories are derived after collecting data. Data driven research in metabolome analysis is also used to search for compounds that could be indicators from metabolite data. Physicochemical changes during fruit development and maturation contribute to determine the optimal harvesting time (Candir et al. 2009; Shwartz et al. 2009) and to investigate changes in specific compounds during fruit maturation (Chapman and Horvat 1990; Ueda et al. 2000; Wu et al. 2005) in temperate, tropical, and sub-tropical fruit.

In this study, metabolome analysis was conducted to comprehensively understand fruit maturation and to search compounds as new maturation indicators. Thereafter, physicochemical changes including potential indicator compounds during fruit development were evaluated. This study

might well contribute to the comprehensive understanding of metabolism during fruit maturation and make use of maturation indicators in jaboticaba to clarify the harvesting time more clearly.

MATERIALS AND METHODS

Plant materials. Trees of 'Sabara' jaboticaba were cultivated in a greenhouse (winter minimum temperature > 10 °C) at Tokyo University of Agriculture (35.6° N, 129.6° E) in Japan.

Metabolome analysis at the three different maturation stages. Metabolite analysis of fruit was done based on its ripening stages such that: unripe fruit, ripe fruit, and overripe fruit, as shown in Fig 1. The fruits were classified into the three different maturities, based on the subjective evaluation of the texture of the fruit, skin colour, and firmness. After harvest, jaboticaba fruits were measured of its firmness, skin colour and acetaldehyde and ethanol production. Then those fruits were frozen in liquid nitrogen for the analysis of sugars, organic acids, and amino acids by HPLC and GC-MS.

Changes in physicochemical composition during fruit development. This experiment was conducted from November to December 2017. The fruits were collected at 2 or 4 days intervals. Jaboticaba fruits were measured their length, diameter, pericarp colour, firmness, respiration rate, acetaldehyde, and ethanol production.

Fruit size, skin colour, and firmness. Fruit length and diameter were measured with a caliper according to Al-Maiman and Ahmad (2002). The measurement of length was made on the polar axis of fruit, i.e. between the apex and stem. The maximum width of the fruit, as measured in the direction perpendicular to the polar axis, is defined as the diameter. The L, a, and b values of pericarp colour were measured three times per fruit using the Handy Colourimeter (NR- 3000, Nippon Denshoku IND. CO., LTD, Osaka, Japan) according to Jia et al (2005). The colour meter was calibrated with a dedicated calibration board before use. Fruit firmness was measured three times per fruit with a non-destructive firmness meter Multilateral Tester (Model 2519-104, INSTRON Company, Kanagawa, Japan) indicating the force (N) required for pressing the fruit skin at 1 mm/second using Φ 1 cm metal plug (Poyesh et al. 2018).

Ethylene, acetaldehyde and ethanol analysis. Ethylene, acetaldehyde, and ethanol were analyzed after 2 h of incubation under dark condition at room temperature. One ml of headspace gas was taken out using a plastic syringe, and acetaldehyde and ethanol were analyzed with GC-FID (GC-14B, Shimadzu, Japan), which was equipped with a Sunpack A column (Shinwa Kako, 2.1 m×3.2 mmφ, glass column filled with porous poly beads). The injector and FID were subjected to 180 and 200 °C, respectively. The initial column oven temperature was set at 80 °C for 1 minute, then increased by 10 °C per minute until 160 °C. The identification of acetaldehyde and ethanol was confirmed using standard gas.

Metabolome analysis. Metabolites were measured with GCMS-QP2010 Plus (Shimadzu, Japan), using a electron ionization, on a nonpolar phase column (DB-5, Agilent Technologies, USA) according to Yin et al (2010) and Ijima and Aoki (2009) with some modifications. Each sample (0.1 g of frozen tissue powder) was extracted with 250 µl of methanol and chloroform, one after another. After adding 50 µl of 2.0 mg/ml ribitol solution as an internal standard and 175 µl of ultrapure water, the samples were vigorously mixed. These samples were centrifuged at 12000 rpm for 10 min at room temperature. Then 80 µl of the supernatant fluid of each sample was corrected into a 1.5 ml plastic tube. These samples were evaporated to dryness for 3 h in a centrifuge evaporator (CVE-200D, TOKYO RIKAKIKAI CO, LTD, Japan). Afterwards, these samples were freeze-dried overnight using a lyophilization container (Modulyo 4K, Edwards, USA). For methylation, 40 µl of methoxylamine (20 mg/ml pyridine) was added to the samples and incubated for 90 min at 37 °C. Trimethylsilylation was performed by adding 50 µl of N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) solution for 30 min at 37 °C. The

initial column oven temperature was set at 100 °C for 4 minutes, then increased by 4 °C per minute until 320 °C. After that, it was kept for 10 minutes at 320 °C. Metabolites can be identified by comparing fragment patterns and retention indices with those of standard compounds in compound databases. The identification of lactic acid, pyruvic acid, succinic acid, α-ketoglutaric acid, Shikimic acid, glutamic acid, GABA, phenylalanine was confirmed using standard solutions; a match similarity of over 70% was accepted.

Sugar and organic acid analysis. For sugars (sucrose, glucose, and fructose) and organic acids (citric acid and malic acid), each sample (0.5 g freeze-dried powder) was extracted with 1.5 ml of ultrapure water. These samples were centrifuged at 9000 rpm for 10 min at room temperature. Then the supernatant fluid of each sample was collected into another micro-tube, and this work was repeated three times. Then, these samples were filtered through filters (Sep-Pak® Plus C18, Waters, USA, 0.45µm cellulose acetate syringe filter, Membrane Solutions Limited, USA) to prepare as organic acid analysis samples of all stages and sugar analysis samples of unripe fruit. Sep-Pak cartridge filter was activated with methanol. In addition, those filters were washed three times with the extraction sample before use. Organic acid analysis samples of ripe and overripe fruit were diluted 5-fold with ultrapure water, which were used for sugar analysis.

Sucrose, fructose, and glucose were determined using an HPLC with a differential refractometer (RID-10A, Shimadzu, Japan), on a KS-801 (8.0 mmI.D.×300mm, Shodex, Japan) and KS-G (6.0 mm I.D. ×50mm) column. Ultrapure water was used as mobile phase with a flow rate of 0.7 ml/min, and the injection volume was 5 µl. The column oven temperature was set at 80 °C. The identification of sucrose, fructose, and glucose was confirmed by using standard solutions.

Citric acid and malic acid were determined using an HPLC with a conductivity detection (CDD-10A vp, Shimadzu, Japan), equipped with two KC-811 (8.0 mmI.D. ×300mm, Shodex, Japan) and KC-G 6B (6.0mmI.D. ×50mm, Shodex, Japan) columns. $HClO_4$ aq of 3mM was used as mobile phase solution with a flow rate of 1.0 ml/min, and the injection volume was 5 µl. The column oven temperature was set at 40 °C. The identification of citric acid and malic acid was confirmed using standard solutions.

Respiration analysis. Respiration rate, measured as CO_2 released, was analyzed after two h incubation in a glass jar under the dark conditions at room temperature. One ml of headspace gas was taken out using a plastic syringe, and the amount of CO_2 was analyzed with GC-TCD (GC-14B, Shimadzu Japan), which was equipped with a column (Shinwa Kako, 2.1 m×3.2 mmφ, glass column filled with porous poly beads). The column, injector, and TCD were set at 40, 150, and 150 °C, respectively. The identification of CO_2 was confirmed using standard CO_2 gas.

Statistical analysis. Fruit samples were collected from 3 individual plants and 10 fruits were harvested per measuring day. In addition, if the conditions in the green house are constant, the metabolome is stable, and annual fluctuations and seasonal changes could be ignored. One-way ANOVA was conducted for statistical analysis of physicochemical components between the different maturation stages followed by Fisher's LSD ($P < 0.05$) in the open-source statistical language R environment (Version 4.0.0., R Development Core Team 2020). Principal component analysis (PCA) was used to investigate a relationship between different maturation stages and fruit colour, firmness, and metabolites with a commercial program (Pirouette version 4.5, Infometrix, USA).

RESULTS AND DISCUSSION

Metabolite analysis. Metabolite analysis of 'Sabara' fruit was done based on three maturation stages such that: unripe fruit (24~26 DAF), ripe fruit (36~38 DAF), and overripe fruit (56~58 DAF), as shown in Fig 2. The skin colour and firmness of each stage are shown in Table 1.

Fig 2. 'Sabara' jaboticaba fruit maturation used for metabolome analysis.

Table 1. Fruit color and firmness of 'Sabara' jaboticaba fruits used for metabolome analysis.

		Unripe fruit	Ripe fruit	Overripe fruit
Fruit color	L-value	50.50 ± 2.24 a	26.92 ± 0.68 b	22.86 ± 0.90 c
	a-value	1.09 ± 2.39 c	34.22 ± 3.96 a	26.75 ± 3.58 b
	b-value	20.99 ± 1.19 a	1.18 ± 0.79 b	1.40 ± 0.91 b
Fruit firmness (N)		6.67 ± 0.77 a	2.21 ± 0.40 b	0.48 ± 0.11 c

Data shown are the means of ten replicates ± standard error. The different letters indicate statistical significance of means in the different fruit maturation stages estimated by Fisher's LSD test ($P < 0.05$).

Sugars, organic acids, and amino acids determined in jaboticaba at each stage using GC-MS and HPLC are shown in Table 2.

Table 2. Metabolite levels of representatives of sugars, organic acids, amino acids, acetaldehyde, and ethanol in 'Sabara' jaboticaba unripe, ripe, and overripe fruits.

Metabolites	Unripe fruit	Ripe fruit	Overripe fruit
Fructose (mg/gFW)	15.14 ± 0.61 c	31.39 ± 1.43 b	84.34 ± 2.10 a
Glucose (mg/gFW)	7.23 ± 0.35 c	22.22 ± 0.99 b	51.77 ± 2.66 a
Sucrose (mg/gFW)	18.14 ± 1.95 c	62.34 ± 3.37 b	72.02 ± 3.13 a
Citric acid (mg/gFW)	30.52 ± 0.77 a	25.83 ± 1.04 b	18.57 ± 0.76 c
Glutamic acid (µmol/gFW)	1.56 ± 0.22 a	0.19 ± 0.04 b	-
Ketoglutaric acid (nmol/gFW)	172.14 ± 4.70 a	77.32 ± 8.15 b	216.50 ± 43.00 a
Malic acid (mg/gFW)	3.65 ± 0.17 a	0.95 ± 0.09 b	0.31 ± 0.02 c
Lactic acid (µmol/gFW)	2.94 ± 0.17 c	4.42 ± 0.31 b	12.17 ± 0.48 a
Pyruvic acid (nmol/gFW)	538.07 ± 13.46 a	200.16 ± 11.92 b	82.93 ± 6.66 c
Shikimic acid (µmol/gFW)	8.28 ± 0.76 a	2.07 ± 0.13 b	1.12 ± 0.07 b
Succinic acid (nmol/gFW)	70.51 ± 3.97 b	105.32 ± 5.55 b	278.36 ± 30.18 a
Aminobutyric acid (µmol/gFW)	0.12 ± 0.02 b	0.44 ± 0.06 b	4.18 ± 0.35 a
Phenylalanine (nmol/gFW)	9.79 ± 0.06 a	-	12.67 ± 1.95 a
Acetaldehyde (nl/g/hr)	0.52 ± 0.05 c	0.79 ± 0.03 b	29.00 ± 3.17 a
Ethanol (nl/g/hr)	1.47 ± 0.07 c	3.34 ± 0.26 b	1149.79 ± 152.92 a

Data are shown in the means of ten replicates ± standard error. The different letters indicate statistical significance of means in the different fruit maturation stages estimated by Fisher's LSD test ($P < 0.05$); - indicates not detected.

The maturation process from unripe into ripe fruit is accompanied with the increase of sugars and the decrease of organic acids. Sucrose (3.4-fold), fructose (2.1-fold), glucose (3.1-fold), acetaldehyde (1.5-fold), ethanol (2.3-fold), and lactic acid (1.5-fold) show significant increased from unripe to ripe fruit. Citric acid (0.9-fold), malic acid (0.3-fold), α-ketoglutaric acid (0.5-fold), glutamic acid (0.1-fold), pyruvic acid (0.4-fold), and shikimic acid (0.3-fold) showed significant decreased from unripe to ripe fruit. On the other hand, phenylalanine could not be detected at ripe fruit. GABA (3.5-fold) and succinic acid (1.5-fold) increased from unripe to ripe, but significant differences were not found between unripe and ripe fruit.

The maturation progress from ripe into overripe fruit showed the increase of sugars and several organic acids as well as acetaldehyde, ethanol, and lactic acid. Sucrose (1.1-fold), fructose (2.7-fold), glucose (2.3-fold), α-ketoglutaric acid (2.8-fold), succinic acid (2.6-fold), GABA (9.6-fold), lactic acid (2.8-fold), acetaldehyde (37-fold), and ethanol (340-fold) showed significant increased from ripe to overripe fruit. Citric acid (0.7-fold), malic acid (0.4-fold), and pyruvic acid (0.4-fold) showed significant decrease from ripe to overripe fruit. Shikimic acid (0.5-fold) decreased from ripe to overripe fruit, but no significant difference was found between ripe and overripe fruit. Glutamic acid could not be detected at overripe fruit.

Acetaldehyde and ethanol production increased more than sugars, organic acids, and amino acids from ripe to overripe fruit. metabolites with rapid changes during fruit maturation should be used as maturation indicators because jaboticaba fruit has an extremely short optimal harvesting time. Acetaldehyde and ethanol appear to be better than sugars or organic acids as maturation indicators.

Principal component analysis (PCA) on physicochemical parameters was conducted to compare their metabolites at three different maturation stages (Fig 3). The maturation process is divided into three stages, and two principal components could explain 98.2% of the overall variance of the metabolome analysis, with 72.5% and 25.7% for PC1 and PC2, respectively.

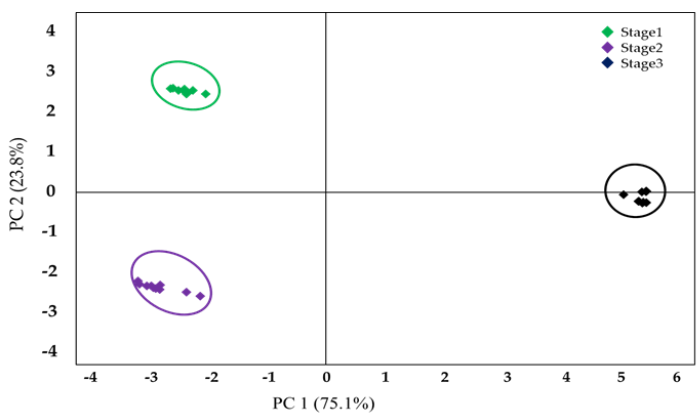

Fig 3. Score plots on principal component analysis (PCA) of physicochemical parameters derived from metabolome analysis at the different maturation stages of jaboticaba fruit. Color in green, purple, and black indicate unripe, ripe, and overripe fruit, respectively. Data of ten replicates for each stage are plotted.

The contribution of each metabolite against main principal component loading is shown in Fig 4, referring to the correlation coefficient between original variables and the PC1. The positive value indicates a positive correlation and vice versa. The strongest correlation is shown in glutamic acid (negative), followed by phenylalanine (positive), acetaldehyde (positive), ethanol (positive), and GABA (positive).

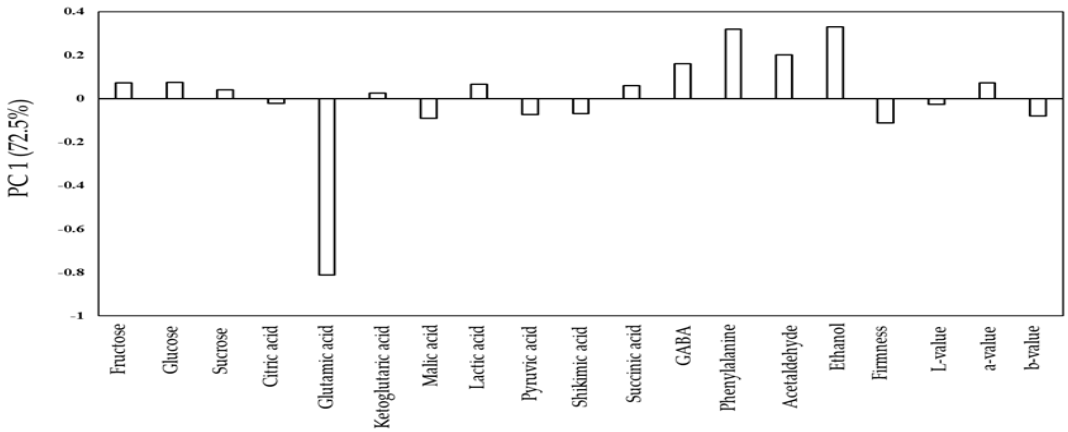

Fig 4. Contribution of each metabolite against main principal component loadings of physicochemical parameters derived from metabolome analysis at the different maturation stages of jaboticaba fruit.

A metabolic map constructed according to the results, highlighting the primary metabolic processes: glycolysis, TCA cycle, GABA shunt, shikimate pathway, and anaerobic respiration by referring to the literature (Iijima and Aoki 2009; Ji et al. 2020; Thimm et al. 2001; Tohge et al. 2013; Yin et al. 2010; Zhang et al. 2010). There observed significant metabolic changes (P <0.05) among unripe, ripe and overripe fruit as highlighted on the metabolic map (Fig 5).

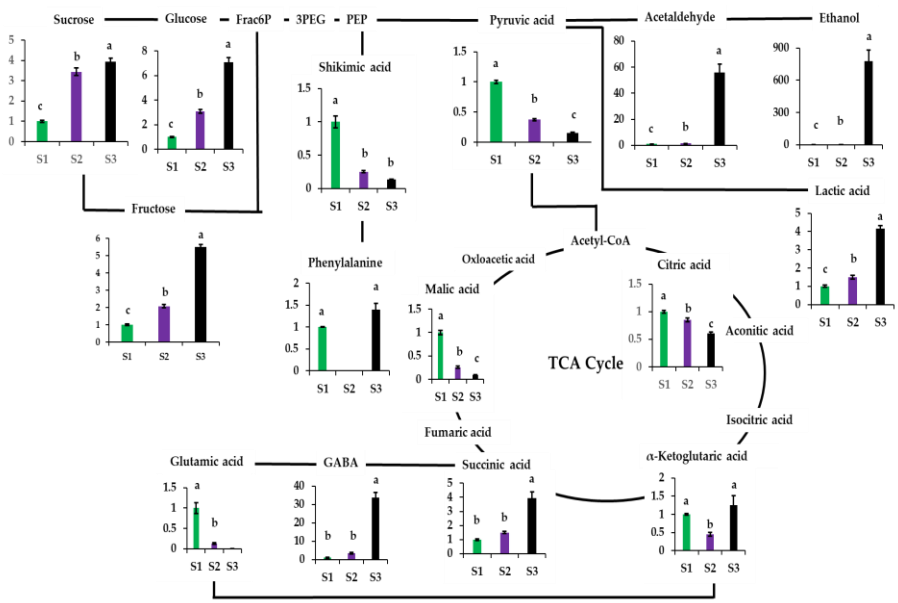

Fig 5. The changes in metabolites' level from unripe to overripe fruit are shown according to the primary metabolism such as glycolysis, TCA cycle, shikimic pathway, GABA shunt. Names of metabolites in black or grey indicate detected or not detected, respectively. The bar graphs of the three different maturation stages in each component are calculated as relative values against unripe fruit. The different letters in bar graphs indicate the statistical significance of means by Fisher's LSD test (P < 0.05). Abbreviations are as follows: S1, unripe fruit; S2, ripe fruit; S3, overripe fruit; Frac6P, fructose-6-phospate; 3PGA, 3-phosphoglycerate; PEP, phosphoenolpyruvic acid; and GABA, γ- aminobutyric acid.

The increase in sugars and decrease in citric and malic acids were observed throughout maturation. Sucrose has the highest amount, followed by fructose and glucose in case of unripe and ripe fruit. In overripe fruit, fructose has the highest amount, followed by sucrose and glucose. Sucrose increases sharply from unripe to ripe fruit, while it is not significant between ripe and overripe fruit. Citric acid and malic acid decrease significantly throughout the maturation. The results reflect the taste changes, with increase in sweetness and decrease in sourness.

Succinic acid increased throughout maturation. There was a significant difference between ripe and overripe fruit, but not between unripe and ripe fruit. GABA increases while glutamic acid decreases from ripe to overripe fruit. It has been reported increase GABA throughout fruit maturation in mangosteen (Parijadi et al. 2018) and grape (Ali et al. 2011). From the viewpoint of human health, GABA had positive effects such as reducing stress and sleep induction (Dhakal et al. 2012; Okada et al. 2000). The amount of GABA in overripe fruit of jaboticaba is 4.18 μmol/gFW which is higher than tomato, apple, and peach (Akihiro et al. 2008; Shang et al. 2011; Trobacher et al. 2013).

The shikimate pathway links the metabolism of carbohydrates such as tyrosine, phenylalanine, and tryptophan (Schmid and Amrhein 1995). Furthermore, anthocyanins are produced via the phenylpropanoid pathway with phenylalanine as a precursor (Cheng and Breen 1991). Phenylalanine decreased from unripe to ripe fruit but increased from ripe to overripe fruit. Therefore, the changes of phenylalanine content might reflect the anthocyanins accumulation and decomposition.

Alcohol and lactic acid fermentation are regarded as a typical anaerobic metabolism (Du et al. 2018). Acetaldehyde, ethanol, and lactic acid increased sharply from ripe to overripe fruit. Ethanol is produced from pyruvic acid via acetaldehyde, involving the enzymes; pyruvate decarboxylase (PDC, EC 4.1.1.1) and alcohol dehydrogenase (ADH, EC 1.1.1.1), respectively (Ke et al. 1994; Tadege et al. 1999). In case of lactic acid fermentation, it is involved with the enzyme; lactate dehydrogenase (LDH, EC 1.1.1.27) (Pegoraro et al. 2012). In both courses, pyruvic acid is the first component in alcohol and lactic acid fermentation. In this experiment, pyruvic acid has decreased throughout maturation. Therefore, it is suggested that fermentation processes from pyruvic acid to acetaldehyde, ethanol, and lactic acid work unceasingly. They have been shown to accumulate during maturation in various fruit in temperate, tropical, and subtropical fruit tree (Beltrán et al. 2015; Chervin et al. 1999; Fuggate et al. 2010).

It was suggested that if acetaldehyde and ethanol could be established as new maturation indicators, these compounds could be applied to other fruits. In the case of some citrus fruits, the ethanol content of fruit juice is known as a maturation indicator (Pesis 2005). This might as well contribute to distinguish jaboticaba fruit that are ready to be eaten fresh. Based on such metabolic changes, future experiments should focus on acetaldehyde and ethanol production to check the possibility of their use as maturation indicators.

Changes in physicochemical composition in fruits. The changes in fruit size of jaboticaba were shown in Fig 6. The transverse and longitudinal of fruit were similarly developed. The fruit size hardly changed from 8 to 10 DAF, then increased constantly from 10 to 30 DAF. Fruit size did not change significantly until 52 DAF. The period of complete fruit growth has been reported as about 160 DAF in pear (Pei et al. 2020), 85 DAF in peach (Ishida et al. 1973), 90 DAF in blueberry (Shimura et al. 1986), 60 DAF in grape (Minemura et al. 2009), 45 DAF in strawberry (Pei et al. 2020), 40 DAF in cherry (Ren et al. 2011), and 25 DAF for pitaya (Jamaludin et al. 2011). Jaboticaba fruit reached its maximum growth at 34 DAF. Jaboticaba fruit growth was shown to be significantly faster than those fruits except for pitaya. Three types of fruit growth patterns have been reported: single sigmoid curve such as avocado, pear, and pineapple, double sigmoid curve such as blueberry, grape, and peach, and triple sigmoid curve such as kiwifruit (Coombe et al. 1976; Pratt and Reid 1974). Jaboticaba fruit grew in a way to form a single sigmoid curve during fruit development in accordance with the observation of

previous studies (Teixeira et al. 2011b).

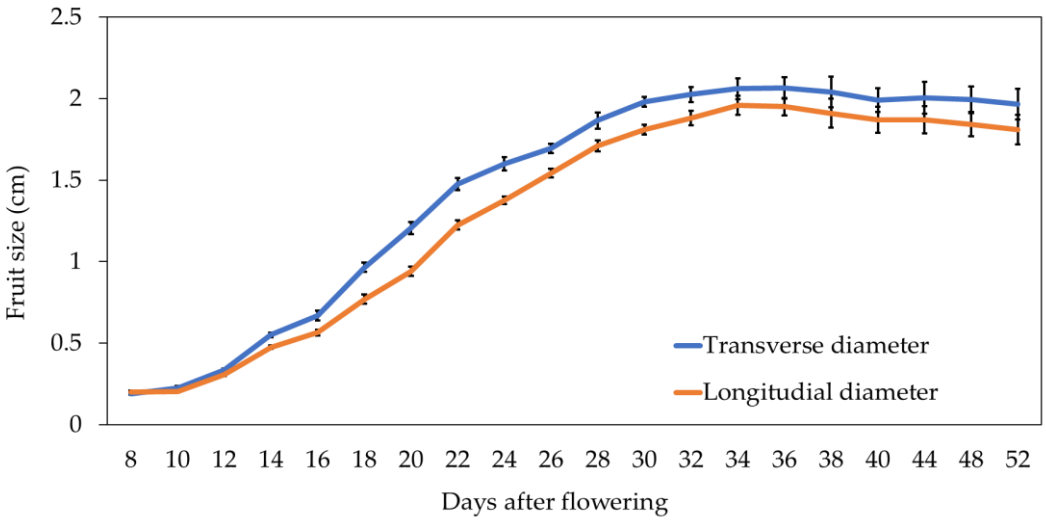

Fig 6. Changes in fruit size of 'Sabara' jaboticaba from 8 DAF to 52 DAF. Data are shown in the means of ten replicates and vertical bars represent standard errors.

The coloration parameters of the fruit skin during fruit development and maturation were shown in Fig 7. There were no significant changes in a value from 24 to 26 DAF. Then, it increased dramatically until 32 DAF. Thereafter, it decreased significantly from 40 to 52 DAF. The L and b values showed no significant changes from 24 to 30 DAF. Consequently, the values decreased quickly from 30 to 34 DAF. After that, the values gradually decreased until 52 DAF.

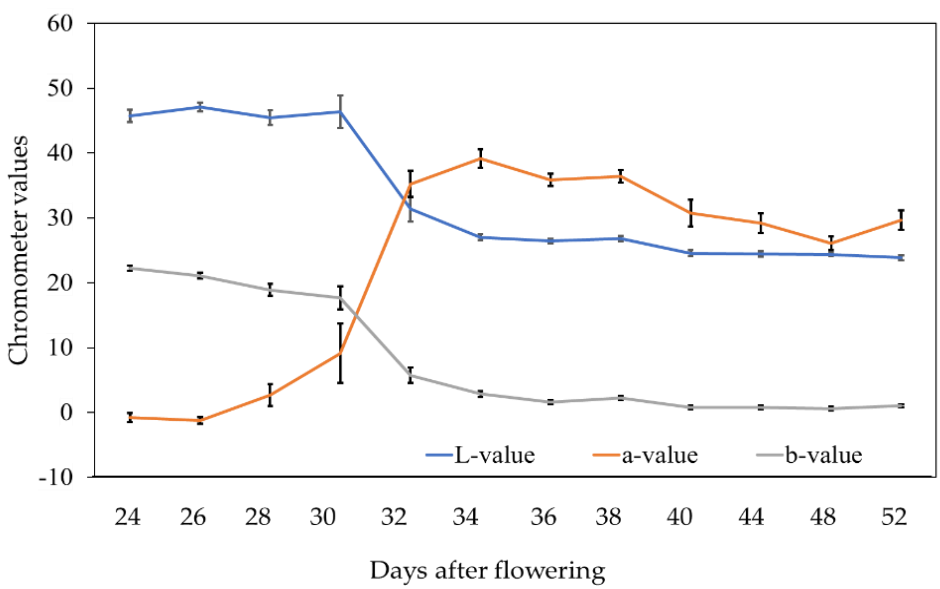

Fig 7. Changes in fruit skin color (L, a, and b value) from 24 DAF to 52 DAF. Data are shown in the means of ten replicates and vertical bar represents standard error.

The colouration period has been reported as about 45 days in grape (Minemura et al. 2009; Coombe and McCarthy 2000.), 20 days in guava (Deepthi. 2017), 15 ~ 20 days in cherry (Muskovics et al. 2006), 14 days in blueberry (Shimura et al. 1986), 6 days in strawberry (Yoshida et al. 2002), 3~5 days in pitaya (Jamaludin et al. 2011; Wu et al. 2019). The colouration period in jaboticaba fruit was about 10 days, almost the same as strawberry. It was considered that the increase of a-value and the decrease of b-value in jaboticaba might be reflective of anthocyanin accumulation and chlorophyll decomposition at the ripening stage (Becker et al. 2015). In addition, phenylalanine decreased from unripe to ripe fruit, and increased from ripe to overripe fruit in the metabolome analysis. Phenylalanine is closely related to the synthesis of anthocyanins. In particular, the decrease in a-value and b-value might be related to the decrease or restriction of anthocyanin synthesis after 40 DAF, since it is reported that anthocyanin content decreases at the late maturation stage in temperate, subtropical, and tropical fruits (Bureau et al. 2009; Faragher and Brohier 1984; Kulkarni and Aradhya.2005; Rogez et al. 2011).

The changes in respiration rate during fruit maturation in jaboticaba were shown in Fig 8. Respiration rate decreased dramatically from 24 to 28 DAF. Then, it decreased gradually from 28 to 52 DAF. Although climacteric fruits exert a peak in respiration during fruit ripening (Faragher and Brohier 1984; Rooban et al. 2016), the peak cannot be detected in jaboticaba. Therefore, it was concluded that jaboticaba is a non-climacteric fruit as in the previous studies (Duarte 2007; Teixeira et al. 2011a). Jaboticaba fruit ripening during postharvest is difficult as in the climacteric type, such as tomato and banana (Giovanelli et al. 1999; Kulkarni et al. 2011). Thus, it requires full ripening on the tree.

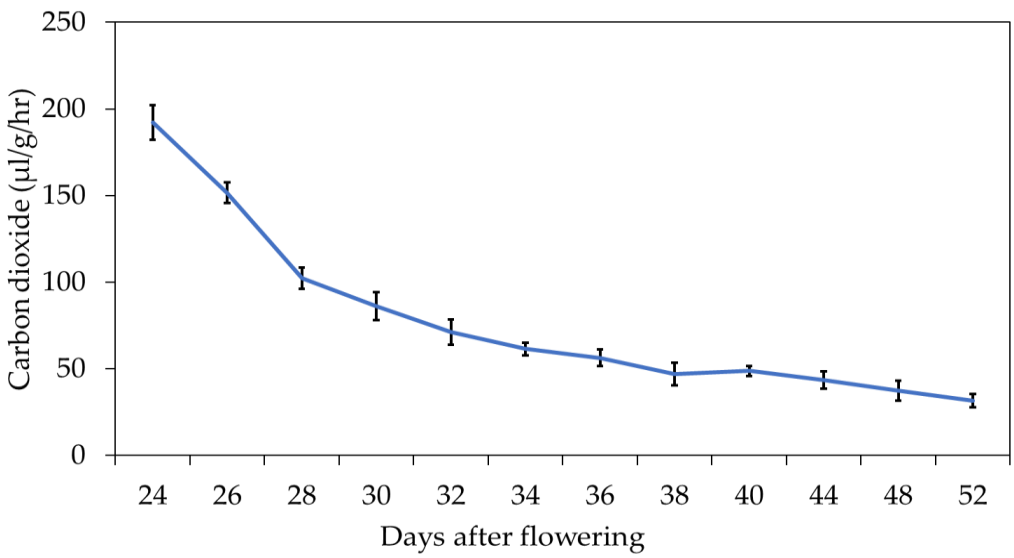

Fig 8. Change in respiration of 'Sabara' jaboticaba from 24 DAF to 52 DAF. Data are shown in the means of ten replicates and vertical bar represents standard error.

The changes in fruit firmness of jaboticaba during fruit development and maturation are presented in Fig 9. Fruit firmness remained high from 24 to 26 DAF. Then dramatic softening from 26 DAF to 32 DAF was recorded. Thereafter, a gradual decrease was observed from 32 DAF to 52 DAF. The period from the start of the decline in fruit firmness to the completion of coloration has been reported as about 30 days in cherry (Muskovics et al. 2006), 20 days in blueberry (Forney et al. 2012), 10 days in guava (Bashir and Abu-Goukh 2003), 7 days in pitaya (Wanitchang et al. 2010). Jaboticaba completed coloration 8 days after fruit firmness began to decline.

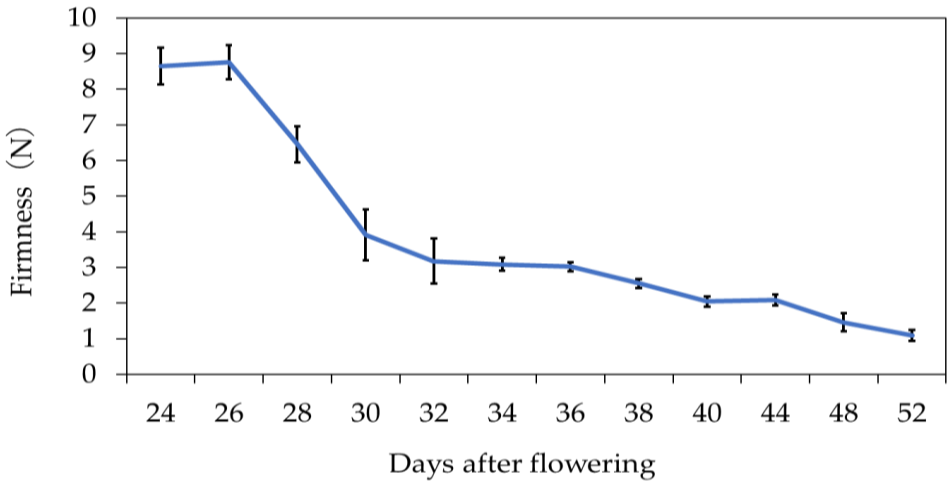

Fig 9. Changes in firmness of 'Sabara' jaboticaba fruits from 24 DAF to 52 DAF. Data are shown as the means of ten replicates and vertical bar represents standard error.

The changes in acetaldehyde and ethanol in fruits during maturation were shown in Fig 10 A and B. Acetaldehyde remained low until 34 DAF. However, it increased constantly from 34 DAF to 52 DAF. Jaboticaba fruit produces 5 nl/g/hr of acetaldehyde at 52 DAF. Ethanol remained low in concentration until 38 DAF. Then it increased rapidly from 38 DAF to 52 DAF. Jaboticaba fruit produced 85 nl/g/hr of ethanol at 52 DAF. Acetaldehyde and ethanol increased after 34 DAF and 40 DAF, respectively. The high amount of ethanol is known to affect the fruit taste significantly (Ueda et al. 2019) and the overripe fruit may not be suitable to be eaten fresh. Since the induction of acetaldehyde release precedes ethanol production by four days, acetaldehyde release would be a more accurate indicator for harvest. The maturity of jaboticaba fruit would be distinguished by the detection of acetaldehyde or ethanol using simple and non-destructive methods such as the test seal. Test seal has been reported to detect acetaldehyde and ethanol to predict abnormal fruits of melon (Agatsuma and Oshima 1981). The color of the test seal changes when fruit produces acetaldehyde or ethanol. Therefore, acetaldehyde and ethanol might be detected easily without using an analytical instrument such as GC.

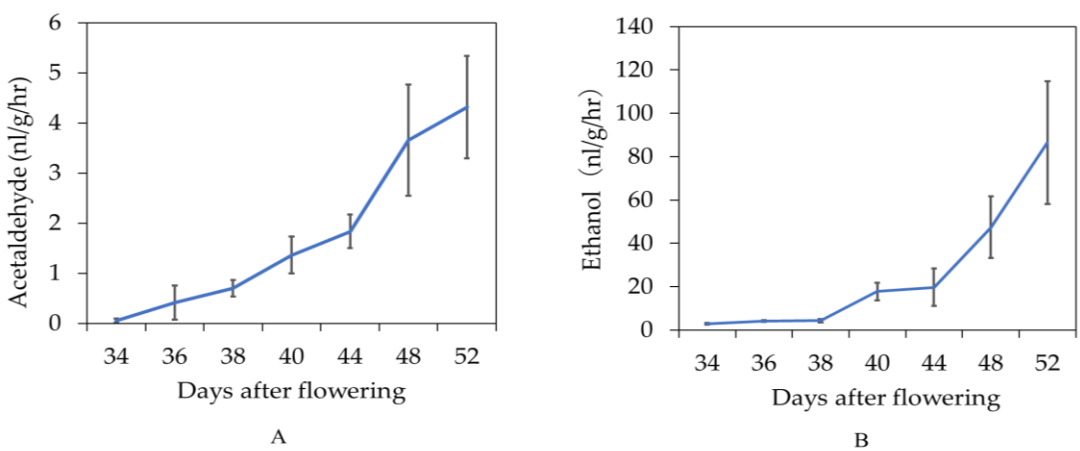

Fig. 10. Changes in acetaldehyde (A) and ethanol (B) production rate of 'Sabara' jaboticaba from 34 DAF to 52 DAF. Data are shown in the means of ten replicates and vertical bar represents standard error.

The maturation of jaboticaba fruit can be classified into 4 stages. The unripe stage is until 26 DAF, when fruit skin color is still green, and fruit firmness is high. The ripening stage is from 26 to 34 DAF, when fruit skin color changes from green to dark-purple, and fruit firmness starts to decrease. The mature stage is from 34 to 40 DAF, when fruit skin color and fruit firmness have settled down and acetaldehyde begin to increase. The overmature stage is after 40 DAF, when fruit firmness is more reduced and acetaldehyde and ethanol increase dramatically.

CONCLUSION

Metabolome analysis at three maturation stages and monitored physicochemical changes during fruit maturation in jaboticaba revealed that sugars increase, and organic acids decrease as the fruit matures. GABA shunt and anaerobic respiration such as alcohol and lactic acid fermentation can be activated from the ripe to the overripe stage. The maturity of jaboticaba fruit might be distinguished through its acetaldehyde and ethanol content. Although the overripe fruit of jaboticaba is not suitable to be eaten fresh because of the higher ethanol content, it has the potential as a healthy food due to its high amount of GABA when a suitable processing is developed. This study provides insights on how to distinguish fruit maturity and understand the comprehensive fruit maturation in jaboticaba. In order to further elucidate the metabolism of fruit maturation, enzyme activities, genetic information, and secondary metabolites should be investigated.

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NUCLEOTIDE SEQUENCE-BASED IDENTIFICATION OF *Lentinus* ISOLATES FROM LUZON ISLAND, PHILIPPINES

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ABSTRACT

Lentinus species (Polyporaceae) are saprophytic, wood-rotting basidiomycetous mushrooms with significant nutritional and pharmacological values. In this study, the molecular identity and variability of 15 *Lentinus* isolates collected from different areas in Luzon Island, Philippines were investigated. Genomic DNA from fresh *Lentinus* mycelia was extracted, PCR amplicons-amplified using ITS1F and ITS4BR primers were sequenced and the consensus sequences were compared against NCBI GenBank database. Phylogenetic trees were constructed using Maximum Likelihood (ML) method with Kimura-2-parameter model in Molecular Evolutionary Genetics Analysis (MEGA X). Results of BLASTn analysis of ITS consensus sequences confirmed the molecular identities of seven *Lentinus* species including *L. tigrinus*, *L. squarrosulus*, *L. sajor-caju*, *L. strigosus*, *L. swartzii*, *L. glabratus* and *Panus* (*Lentinopanus*) *conchatus* showing similarities that ranged from 97.90% to 100% (with 99% to 100% query coverage) with their corresponding GenBank reference sequences. The ML phylogenetic tree supported the distinct grouping of isolates of each *Lentinus* species, showed the separation of *L. squarrosulus* CPS5 from the other two *L. squarrosulus* isolates (LSQBot and LSQOs), and the separation of both *L. strigosus* isolates and *P. conchatus* from other *Lentinus* taxa, indicating their own separate lineage. In conclusion, ITS rDNA is a useful genetic marker for molecular identification and species differentiation of Philippine *Lentinus* isolates.

Key words: *Lentinus* species, wild edible mushrooms, phylogeny, ITS rDNA, CPS5.

INTRODUCTION

Mushrooms are important resources of functional food and bioactive metabolites.They have a wide variety of bioactive compounds, which have been shown to exhibit several biological activities including antioxidant, anticancer, antitumor, anti-inflammatory, immunomodulatory, anti-aging, anti-diabetic, anti-hypertension, anti-obesity, anti-atherosclerotic, anticoagulant, anti-hyperlipidemia, antimicrobial, antiviral, anti-hepatitis, antimalarial, anti-schistosome infection, hepatoprotective, neuroprotective, cardiovascular-protective, hypo-sexuality, anti-osteoporotic, anti-arthritic, improve metabolic syndrome, immune functions, and gastrointestinal health (Ashraf et al. 2020; Samarasinghe and Waisundara 2020; Su et al. 2020; Blumfield et al. 2020; Lin et al. 2019). Polysaccharides such as lentinan, grifolan, pleuran, ganoderan, schizophyllan, krestin, and polysaccharide peptide (PSP) isolated from *L. edodes*, *G. frondosa*, *P. ostreatus*, *G. lucidum*, *S. commune*, and *T. versicolor*, respectively,

have been well-characterized and widely reported for their medicinal properties, particularly anticancer and immunomodulatory activities (Meng et al. 2016; Rathore et al. 2017; Zhang et al. 2019; Chakraborty et al. 2021).

In the exploration and exploitation of the numerous advantages of mushrooms, accurate identity is very important. Many studies have been made to molecularly identify and distinguish variations among closely related mushrooms using different genetic barcodes. Schoch et al. (2012) reported the use of six genes namely, *RPB1*, *RPB2*, *nLSU*, *nSSU*, *mSSU*, and *TEF1* by the consortium of Assembling the Fungal Tree of Life (AFTOL). However, the International Fungal Barcoding Consortium formally recommended that the internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA gene cluster be used as the primary barcode for fungal identification (Schoch et al. 2012). Analysis of ITS regions confirmed the identity of 38 macrofungal isolates from Iran belonging to 22 species, 19 genera, 10 families and 5 orders (Alimadadi et al. 2019) and *Hericium* species, *H. alpestre*, *H. coralloides* and *H. erinaceus*, collected in Italy (Cesaroni et al. 2019). Moreover, ITS1 and ITS4 sequence analysis revealed the identity of 3 *Termitomyces aurantiacus*, 8 *Tricholoma matsutake*, 2 *Tricholoma robustum*, 4 *Pleurotus ostreatus*, *Schizophyllum commune* and *Pleurotus pulmonarius* (Adeniyi et al. 2018) and non-gilled mushrooms *Auricularia polytricha*, *Ganoderma lucidum*, *Auricularia auricula-judae*, and *Trametes elegans* collected from North Western Himalayas (Singh and Tripathi 2018).

Lentinus species (Polyporaceae, Basidiomycota) are normally wood-decaying mushroomsgrowing solitary or more often in groups on water-soaked logs or trunks of trees, which are morphologicallycharacterized by firm to tough round pileus with light brown to blackish scales, saw-toothed edges, white to yellowish underside gills, and with scaly white stipe fruiting bodies (Dulay et al. 2020a). They are widely distributed in lowland and upland areas in the Philippines, mostly during the months of May to October. They have been recorded and documented in various ethnomycological and species listing studies conducted in different areas of Luzon Island, including six Aeta tribal communities in Pampanga, Zambales, Tarlac (De Leon et al. 2013), Gaddang communities in Nueva Vizcaya (Lazo et al. 2015), Bazal-Baubo Watershed, Aurora (Tadiosa et al. 2011), Mt. Bangcay, Cuyapo, Nueva Ecija (Dulay and Maglasang 2017), Central Luzon State University Campus, Science City of Muñoz, Nueva Ecija (Culala and Dulay 2018), Sitio Canding, Barangay Maasin, San Clemente, Tarlac (Dulay et al. 2020b), Mt. Makiling Forest Reserve, Los Baños, Laguna (De Castro and Dulay 2015), and Mt Palay- Palay / Mataasna Gulod Protected Landscape, Southern Luzon (Arenas et al. 2015).

The Central Luzon State University (CLSU) is one of the 27 Tuklas Lunas Development Centers (TLDC) in the Philippines, and the only one that focuses on the exploration of Philippine wild mushrooms for their medicinal properties and development of functional foods. The CLSU-TLDC has established its culture collections of successfully isolated wild mushrooms collected from the different areas of Luzon Island, Philippines. However, most of the isolated mushroom species were identified based on their morphology, which is sometimes not conclusive. Thus, the present study investigated the molecular identification of these wild mushrooms using the internal transcribed spacer (ITS) region of the rRNA genes in our intention to establish their accurate identity, which is essential for further exploration of their significant position in various applications.

MATERIALS AND METHODS

Mushroom culture. Pure cultures of the different isolates of *Lentinus* species were acquired from the culture collections of the Center for Tropical Mushroom Research and Development, and Tuklas Lunas Development Center, Department of Biological Sciences, College of Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. The fruiting bodies of *Lentinus* species growing in their natural habitat are shown in Figure 1.

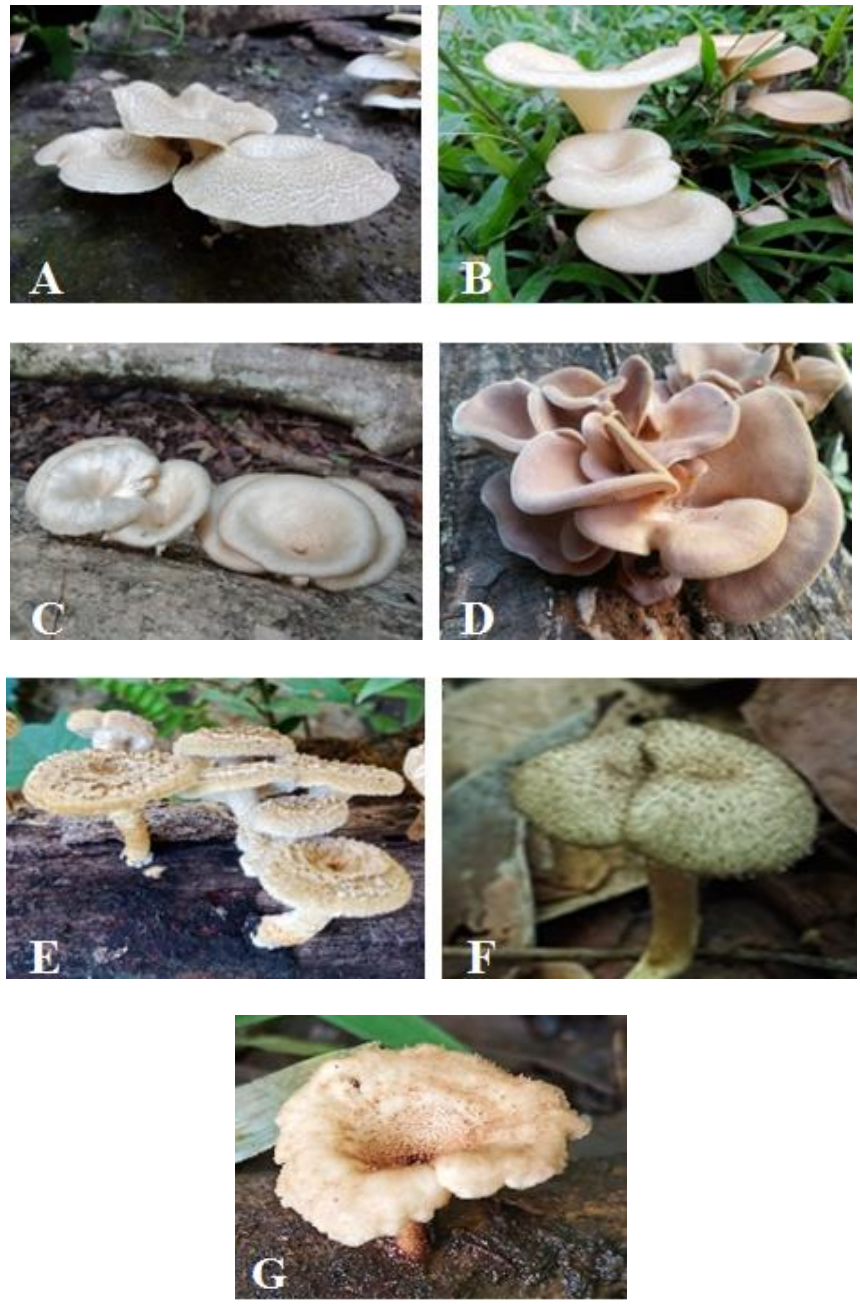

Fig. 1. Wild fruiting bodies of *Lentinus tigrinus* AS21 (A) from Poblacion, Alilem, Ilocos Sur; *L. squarrosulus* LSQOs (B), *L. sajor-caju* C005 (C), *L. strigosus* BIL1324 (D), *L. swartzii* BIL4618 (E), and *L. glabratus* CVS22 (F) from Science City of Muñoz, Nueva Ecija; and *P. conchatus* (G) from So. Canding, Maasin, San Clemente, Tarlac.

The presumptive identity based on the morphological characteristics, culture code, and place of origin of the 15 *Lentinus* isolates are summarized in Table 1. Agar blocks of mycelia were sub-cultured on potato dextrose agar (PDA) plates and incubated at 28°C, alternating light and dark

condition for seven days to allow mycelial growth. These cultures were used as source of the DNA samples for molecular identification.

Table 1. The different isolates of *Lentinus* collected from the different areas of Luzon.

Presumptive Identity*	Isolate code	Place of origin
Lentinus tigrinus	DQS75	Dolores, Quezon
	BP32	Binanuaanan, Camarines Sur
	AS21	PoblacionAlilem, Ilocos Sur
Lentinus squarrosulus	LSQBot	Botolan, Zambales
	CPS5	Patagueleg, Cagayan
Lentinus sajor-caju	LSCBot	Botolan, Zambales
	C005	Lingap Kalikasan, CLSU Campus, Muñoz, Nueva Ecija
Lentinus strigosus	BIL1324	RM Cares, CLSU Campus, Muñoz, Nueva Ecija
	CL-01	Trailer House, CLSU Campus, Muñoz, Nueva Ecija
Lentinus swartzii	CL-02	Trailer House, CLSU Campus, Muñoz, Nueva Ecija
	BIL4618	Bioassay, CLSU Campus, Muñoz, Nueva Ecija
Lentinus glabratus	CVS 22	Lingap Kalikasan, CLSU Campus, Muñoz, Nueva Ecija
	CVS 29	Lingap Kalikasan, CLSU Campus, Muñoz, Nueva Ecija
Lentinopanus conchatus	A52	So. Canding, Maasin, San Clemente, Tarlac
Lentinus sp.	LSQOs	Osmeña, CLSU Campus, Muñoz, Nueva Ecija

*Presumptive identity was based on the morphological characteristics of the 15 *Lentinus* isolates.

Genomic DNA extraction. The genomic DNA was extracted from the fresh mycelia of mushrooms. A 100 mg mycelial sample from each mushroom was crushed using mortar and pestle with liquid nitrogen, and the ground sample was transferred into 2 mL-capacity microtube. A 600 µL volume of pre-warmed 2× cetyltrimethylammonium bromide (CTAB) buffer (20 g of CTAB dissolved in 860 mL sterile double distilled water, 100 mL of 1M Tris pH 8.0, 81.82 g of NaCl, and 40 mL of 0.5M EDTA pH 8.1) and 70 µL of 20% sodium dodecyl sulfate (SDS) were added into each sample, mixed using a vortex, and incubated at 65°C for 45 min in a dry bath (LabnetAccublock™ Digital Dry Bath, USA). After cooling, 600 µL of chloroform (1 isoamyl alcohol: 20 chloroform) were added, mixed thoroughly using a vortex, and centrifuged (Centurion Scientific Refrigerated Centrifuge, UK) at 10,000 rpm for 30 min. The upper layer was transferred into 1.5 mL-capacity microtube. Then, 600 µL of ice-cold isopropanol were added and incubated overnight at -20°C. After incubation, the mixture was centrifuged at 10,000 rpm for 10 min, and the isopropanol was decanted. The pellet was washed with 500 µL of 70% ethanol and centrifuged at 10,000 rpm for 3 min. Washing was done twice. Ethanol was decanted and the microtubes were inverted on a clean paper towel for about 10 min to completely remove any traces of ethanol. The DNA pellet was re-dissolved in 100 µL 1× Tris EDTA buffer (10 mM Tris, pH 8.0 and 1 mM EDTA pH 8.0) and allowed to stand at room temperature for 3-4 hrs. To check the quality of the DNA, a mixture of 1 µL of DNA and 1 µL of 2× loading dye was prepared and loaded onto 1% agarose gel with 1 µL of FloroSafe DNA stain (Axil Scientific Pte Ltd, Singapore), run in gel electrophoresis system (Mupid® - One Electrophoresis, Japan) at 100 V for 30 min, and visualized using Labnet GDS-1302 Enduro Imaging System, USA. The fungal genomic DNA samples were quantified using spectrophotometer (Thermo Scientific™ Multiskan™ GO Microplate Reader), and the final concentration was adjusted to 100 ng/µL using 1× Tris EDTA buffer and stored at 4°C.

PCR amplification. PCR amplification of the ITS rDNA was carried out using primer pair ITS1 Forward (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4B Reverse (5'-CAGGAGACTTGTACACGGTCCAG-3') (Gardes and Bruns 1993). The ITS1F/ITS4BR primer pair has been shown to be particularly useful for detection and analysis of the basidiomycete component

(Gardes and Bruns 1993), and has been widely used for the molecular identification of several mushrooms (Adeniyi et al. 2018; Alimadadi et al. 2019). The 25 µL PCR reaction mixture contained the following: 4 ng of fungal genomic DNA, 0.4 µM of each ITS primer, 1 mM of deoxynucleoside triphosphates (dNTPs) mix, 1× PCR buffer, 1.5 mM of MgCl2, 0.5 U of KAPA Taq DNA polymerase (KAPABiosystems Manufacturing, South Africa), and 16.9 µL of nuclease-free water. The PCR Applied Biosystem® 2720 Thermal Cycler (Applied Biosystems, California, USA) conditions were programmed as follows: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 51°C for 30 sec, extension at 72°C for 60 sec, and final cycle of extension at 72°C for 10 min. The quality of PCR amplicons were checked following the same method described in the preceding section. The expected amplicon size was approximately 850 bp.

DNA sequencing and sequence alignment. PCR amplicons were sent to Apical Scientific SdnBhd in Malaysia for outsourced DNA purification and double pass DNA sequencing. The sequence data generated using the forward primer and reverse primer were edited, aligned, and the consensus sequences were determined using BioEdit 7.2 (Hall 1999). The nucleotide sequence comparisons were performed using the standard nucleotide Basic Local Alignment Search Tool (BLASTN) against the National Center for Biotechnology Information (NCBI) GenBank database.

Phylogenetic analyses. Phylogenetic analysis was conducted in Molecular Evolutionary Genetics Analysis (MEGA) version X (Kumar et al. 2018). The nucleotide sequences were aligned using sequence alignment program MUSCLE algorithm. The ML best-fit substitution model was generated with maximum likelihood statistical method and selected based on the Bayesian Information Criteria (BIC) score. The phylogenetic tree was constructed by using maximum likelihood method with Kimura-2-parameter (K2P) substitution model (Kimura 1980). A discrete gamma distribution was used to model evolutionary rate variation among sites (5 categories). The alignment gaps were treated using Use All Sites method. Bootstrap analysis was performed with 1,000 replicates to evaluate confidence levels of the clades in the phylogenetic tree (Felsenstein 1985). Tree was rooted using *Pleurotus ostreatus* as the outgroup taxon.

RESULTS AND DISCUSSION

Mushrooms are high-valued mycoresources of nutritious food and bioactive metabolites for nutraceutical and pharmacological applications. With the high interest to the numerous benefits of mushrooms, it is imperative to establish their accurate identity especially the unrecorded and underutilized naturally occurring mushrooms. Mushrooms are usually identified based on their morphological structures and unique additional features such as annulus or ring, volva or cup, and veil present in the fruiting body. However, this method is sometimes not conclusive because of the inconsistent morphology.

DNA barcoding is a novel diagnostic tool in providing accurate identity of the species of Basidiomycota (Badotti et al. 2017). Several studies reported the use of this tool to identify wild and cultivated mushrooms, grocery-store fresh and powdered mushrooms, and mushroom-based dietary supplements, to confirm misidentified mushroom species, and to compare different genetic markers and establish the most suitable target markers for specific mushroom group (Adedokun et al. 2016; Raja et al. 2017; Badotti et al. 2017; Fernández-López et al. 2018; Li et al. 2018; Wang et al. 2019; Gunnels et al. 2020; Dulay et al. 2020c).

PCR-amplified products, ITS sequences, and BLASTn identities of *Lentinus* isolates. In the present study, the molecular identities of 15 *Lentinus* isolates collected from Luzon Island, Philippines using the ITS region of rDNAs were established. PCR amplification using the ITS1F and ITS4BR primer pair produced single bands of approximately 850 bp.

BLASTn analysis of the nucleotide sequences of the ITS rDNA amplicons generated using ITS1F and ITS4BR primers through homology search in GenBank database confirmed that the PCR-amplified products were *Lentinus* mushrooms. The morphological identities and results of the BLASTn analysis of rDNA-ITS sequences of the different isolates of *Lentinus* species are summarized in Table 2. The nucleotide sequences of all *Lentinus* isolates showed 97.90% to 100.00% similarity to their respective GenBank sequences with 99% to 100% query coverage. It can be noticed that most *Lentinus* nucleotide sequences showed <100% similarity to their GenBank accession sequence equivalent, which indicates sequence divergence between Philippine *Lentinus* and the deposited *Lentinus* sequences in the GenBank repository.

Table 2. Morphological identities and BLASTn analysis results of the rDNA-ITS sequences of the different isolates of *Lentinus* species and the reference sequences from GenBank.

Isolate / Strain Code	Morphological Identity	BLASTn Identity	Accession Code	Identity (%)	Query Cover (%)
DQS75	*L. tigrinus*	*L. tigrinus*	OM102521	99.86	100
BP32	*L. tigrinus*	*L. tigrinus*	OM102522	99.71	100
AS21	*L. tigrinus*	*L. tigrinus*	OM102523	99.29	100
LSQBot	*L. squarrosulus*	*L. squarrosulus*	OM102524	99.03	100
CPS5	*L. squarrosulus*	*L. squarrosulus*	OM102525	98.44	99
LSQOs	*Lentinus* sp.	*L. squarrosulus*	OM102526	99.03	100
LSCBot	*L. sajor-caju*	*L. sajor-caju*	OM102527	98.60	100
C005	*L. sajor-caju*	*L. sajor-caju*	OM102528	97.90	100
BIL1324	*L. strigosus*	*L. strigosus*	OM102529	99.58	100
CL-01	*L. strigosus*	*L. strigosus*	OM102530	99.58	100
CL-02	*L. swartzii*	*L. swartzii*	OM102531	99.39	100
BIL4618	*L. swartzii*	*L. swartzii*	OM102532	99.39	100
CVS 22	*L. glabratus*	*L. glabratus*	OM102533	100.00	99
CVS 29	*L. glabratus*	*L. glabratus*	OM102534	100.00	99
A52	*L. conchatus*	*P. conchatus*	OM102535	100.00	100
LE214778	-	*L. tigrinus*	KM411459.1	-	-
JZ26	-	*L. squarrosulus*	MG719283.1	-	-
MEL:2382718	-	*L. sajor-caju*	KP012899.1	-	-
LE5829	-	*L. strigosus*	KM411451.1	-	-
EB1101	-	*L. swartzii*	KT956124.1	-	-
AP8	-	*L. glabratus*	KF860882.1	-	-
UOC SIGWI S24	-	*P. conchatus*	KR818817.1	-	-

Raja et al. (2017) mentioned that it is reasonable to start with ≥97% to 100% sequence similarity (i.e., <3% sequence divergence) and ≥80% query coverage for assigning species name based on the results of BLASTn analysis in the GenBank database, since the calculated average weighted infraspecific ITS variability in fungi was 2.51±4.57% (Nilsson et al. 2008). Therefore, the percentage identities and the query coverage values of all *Lentinus* isolates obtained in the present study are within the above-mentioned values, confirming their acceptable and valid molecular identities.

Interestingly, the morphological identities of 13 *Lentinus* isolates conformed with their molecular identities. However, the LSQOs isolate, which was morphologically identified down to the genus level only (*Lentinus* sp.), was molecularly identified down to the species level as *Lentinus squarrosulus*. On the other hand, the A52 isolate, which was morphologically identified as *Lentinopanus conchatus*, showed 100% homology with *Panus conchatus*. According to the Mycobank database

(www.mycobank.org), *P. conchatus* (Bull.) Fr. 1838 (MB#160358) is synonymous to *Lentinus conchatus* (Bull.) J. Schrot. 1889 (MB#456032) and *Lentinopanus conchatus* (Bull.) Pilat 1941 (MB#333101). Accordingly, *P. conchatus* was also regarded as one of the species of *Lentinus* by some mycotaxonomists.

Previously, the molecular identities of other Philippine wild mushrooms including *Agaricus erectosquamosus*, *Agaricus haematinus*, *Agaricus parvibicolor*, *Auricularia polytricha*, *Auricularia asiatica*, *Chaetocalathus conchatus*, *Chlorophyllum molybdites*, *Clitopilus prunulus*, *Coprinopsis clastophylla*, *Cyathus crassimurus*, *Cymatoderma elegans*, *Dacryopinax spathularia*, *Deconica coprophila*, *Earliella scabrosa*, *Favolus acervatus*, *Ganoderma australe*, *Ganoderma gibbosum*, *Gymnopilus dilepis*, *Gymnopus melanopus*, *Hexagonia tenuis*, *Hohenbuehelia grisea*, *Hymenagaricus taiwanensis*, *Marasmius leveilleanus*, *Marasmius tenuissimus*, *Oudemansiella canarii*, *Panus conchatus*, *Phallus merulinus*, *Polyporus tenuiculus*, *Polyporus thailandensis*, *Pterula echo*, *Schizophyllum commune*, *Trametes hirsuta*, *Xanthagaricus taiwanensis*, *Xylaria anisopleura* and *Xylaria escharoidea* were confirmed with 97.16% to 100% similarity using ITS region of rDNA (Dulay et al. 2020c). Similarly, molecular identification using ITS region confirmed the identities of Philippine mushrooms such as *Lentinus strigosus*, *Cookeina insititia*, *Psathyrella typhae*, *Panus conchatus*, *Pleurotus pulmonarius*, *Xylaria papulis*, *Ganoderma lucidum*, *Schizophyllum commune*, *Coprinellus aureogranulatus*, *Lentinus swartzii*, and *Panaeolus foenisecii* (Ramel 2018; Undan et al. 2016; Lopez et al. 2016). The ITS region is a good DNA marker for identification of *Lentinus* spp.

Phylogeny of *Lentinus* species. Maximum likelihood tree showed the distinct clades of isolates of each *Lentinus* species with 99% to 100% bootstrap support (Fig. 2). The first clade showed the grouping of *L. tigrinus* isolates (BP32, DQS75 and AS21) with *L. tigrinus* (KM411459.1) reference sequence, which is found more related to the second clade, the group of two *L. glabratus* isolates (CVS29 and CVS22) and reference sequence (KF860882.1). The other side branch revealed the closer relationship of *L. swartzii* isolates (BIL4618 and CL-02) and *L. squarrosulus* isolates (LSQBot and LSQOs) and their GenBank reference sequences, the third and fourth clade. However, it can be noticed that *L. squarrosulus* CPS5 separated from these two *L. squarrosulus* isolates. These four clades were closely related to the group of *L. sajor-caju* isolates (LSCBot and C005) as the fifth clade. Both *P. conchatus* and the two *L. strigosus* isolates separated from the other *Lentinus* taxa. The ML phylogenetic tree rooted in *Pleurotus ostreatus*, which was used as an outgroup taxon.

The separation of CPS5 from the other two isolates of *L. squarrosulus* and the similarity of *L. squarrosulus* isolates with the two different GenBank accession codes (MG719283.1 and JQ868749.1) in the BLASTn analysis strongly suggest that there are two different strains of *L. squarrosulus* present in the isolates studied. In the alignment of sequences of *L. squarrosulus* isolates, several nucleotide variations were found including 30 positions of basepair substitutions, six positions of adenine and cytosine deletions and three positions of thymine, adenine and cytosine insertions. This shows that nucleotide variations in the ITS rDNA region exist among *Lentinus* isolates belonging to the same species. This nucleotide variation may be attributed to the differences on the climatic condition of the specific regions where the mushrooms were collected. The Philippines has four types of climates depending on the distribution of rainfall and the period of the dry season. The Provinces of Nueva Ecija and Zambales, where *L. squarrosulus* isolates LSQOs and LSQBot originated, respectively, are under climate type 1, while Cagayan Valley, where CPS5 isolate was found, is under the type 3 climate zone, which has a yearlong warm condition. Similarly, *Hericium* mushrooms, including six species and 23 isolates from different geographic origin showed nucleotide sequence variation in both ITS1 and ITS2 regions (Park et al. 2004). Oyetayo (2014) suggested that variations in the ITS nucleotide sequences might be due to the different ecological regions where the mushrooms are growing. In addition, the shared similarity can be diminished or erased after some time because of the changing environmental conditions (Konstantinidis and Stackebrandt 2013).

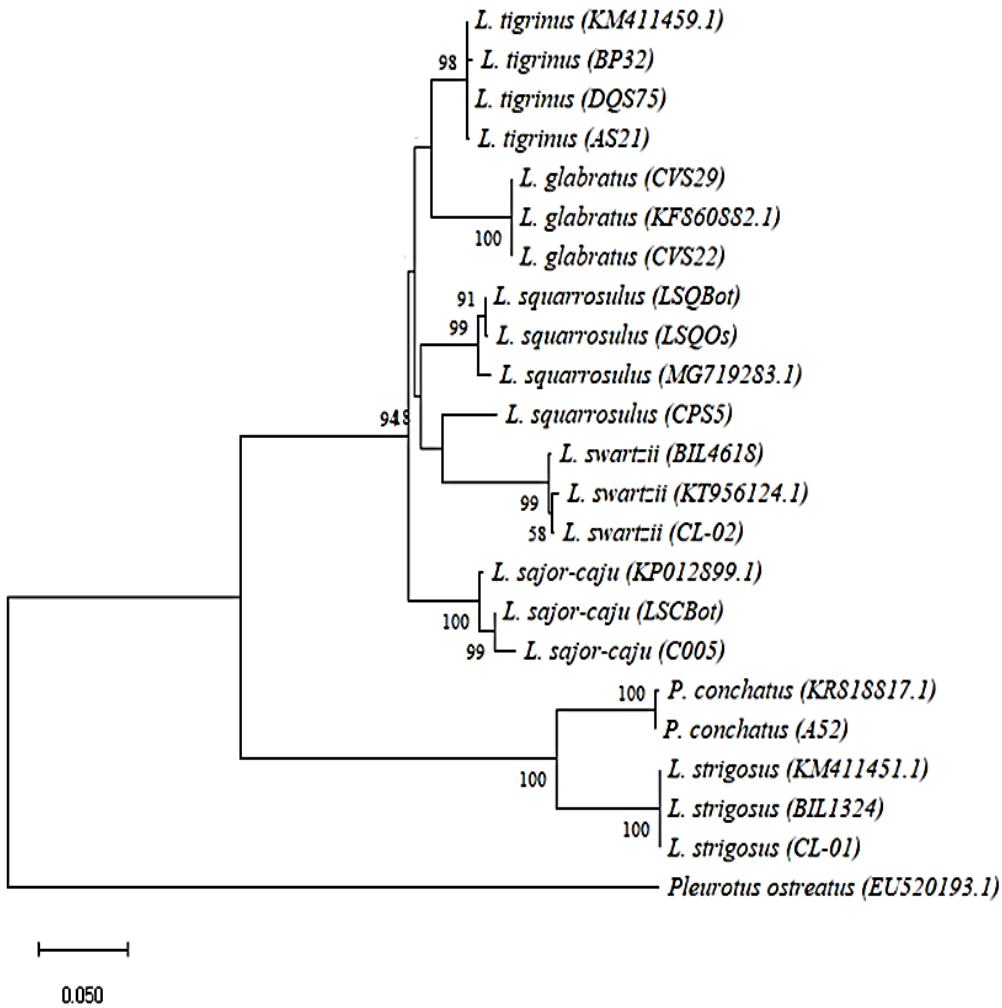

Fig. 2. Phylogenetic tree of 15 *Lentinus* isolates recovered from the Maximum Likelihood analysis using MEGA X. Bootstrap values were based on the 1000 replicates. Scale bar represents the substitution per site. *Pleurotus ostreatus* was used as an outgroup taxon.

Moreover, the separation of both *P. conchatus* and the two *L. strigosus* isolates from the other *Lentinus* taxa observed in the ML tree suggests their own separate lineage. In the BLASTn analysis, one of the equivalent GenBank sequences of *L. strigosus* isolates CL-01 and BIL1324 nucleotide sequences was *Panus lecomtei* (JQ955726.1) with 99.71% similarity and 96% query cover. Therefore, *L. strigosus* was also regarded as *Panus* species. The phylogenetic relationships between *Lentinus* and *Panus* and their allies have been controversial. On the basis of their hyphal morphology, some mycologists consider *Lentinus* and *Panus* as separate genera (Corner 1981), while other authors treat *Panus* as a subgenus of *Lentinus* (Pegler 1983). However, Binder et al. (2013) consider *Lentinus* within the core polyporoid clade and *Panus* within the residual polyporoid clade based on the phylogenetic and phylogenomic analyses.

Altogether, the molecular identities of 15 Philippine *Lentinus* isolates up to the species level and their phylogenetic relationships were established in the study.

CONCLUSIONS

ITS rDNA is a suitable genetic marker for molecular identification, characterization, and species differentiation of the 15 *Lentinus* isolates, and for the establishment of their phylogenetic relationships. With the observed degree of divergence in the ITS rDNA sequences of *Lentinus* isolates with those previously deposited GenBank sequences, there is a need to deposit the unique nucleotide type culture of indigenous Philippine *Lentinus* into the GenBank database to sufficiently load the database with Philippine mushroom sequences. The molecular identities of *Lentinus* species are very useful in the evaluation of their growth performance, biomass production and biological activities.

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BIOCHEMICAL CHARACTERIZATION OF A LANTHANUM-ELICITED INSECTICIDAL COMPOUND IN ACTINOBACTERIA

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ABSTRACT

This study sought to determine the effect of Lanthanum elicitation on the insecticidal activity of *Streptomyces angustmyceticus* strain CGS B11 on the Asian corn borer (ACB), *Ostrinia furnacalis*. Lanthanum belongs to one of the rare earth elements and is found effective in eliciting secondary metabolite production in Actinobacteria. CGS B11 was isolated from volcanic soil collected in Daraga, Albay Province, Philippines. Bioassay-guided fractionation of crude extracts from CGS B11 revealed two compounds that were of comparable insecticidal activity to Spinosad. Lanthanum elicitation in CGS B11 produced one unique compound with Retention factor= 0.33 as detected by thin-layer chromatography. The compound has significantly enhanced insecticidal activity with median lethal concentration (LC_{50}) = 0.41 ng/cm^2 to neonate larvae of *O. furnacalis*. The relative potency of the Lanthanum-elicited insecticidal compound is more than 20 times greaterthan Spinosad (LC_{50}= 9.25 ng/cm^2). Further analysis by Fourier Transform Infrared Spectroscopy detected a peak at 3365.93 cm^{-1}, which indicated the presence of hydroxyl group in crude extract derived from Lanthanum-treated CGS B11. Taken together, the results of this study showed that bioprospecting from volcanic soil coupled with the use of rare earth elicitation with Lanthanum proved to be a very effective approach in enhancing potency of a potentially new insecticidal compound in strain CGS B11. The new information revealed by this study opens the avenue of using rare earths as a feasible method of eliciting new mode of action insecticides from Actinobacteria for application in insect resistance management.

Key words: *Streptomyces*, activation, fermentation, Fourier Transform Infrared Spectroscopy, bioassay

INTRODUCTION

Actinobacteria are a group of Gram-positive bacteria found in terrestrial and aquatic ecosystems (Anandan et al. 2016). They play important ecological roles such as nutrient recycling, degradation of complex polymers and production of bioactive molecules (van der Meij et al. 2017, Bhatti et al. 2017). Most of the reported bioactivities of Actinobacteria focusedon natural products with pharmaceutical and other industrial applications (Thatoi et al. 2013). However, Actinobacteria are also capable of producing environment-friendly insecticidal compounds (Kirst 2010; Barka et al. 2016). Avermectin from *S. avermitilis* (Lasota and Dybas 1991) and spinosyns from *Saccharopolyspora spinosa* (Kirst 2010) are classic examples. In the Philippines, Actinobacteria is a neglected resource of natural products for insect pest management. To date, only a few strains collected from various sources in the country were reported to contain insecticidal compounds (Bayot-Custodio et al. 2014; Creencia et al.

2021). Other reported local studies are focused on antimicrobial properties of Actinobacteria (Basilio et al. 2003; Parungao et al. 2007; Zulaybar et al. 2017).

Bioprospecting for culturable Actinobacteria from neglected or extreme habitats is reported to be an effective strategy for discovery of novel bioactive compounds (Goodfellow and Fiedler2010). Such approach reduces the chance of rediscovery of previously known compounds from commonly sampled environments (Hassan et al. 2019). High actinobacterial diversity was observed in volcanic caves (Riquelme et al. 2015). It is a specific habitat that has high potential for the isolation of new bioactive compounds (Selim et al. 2021).

Antimicrobial compounds were also discovered from Actinobacteria isolated from volcanicsoil (Cheah et al. 2015, Jia et al. 2016). Chemical elicitation with rare earth elements (REEs) was used mainly for elicitation and overproduction of antimicrobial compounds in Actinobacteria (Antoraz et al. 2015, Begani et al. 2018). The REEs consist of 17 elements including Lanthanum in the periodic table (Ochi et al. 2014). Lanthanum is effective in activating silent and poorly expressed secondary metabolite biosynthetic genes in Actinobacteria (Ochi and Hosaka 2013). The merit of the said approach is that rare earth elicitation does not require genetic manipulation to activate genes responsible for coding secondary metabolite production in Actinobacteria (Ochi 2016). Rare earths were not used for elicitation of natural insecticides in Actinobacteria and thus, should be a feasible approach to facilitate the discovery of new mode of action insecticides.

The Asian corn borer (ACB) is a major insect pest of corn in the Philippines. Reported yield losses from ACB infestation in traditional (i.e., non-*Bacillus thuringiensis* (Bt) corn) ranged from 20% to 80% (Mutuc et al. 2011). Farmers continue to use chemical insecticide application regardless of corn variety they are growing for fear of ACB or other insect pest damage (Afidchao et al. 2014). This practice, if not done judiciously could lead to insecticide resistance in ACB.

The present study sought to determine the effectiveness of Lanthanum elicitation on the production of insecticidal compound in *S. angustmyceticus* CGS B11 and to measure improvement in insecticidal activity of elicited insecticidal compound to ACB larvae.

MATERIALS AND METHODS

Isolation of Actinobacteria. *S. angustmyceticus* strain CGS B11, henceforth referred to as CGS B11 was isolated from volcanic soil in Albay Province in 2018. Briefly, approximately 100 g soil sample was collected from each of ten sites situated in one location in Cagsawa, Albay, Philippines (13º.09’55.29”N, 123º42’03.2”E). The distance between any two sites was at least one kilometer. Soil samples were collected using hand shovel and immediately placed inside sterile plastic bags. All soil samples were taken from the superficial horizon (0-30 cm depth) because the most intense microbial activity is present within this range of soil depth (Joux et al. 2015). Afterwards, the soil samples were transported to the laboratory for aseptic isolation of Actinobacteria. One gram of each soil sample was suspended in 9 ml of sterile distilled water. The suspension was thoroughly mixed using a vortex mixer and serially diluted up to 10^{-4}. An aliquot of 10^{-4} dilution was spread on yeast malt extract agar plates and incubated at 30ºC for 7 days. The composition of YMA is as follows (g/L): yeast extract 4; malt extract 10; dextrose 4; agar 20. YMA medium was sterilized by autoclaving for 25 min at 121ºC (Gao et al. 2009). To ascertain purity, colonies of CGS B11 appearing on the Petri plate after three days of incubation were re-streaked on fresh Petri plates containing the same culture medium. Each pure colony was picked from the Petri plate and streaked onto YMA test tube slant and afterwards stored at 4ºC. The identification of CGS B11 was reported in a previous study (Jimenez et al. 2021).

Chemical elicitation of insecticidal activity. A 10% inoculum was prepared by aseptically transferring a loopful of CGS B11 from YMA test tube slant into 100 ml of yeast malt extract broth (YMB), pH 7.0.

YMB is composed of the following ingredients (g/L): yeast extract 4; malt extract 10; dextrose 4; distilled water 1 L. Before use, YMB was sterilized as described above. The inoculum was cultivated in a shaker with 250 rpm agitation speed for 5 days under ambient temperature. Afterwards, the 5 day-old CGS B11 inoculum was aseptically transferred into 900 ml of YMB, pH 7.0 containing 200 µM Lanthanum which was cultivated for another 5 days at 30ºC in a shaker with 250 rpm agitation speed. Simultaneous setup with similar cultivation conditions was provided as negative control except that CGS B11 was cultivated in YMB without Lanthanum. After 5 days of cultivation, crude extract from fermentation broth from each of the two treatments was prepared by organic solvent (ethyl acetate) extraction.

Preparation of crude extracts. The fermentation broths from the two treatments (i.e., Lanthanum-treated and untreated CGS B11) were separately mixed with equal volume of ethyl acetate. The mixture was poured into a separatory funnel and manually shaken vigorously for one hour for complete extraction. The mixture was allowed to stabilize for 24 h for the separation of both the aqueous and organic phase. Afterwards, the ethyl acetate layer was collected and concentrated by evaporation using a rotary evaporator. The dried crude extract was resuspended in 2 ml methanol and transferred into amber glass vial for storage at -20°C. The methanol solution of crude extracts prepared from Lanthanum-treated and untreated CGS B11 were separately divide into equal amounts and subjected to thin layerchromatography (TLC) for secondary metabolite profiling and insecticide assay of ACB larvae. For the insect bioassay, the methanol solution of crude extract was subjected to drying using a rotary evaporator and resuspended into 2 ml distilled water containing 0.5% dimethyl sulfoxide (DMSO).

Thin-Layer Chromatography (TLC). The mobile phase (toluene: acetone: methanol, 7:3:1 v/v/v) was poured into the TLC chamber to a level a few centimeters above the chamber bottom. Individual aliquot of ethyl acetate crude extract solution was separately blotted on a TLC plate precoated with silica gel 60 as adsorbent (Merk Millipore). The TLC plate with a thickness of 200 µm in 20 cm x 20 cm format was then placed in the TLC chamber such that the sample spots were well above the level of the mobile phase. The chamber was then covered with a lid. Sufficient time was allowed for the separation of compounds contained in each spotted sample. The plate was then removed and allowed to dry. Separated sample components were viewed under the UV light at wavelength 254 nm and 366 nm.

Fourier transform infrared (FTIR) spectral analysis. Ethyl acetate crude extracts were prepared as described above. The freeze-dried samples were separately analyzed with a Shimadzu IR Prestige-21 FTIR spectrophotometer with attenuated total reflectance accessory. The spectrum was plotted as percent transmittance (% T) versus intensity of infrared spectra. Analysis was conducted at the Forest Products Research and Development Institute, Los Baños, Laguna. IR regions were analyzed using standards available at http://www.spec-online.de.

Insect bioassays. Laboratory-reared neonate ACB larvae were used for the concentration-mortality bioassays. The samples used for bioassays were the two TLC-fractionated compounds from Lanthanum-treated and untreated CGS B11, respectively. The commercial insecticide, Spinosad was included in a separate concentration-mortality bioassay to serve as reference. Six serial dilutions in logarithmic scale of each sample were prepared in 0.5% DMSO. Each of the concentration-mortality bioassays was also provide with blank buffer containing only 0.5% DMSO as negative control. An aliquot (40 ul) of each dilution was applied on the air-dried surface of artificial diet contained inside a 20-ml glass drum vial. One neonate larva was carefully placed with camel hair-brush inside the glass drum vial containing the insecticide treated artificial diet. Ten larvae were used for each concentration (i.e, dilution) . Each concentration was prepared in duplicate (Park et al. 2009; Horstmann and Sonneck 2016). Mortality was recorded after24 h. PriProbit software (Sakuma 1998) was used to estimate median lethal concentration (LC_{50}) and relative potency. When applicable, data were corrected for control mortality using Abbot's formula (Abbot 1925).

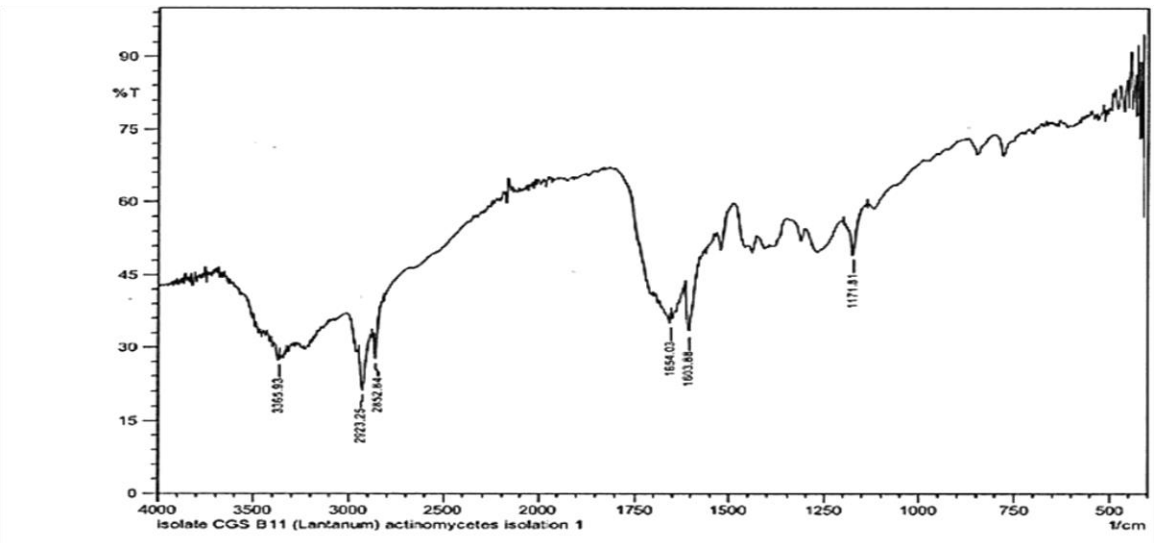
RESULTS AND DISCUSSION

Source and description of Actinobacteria. The identification of insecticidal strains of Actinobacteria is very important as it will lead to the discovery and development of these new strains as additional tool for the management of an economically important insect pest such as the ACB. One promising strain, CGS B11, previously identified as *Streptomyces angustmyceticus* (Jimenez et al. 2021) was isolated from volcanic soil collected from Cagsawa, Albay Province. Previous studies have reported that the genus *Streptomyces* is the dominant taxa in Actinobacteria (Anandan et al. 2016). The morphological features of CGS B11 is shown in Figure 1. Colonies growing on solid medium appeared as opaque, irregularly shaped, alabaster colored colonies with rough surface, umbonate elevation and wavy margin. Culture conditions greatly affect colony morphology in Actinobacteria (Li et al. 2016).

Fig. 1. Colonies of *Streptomyces angustmyceticus* strain CGS B11 growing on yeast malt extract agar medium.

Fourier Transform Infrared Spectroscopy (FTIR) analysis. FTIR spectra of ethyl acetate crude extract prepared from Lanthanum-treated CGS B11 is shown in Figure 2. The spectra revealed the presence of various characteristic functional groups through the absorption bands of Lanthanum-treated crude extract which were not observed in the untreated crude extract sample.

A



B

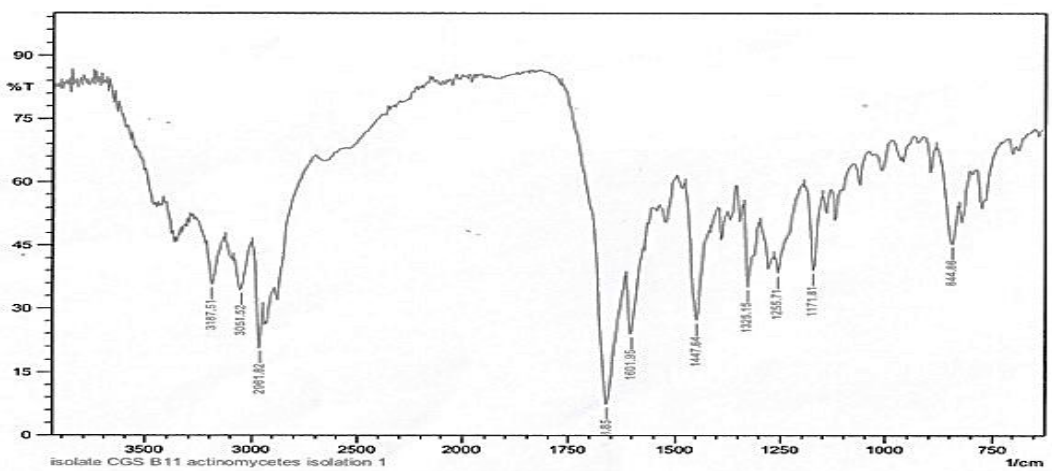


Fig. 2. Fourier transform infrared spectroscopy spectra of ethyl acetate crude extracts prepared from 5-day old fermentation broth of *Streptomyces angustmyceticus* strain CGS B11. A, Lanthanum-treated; B, untreated control.

The spectrum of Lanthanum-treated sample showed characteristic absorption bands for hydroxylgroup (H-bonded OH stretch) at 3,365.93 cm^{-1}, alkenyl group at 1,654.03 cm^{-1} (C=C stretch), methylene group (C-H asym./sym. Stretch), and primary amine at 1,603.88 cm^{-1} (Table 1).

Table 1. FTIR peaks and their respective assigned functional groups detected from dried crude extracts prepared from fermentation broh of *Streptomyces angustmyceticus* strain CGS B11.

Sample: Untreated crude extract		
Peak (cm^{-1})	Stretching and bending vibration	Assigned functional group
3187.51	Ammonium ion	
3051.52	Ammonium ion	
2961.82	C-H asym./sym. stretch	Methyl
1658.85	C-C stretch	Alkenyl
1601.95	C=C-C aromatic ring stretch	Aryl
1447.64	C-H bend	Methylene
1325.15	OH bend	Phenol or tertiary alcohol
1255.71	Aryl -O stretch	Aromatic ether
1171.81	CN stretch	Secondary amine
844.86	C-H 1,4-Distribution (para)	Aromatic ring
Sample: Lanthanum-treated crude extract		
Peak (cm^{-1})	Stretching and bending vibration	Assigned functional group
3365.93	H-bonded OH Stretch	Hydroxyl
2923.25	C-H asym./sym. Stretch	Methyl/Methylene
2852.84	C-H asym./sym. Stretch	Methyl/Methylene
1654.03	C=C stretch	Alkenyl
1603.88	NH bend	Primary amine
1171.81	CN stretch	Secondary amine

Functional groups detected only in untreated sample were as follows: aryl (C=C-C aromatic ring stretch) at 1,601.95 cm^{-1}, phenol or tertiary alcohol (OH bend) at 1,325.15 cm^{-1}, aromatic ether (-O stretch) at 1,255.71 cm^{-1}, aromatic ring (C-H 1,4-Distribution para) at 844.86 cm^{-1}, and methylene (C-H bend) at 1,447.64 cm^{-1}. FTIR analysis had been used in combination with other analytical techniques to study the antimicrobial property of Actinobacteria (Devi and Usha 2020). Similarly, the results reported here highlighted the effectiveness of FTIR as a powerful analytical method in detecting and differentiating the presence of unique functional group found only in the highly potent natural insecticide from CGS B11. Further, the results obtained from FTIR analysis support the findings of thin layer chromatography and insect bioassays of the Lanthanum-treated CGS B11 crude extracts.

Thin Layer Chromatography (TLC) Analysis. TLC profile of two ethyl acetate crude extracts of CGS B11 is shown in Figure 3. Two compounds are clearly visible in Lane 1 (control) and Lane 2 (treated) of the TLC plate chromatogram. The retention factor (Rf) of the two compounds in Lane 1 were Rf= 0.73 and Rf= 0.90, respectively. One compound with Rf= 0.33 (henceforth described as CGSB11-C1La) was detected only in Lanthanum treated crude extract (Lane 2). A second compound with Rf =0.90 (henceforth described as CGSB11-C2La) was also detected in Lanthanum-treated CGS B11. It is unclear if this compound is identical in both samples.

Fig. 3. Thin layer chromatography profile of Lanthanum-treated *Streptomyces angustmyceticus* strain CGS B11. Lane A: R_f1= 0.73, R_f2= 0.90; Lane B: R_f1= 0.33, R_f2= 0.90.

Insect bioassays. Two compounds detected by TLC from untreated crude extract contained insecticidal activity to neonate larvae of ACB (Table 2). The insecticidal activity of both compound 1 (LC_{50}= 1.97 ng/cm^2) and compound 2 (LC_{50}= 3.21ng/cm^2) were not significantly different from that of Spinosad (LC_{50}= 9.25 ng/cm^2). In contrast,the insecticidal activity of CGSB11-C1La (LC_{50} = 0.41 ng/cm^2) and CGSB11-C2La (LC_{50} =1.36 ng/cm^2) were significantly higher than the insecticidal activity of Spinosad (LC_{50}= 9.25 ng/cm^2).

Table 2. Probit analysis of mortality data from bioassay of purified fractions of Actinomycetes strains to Asian corn borer, *Ostrinia furnacalis*.[1]

			Model parameters		Lethal concentrations			Model fit	
Source strain	**Treatment[2]**	**n**	**intercept±SE**	**slope±SE**	**LC_{50} (95% FL) (ng/cm^2)**	**LC_{90} (95% FL) (ng/cm^2)**	**χ^2**	**df**	***P***
Streptomyces angustmyceticus CGS B11	C1-YMA	100	-0.25±0.17	0.85±0.16	1.97 (0.74-4.89)	63.61 (22.88-372.99	0.68	3	0.88
Streptomyces angustmyceticus CGS B11	C1- YMA +La	100	0.37±0.18	0.98±0.21	0.41(0.13-0.96)	8.54 (3.18-59.50)	3.02	3	0.39
Streptomyces angustmyceticus CGS B11	C2-YMA	100	-0.35±0.17	0.69±0.15	3.21(1.06-9.83)	224.76 (48.48-6,725)	1.89	3	0.59
Streptomyces angustmyceticus CGS B11	C2- YMA +La	100	-0.09±0.16	0.68±0.15	1.36 (0.37-3.93)	103.21(24.12-2,604)	0.51	3	0.92
Reference	Spinosad	120	-0.91±0.23	0.95±0.16	9.25 (4.58-18.14)	208.64 (87.77-791.71)	0.88	4	0.93

[1]Neonate (<24h old) larvae were used in artificial diet surface overlay assay. Mortality was scored after 24 h.
[2]YMA, yeast malt extract; La, Lanthanum; C1, compound 1; C2, compound 2.

The relative potency of CGS B11-C1La is more than 20 times greater than Spinosad (Table 3). The relative potency of the other compounds ranged from 2.80 to 6.06 which was not significantly different from that of Spinosad.

Table 3. Potency estimates of thin layer chromatography fractions isolated from ethyl acetate crude extracts prepared from 5-day-old fermentation broth of *Streptomyces angustmyceticus* strain CGS B11.

Treatment[1]	**Relative Potency[2]**	**Fiducial Limits (95%)**
Spinosad (reference)	1.00	0.30-3.32
CGS B11 C1	4.59	1.35-15.48
CGS B11 C2	2.80	0.82-9.41
CGS B11 C1-La (Rf1)	24.07	6.94-85.99
CGS B11 C2-La (Rf2)	6.06	1.79-20.56

[1]C1, compound 1; C2, compound 2; Rf, retention factor

It has been reported previously that REE-mediated activation of cryptic gene clusters in Actinobacteria might contribute to the discovery of novel bioactive compounds (Ochi and Hosaka 2013). This mechanism might also be responsible for the successful production of the insecticidal compound CGS B11-C1La. One possible explanation of the more than 20-fold increase in potency of CGSB11-C1La might be the presence of hydroxyl group in the pharmacophore of the molecule. The hydroxyl group might bedirectly involved in tight binding to a different target receptor site. Tight binding to receptor sight in target insect is an important factor contributing to higher insecticidal activity (Li et al. 2013).

We are not aware of any previous reports on the positive effect of Lanthanum as a chemical elicitation agent in insecticidal Actinobacteria. In another study, Lanthanum together with another rare earth element Scandium was reported to induce the production of actinorhodin in *Streptomyces coelicolor* (Tanaka et al. 2010). The bioassay results also showed that even without chemical elicitation the insecticidal compounds present in CGS B11 were of comparableinsecticidal activity with Spinosad. Aside from toxicity to ACB, CGS B11 crude extract also contained insecticidal activity to mango fruitfly, *Bactrocera philippinensis* and whitefly *Bemisia tabaci* (data not shown). The culture medium and conditions used for cultivation mighthave promoted the production of more than one insecticidal compound in the crude extract of CGS B11which gave it wider spectrum of insecticidal activity. It might be possible that the two compounds detected by TLC in CGS B11 were acting independently or in synergy. These insecticidal compounds could be further investigated for possible synergistic action with the REE-elicited insecticidal compounds to produce even more greater potency for control of ACB and other insect pests. The bioassay results observed in this study is consistent with previous observation that insecticidal compounds derived from Actinobacteria were effective against several insect pests (Kumari et al. 2014).

This is the first report of a strain (CGS B11) of *S. angustmyceticus* having insecticidal activity to insect pests. The scope of this study is limited only to insecticidal effects of Lanthanum-elicited compounds to larvae of ACB. We did not attempt to determine the insecticidal activity of the Lanthanum-elicit compounds to other insect pests. The effect of other rare earth elements was also not explored in this study. It is possible that CGS B11 when treated with other rare earth elements, singly or in combination with Lanthanum might elicit additional new insecticidal compounds with novel mode of action and/or greater potency to larvae of ACB or to other insectpests. The discovery of new insecticidal compounds from CGS B11 by various REE elicitation would be a significant development considering that the number of compounds in new classes ofinsecticides is likely small (Sparks 2013).

Insect bioassay is an important experimental tool to estimate the efficacy of insecticidal compounds. Despite the limitation in the number of insects used in the experiment, the insect mortality data fits well with the probit model as shown by insignificant $\chi 2$ value for each set ofbioassay data. The estimated slopes from the probit analysis indicated that the test insects usedfor the bioassay were homogenous. These favorable characteristics of the performed bioassay could be replicated in a wider number of test insects with calculable dependability in each case because we are following a standardized bioassay protocol for ACB in the laboratory (Hoskins and Craig 1962). Because of limitation in the amount of insecticidal compound obtained from the TLC analysis, samples from crude extracts were directly used for FTIR analysis. FTIR spectroscopy has been used directly to identify biologically active functional groups in crude samples (Sankarganesh and Joseph 2016).

Purification and structure elucidation of the promising insecticidal fractions produced by Lanthanum elicitation will be needed for establishing novelty in terms of structure and mode ofaction. The discovery of a new mode of action insecticide would be very useful for IRM, especially in the design of insecticide rotation for field application (Thompson et al. 2008).

CONCLUSION

Lanthanum elicitation is a highly effective method for the production of insecticidal compound in CGS B11. The elicited insecticidal compound had greatly improved insecticidal activity to ACB larvae. Possible synergism of the insecticidal fractions could also provide an alternative mechanism toenhance potency and at the same time lessen the cost and negative impact of synthetic insecticides. Designing a suitable formulation of the said insecticidal compounds will be required to evaluate the insecticidal potency under field conditions.

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ECONOMIC IMPACTS OF RICE TARIFFICATION LAW ON THE PHILIPPINE RICE DOMESTIC MARKET

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ABSTRACT

Lifting of quantitative restrictions on staple foods allows an influx of supply in the country favoring the consumers but hurting domestic producers because of lowered prices. Using partial equilibrium analysis, this paper examined how the policy shift on rice imports from quantitative restrictions to ad valorem tariffs affects domestic rice supply and household access and how it impacts market efficiency. Simulation results showed that the loss in producer protection resulted to a sizeable reduction in domestic rice production both in the short-run and long-run. On the other hand, food expenditure on rice is greatly reduced due to increased rice affordability and domestic consumption is improved. Inefficiency losses in consumer surplus is diminished, although producer surplus is decreased. But overall, net societal welfare is improved. The author noted that while this may improve the country's food security, food self-sufficiency is threatened, and the country's vulnerability to shocks in the international market is increased. Further, while the policy shift may address nutrition problems especially among low-income groups since reduced rice expenditures can increase their expenditures on meat and poultry products, this may be constrained by the multi-layered supply chain of rice in the Philippines which increases marketing costs, affecting rice retail prices.

Key words: ad valorem tariffs, quantitative restrictions, partial equilibrium analysis, international trade, rice market

INTRODUCTION

A change of trade policies on staple crops has always raised important debates as it has redistributive effects among key players (Swinnen 2021). For a net food importing country, a move to liberalize may improve its food access but this also put pressures on its domestic food producers. This has been the case for the Philippines after the implementation of Rice Tariffication Law (RTL) (RA 11203) in March 2019, which removed the quantitative restrictions on rice imports, replacing them with ad valorem tariffs. Historically, the Philippines adopted a "strict" protectionist trade policy for the rice sector combining quantitative restrictions and tariffs on rice imports with the NFA regulating the rice importation through a system of import licensing. The government allowed a Minimum Access Volume (MAV), which is the minimum volume of imports per year at a lower tariff rate ("in- quota tariff"). Previously, a MAV of up to about 239 thousand metric tons was approved for rice at a binding rate of 50 percent (actual rate applied both in-quota and out-quota) (Bordey et al. 2016; Briones 2019). However, with the government's application for extension of the special treatment for rice, WTO agreed

but the MAV was increased to 350 thousand metric tons until 2012 at an in-quota tariff rate of 40 percent and a special tariff treatment of only 35 percent was provided by the government to Association of Southeast Asian Nations (ASEAN) exporters under the ASEAN Trade in Goods Agreement (ATIGA) of 2009, and this MAV further increased to about 805 thousand metric tons after the government requested to waive its commitment to WTO until 2017 (Bordey et al. 2016; Briones 2019). With the expiration of the special treatment for rice, RA 11203 was passed, quantitative restrictions were repealed, MAV was reverted to its 2012 level at 350 thousand metric tons, and sanitary and phytosanitary import clearance for rice for the sole purpose of ensuring food safety was added among the import requirements (Republic Act No. 11203 2018). The law was also pushed through after the National Food Authority (NFA) ran out of stocks in the last quarter of 2018 and caused surging inflation in rice prices (Tobias 2019). With the passage of the law, the role of NFA changed with the removal of its powers and functions on rice importation and other regulatory functions in the local grains industry (NFA 2019) and this has permitted other private players to come in and so rice imports are expected to surge. However, this would also allow an increase in total domestic food supply (sum of local production and rice imports) and is expected to address the inflation in rice prices, making rice more affordable to the poor households such as the urban poor and the rural landless poor who are net buyers of rice. Nevertheless, this comes at the expense of rice farmers whose incomes are expected to decrease with the lowered paddy price.

Recent studies have been conducted to assess the initial impacts of RTL. In a study conducted by Cororaton and Yu (2019), which used rice policy simulations employing a Computable General Equilibrium (CGE) model, it was found that tariffication generates favorable income distribution and poverty reduction effects as compared to tighter quantitative restriction on rice imports. This was also supported by Balie et. al (2020) and Briones (2018 and 2019) which showed that RTL reduces consumer and producer rice prices affecting households on the production and consumption side, but the overall effects of the reform are beneficial since the benefits are spread widely across the population given that majority of households are net buyers of rice. Unfortunately, they also found that since the rice growers are net sellers, they are negatively impacted by the policy reform; although they argued that tariff revenues can somehow compensate the losses of rice farmers by increasing their productivity and making them more competitive through infrastructure support, as accounted for by the Rice Competitiveness Enhancement Fund (RCEF) which was crafted to improve the competitiveness and income of the domestic producers through its mechanization, seed, credit, and extension services program amidst the policy shift.

This paper validates and complements the initial findings of the previously cited literatures using partial equilibrium analysis proposed by Tsakok (1990). While CGE models (i.e., comparative static analysis) have the advantage of providing economywide analysis as these models encompass intersectoral linkages in contrast to partial equilibrium models which analyze markets in isolation, the latter is employed in the analysis since the main goal of this paper is to provide an in-depth and quantifiable impact analysis of the policy shift on a single market, which is the Philippine's rice domestic market, specifically on the price changes brought about by the policy, the response of the market agents as producers and consumers, and the implications of these responses on various economic and policy interests encompassing financial, welfare, and economic considerations. The study contributes to literature as it gives special attention on the magnitude of the impact of RTL on domestic rice supply and household access (in terms of household food expenditure), which affect the country's food security, and on how it impacts the rice market efficiency (by examining the changes in producer surplus, consumer surplus, and societal welfare). Quantifying these changes serves as a necessary input for the policymakers in determining the scale of response or intervention needed to mitigate the negative effects of the policy shift to the disadvantaged players as the lifting of the quantitative restriction on rice imports is a trade-off that involves transfer of welfare between the producers and the consumers.

Further, by examining the disaggregated welfare impact of the policy reform using previously estimated demand elasticities by income group and supply elasticities by farm size, the study provides understanding on how the policy reform affects equity in the society. While the policy reform may be beneficial in general for the society as concluded by Balie et al. (2020) and Briones (2019), its impact among income-groups and farmer-groups may vary. The disaggregated analysis is therefore essential to determine if the change in the policy will have adverse effects to economically vulnerable group (i.e., small-scale rice farmers) or if it will direct the economic benefits to the group for which the policy is designed to (i.e., low-income consumers) (Tsakok 1990). In addition, while some of the previously mentioned literature have already assessed whether the RTL is pro-poor, they fail to include depth in their analysis with regard to the size of the impact to the small-scale farmers who are expected to be the most negatively affected by the policy reform. Quantifying this impact may serve as basis in estimating the amount of subsidy, cash transfer, or the like necessary to mitigate the impact to this vulnerable group. However, it must be noted that the results of partial equilibrium models may over or understate the true impact of the shock as these models do not account the spillover or feedback effects of the policy change, but these models do indicate the broad magnitude of the problem (Thomsen 2021; Tsakok 1990). The author hypothesized that consumer surplus, producer surplus, and societal welfare have improved after the policy shift, improving the efficiency in the rice market. According to the OECD Trade Policy Paper written by Czaga (2004), although quantitative restrictions are motivated by the government's desire to protect domestic producers from foreign competitors, the costs they imposed outweigh the benefits both for the importing and exporting countries because quantitative restrictions undermine trade and economic efficiency more than tariffs as quota resources are administratively allocated by the state, whereas with tariffs, resources are allocated through price mechanisms. He noted that this harms consumers in the importing country as these create import substitution effects when the policy-induced scarcity of imports increased the price of the good in the domestic market to the maximum amount that the consumers are willing to pay.

On the equity side, it is hypothesized that the policy shift improved equity across income groups since low-income group spend more on rice than high-income group thereby, benefitting more on lower rice prices. Based on Philippine Statistics Authority's (PSA) data, average rice per capita consumption of upper-class group (AB and C) is 120.28 kgs per year while that of lower-class group (D and E) is 111.62 kgs per year but since around 72 percent of the population belong to the lower-class (Lantican et al. 2013), aggregate rice consumption of lower-class group is greater than the aggregate rice consumption of upper-class group. By definition, those belonging to Class AB have monthly income levels of at least PhP100,000 (USD 2,000), Class C have monthly income levels of PhP20,000 to PhP100,000 (USD 400 to USD 2,000), Class D have monthly income levels of PhP10,000 to PhP20,000 (USD 200 to USD 400), and Class E have monthly income levels of less than PhP10,000 (USD200) (Chua and Tiongson 2012). Worsened equity, however, is assumed across farmer groups since based on 2017 rice-based farm households survey conducted by Philippine Rice Research Institute (PhilRice), rice farming is generally small-scale wherein 83 percent of farmers are operating on less than two hectares and only 17 percent are operating on more than three hectares. The results of the study is useful in balancing the dilemma between equity and efficiency. The results also provided information on how the policy shift may affect the attainment of the second goal of the sustainable development goals (SDGs) that is to "end hunger, achieve food security and improved nutrition and promote sustainable agriculture" (United Nations n.d.).

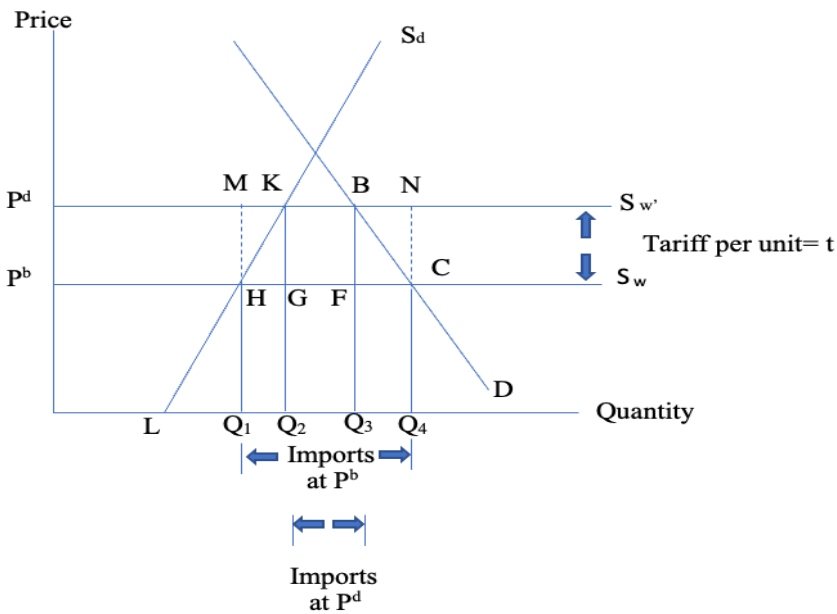
METHODOLOGY

In order to measure the welfare impacts of the policy shift from quantitative restrictions to ad valorem tariffs on rice imports, partial equilibrium analysis proposed by Tsakok (1990) was employed (Fig. 1). This type of analysis, as discussed by Tsakok, is necessary to determine the direction and magnitude of the impact of a change in price policy; although some limitations of the analysis is that it does not account the magnitude of spillover and feedback effects (i.e., interactions of the commodity

with close substitute or complements). The model also assumes that supply is readily available and accessible in the market, that is, the Philippines can buy rice in the international market whenever; although in reality, some of the suppliers in the international market may withhold their rice exports due to domestic market obligation.

Analytical framework. According to Tsakok, using the elasticity estimates and price data, analysts can calculate the financial implications of a change in the price of a commodity, welfare transfers between producers and consumers, and the net gains and losses in economic efficiency. The initial supply and demand conditions are represented by the supply curve S_d, demand curve D, and the supply curve of imports from the rest of the world S_w, which is horizontal because the importing country such as the Philippines is small (i.e., price taker) in the world market. The domestic price is the same as the world price P^b before the tariff is imposed and the consumers are willing to purchase quantity Q_4. At this price, the domestic producers are willing to supply only Q_1 while Q_4-Q_1 will be supplied by imports (Fig.1).

When a tariff is imposed at the ad valorem rate t, domestic price increases above the world price P^b to P^d, and the supply curve of imports is raised vertically to $S_{w'}$ due to the inclusion of the import tax. At this level, consumers will reduce their purchases of the commodity to Q_3, and the producers will respond to the higher price and will increase their output to Q_2. This will in turn reduce the quantity of imports to Q_3-Q_2.



Source: Tsakok (1990)

Fig. 1. Tariff on imports of a food commodity

Numerically, the impacts of the import tariff can be calculated using these formulas (Tsakok, 1990, pp.188-189):

- Change in government revenue (graphically represented by area *GKBF* in Fig. 1):

$$\Delta GR = \left(\frac{NPC-1}{NPC}\right)(W' - V') \quad (1)$$

Where NPC= Nominal Protection Coefficient, which is the ratio of the domestic price that decision makers face given the intervention (P^d) and the border price they would have faced in the absence of the intervention (P^b);
W'= P^dQ_3, the value of consumption at domestic prices;
V'= P^dQ_2, the value of domestic production at domestic prices;

- Change in foreign exchange outlays (graphically represented by Q_1HCQ_4 – area Q_2GFQ_3 in Fig. 1):

$$\Delta FE = -\left(\frac{NPC-1}{NPC^2}\right)(e_sV' - n_dW') \quad (2)$$

Where: n_d = price elasticity of demand and
e_s= price elasticity of supply

- Net economic loss in consumption (NEL_c) (graphically represented by ΔFBC in Fig. 1):

$$\Delta NEL_c = 0.5n_d\left(\frac{NPC-1}{NPC}\right)^2 W' \quad (3)$$

- Net economic loss in production (NEL_p) (graphically represented by ΔHKG in Fig.1):

$$\Delta NEL_p = 0.5e_s\left(\frac{NPC-1}{NPC}\right)^2 V' \quad (4)$$

- Change in consumer surplus (WG_c) (graphically represented by area P^bP^dBC in Fig. 1):

$$\Delta WG_c = -\left[\left(\frac{NPC-1}{NPC}W'\right) + NEL_c\right] \quad (5)$$

- Change in producer surplus (WG_p) (graphically represented by area P^bP^dKH in Fig. 1):

$$\Delta WG_p = \left(\frac{NPC-1}{NPC}\right)V' - NEL_p \quad (6)$$

- Net effect of a tariff on imports (graphically represented by area *HKG* and area *FBC* in Fig.1):

$$Net\ effect = WG_p + WG_c + \Delta GR = -(NEL_p + NEL_c) \quad (7)$$

In this study, two scenarios were compared: (1) the pre-RTL period under quantitative restrictions and (2) post-RTL period under ad valorem tariffs in order to analyze the welfare impacts of the policy shift. In the case of quantitative restrictions, NPC was adjusted to measure the equivalent tariff as proposed by Tsakok (p.69), that is, NPC = (domestic retail price – handling from border to retail market)/ P^b. This was based on the idea that the summation of border price with tariff ($P^b(1+t)$) and handling cost from border to retail market is equal to the domestic retail price so rearranging the formula would yield the adjusted NPC under quantitative restrictions.

In disaggregating the analysis, the same procedure was employed but the market supply curves and market demand curves were disaggregated into the supply curves of large and small farmers and demand curves of low-income and high-income consumers. Various elasticities of supply estimated by Hayami and Herdt (1978) and Hinlo and Cruz (2013) and elasticities of demand estimated by Lantican et al. (2013 were used in the study to provide more sensitivity to the analysis. Although Tsakok (1990) provided some elasticity estimates which can be used by practitioners in the analysis, these were estimated between 1910 to 1974 so the author decided to use the most recent ones available. The results generated using the own-price elasticity of supply estimate of Hayami and Herdt (1978) were shown as Case A while the results generated using the own-price elasticity of supply estimate of Hinlo and Cruz (2013) were shown as Case B. In both cases, the own-price elasticities of demand estimated by Lantican et al. (2013) were used. It was also assumed that the price elasticity of large-scale farmers reaches the

long-run (LR) level as assumed by Hayami and Herdt (1978) due to their greater capacity for investment financing while the price elasticity of small-scale farmers remains at the short-run (SR) level. Both the short-run period (where some factors of production are fixed) and long-run period (all the factors of production are variable) were considered in the analysis to assess the impact of the policy shift in different time horizon. This type of analysis may be relevant because the gravity of the impact may change across time as players tend to better adapt or adjust in the long-term.

Data. Annual rice production, consumption, imports, and rice prices were obtained from the PSA and Food and Agriculture Organization (FAO) websites, and tariff rates and other relevant data were collected from the Bureau of Customs website and other government websites. The annual averages of these data covering 2010 to 2018 were used in the simulations. The CIF (cost, insurance, and freight) prices of milled rice imported from Thailand and Vietnam were used in the analysis as the world prices. This is because Thailand and Vietnam are the biggest trading partners of the Philippines in terms of rice. In 2018, for instance, based on FAO website, 48 percent of the Philippines' total rice imports or about 842.96 thousand metric tons (valued at 355 million USD) were from Thailand while 38 percent or about 674.56 thousand metric tons (valued at 280 million USD) were from Vietnam. The rest of the rice imports (14%) were sourced out from India, Myanmar, China, and Pakistan.

RESULTS AND DISCUSSION

Under RTL, the quantitative restrictions were lifted for rice imports coming from ASEAN countries and were replaced with just ad valorem tariffs. Previously, the minimum access volume (MAV) was at 805 thousand metric tons with varying in-quota and out-quota tariff rates. With the policy shift, the MAV was removed for rice imports coming from ASEAN countries and the MAV for non-ASEAN countries reverted to 350 thousand metric tons (Republic Act No. 11203 2018). Import tariffs imposed are as follows: 35 percent tariff on rice imported from the members of ASEAN under the ASEAN Trade in Goods Agreement (ATIGA), 40 percent in-quota tariff (volumes below 350 thousand metric tons) and 50 percent out-quota tariff (volumes above 350 thousand metric tons) on rice imported from the Most Favoured Nations (MFN), and 180 percent bound tariff rate is imposed on rice imports from non-ASEAN countries above 350 thousand metric tons (Tariff Commission 2021). Rice imports from Vietnam and Thailand are subject to ATIGA tariff rate of 35 percent which is equivalent to NPC of 1.35 (Table 1). Prior to RTL implementation, quantitative restrictions were imposed on rice imports with equivalent tariffs of 65 to 73 percent and NPCs of 1.65 to 1.73, implying a loss in producer protection between 18 to 22 percent.

Table 1. Tariff rates, CIF prices, and nominal protection coefficients for rice by trade agreement

Scenario	Tariff Rate (%) (a)	CIF Thai W/o Tariff (P/mt) [P^b or P^{cif}] (b)	CIF Thai With Tariff (P/mt) [$P^b + t$] (c)	CIF Viet W/o Tariff (P/mt) [P^b or P^{cif}] (d)	CIF Viet With Tariff (P/mt) [$P^b + t$] (e)	Nominal Protection Coefficient (f)
Non-ASEAN country	180					
MFN (out-quota)	50					
MFN (in-quota)	40					
ATIGA	35	9,495	6,318	20,377	27,509	1.35
QR	67 to 71	19,495	33,336	20,377	34,029	1.65 to 1.73

Source: Tariff Commission (2021)

Notes:

- Estimated handling cost from border to retail is assumed to be 10% of the border price (Briones 2019).
- MFN refers to the Most Favored Nations.
- ATIGA refers to ASEAN Trade in Goods Agreement.
- QR means quantitative restrictions.
- P/mt is Philippine pesos per metric ton.
- CIF (cost, insurance, and freight)

Rice produced in Nueva Ecija irrigated systems, a major rice-producing province in the Philippines, would need a 75 percent tariff protection to ensure competitiveness against imported rice from Vietnam, Thailand, or India. Otherwise, they will need to reduce their production cost by about 44 percent from PhP12.41 per kg to PhP6.97 per kg using better seeds/hybrid rice and improved agronomic techniques as well as improved milling efficiency and capacity utilization to maintain current profit margins (Bordey et al. 2016). RTL will result to a reduction in domestic production from 11.61 Mt to as low as 10.88 Mt in the short-run and 10.20 Mt in the long-run or equivalent to one to six percent reduction and nine to 12 percent reduction in domestic production, respectively (Table 2 and Table 3).

Table 2. Effect of the policy shift from quantitative restrictions (Pre-RTL) to Ad Valorem tariffs (Post-RTL)

	Pre-RTL				**Post-RTL**			
	TP: Thailand		**TP: Vietnam**		**TP: Thailand**		**TP: Vietnam**	
	Case A	**Case B**	**Case A**	**Case B**	**Case A**	**Case B**	**Case A**	**Case B**
Supply (million metric tons)								
Market: LR	11.61	11.61	11.61	11.61	10.39	10.20	10.55	10.32
Market: SR	11.61	11.61	11.61	11.61	10.88	11.50	10.94	11.51
Large-scale palay farmer (> than 2 has)	1.97	1.97	1.97	1.97	1.77	1.73	1.79	1.76
Small-scale palay farmer (< or = 2 has)	9.64	9.64	9.64	9.64	9.03	9.54	9.08	9.55
Demand (million metric tons)								
Market: Hicksian	12.79	12.79	12.79	12.79	13.50	13.50	13.44	13.44
Market: Marshallian	12.79	12.79	12.79	12.79	14.15	14.15	14.02	14.02
High-income consumer: Hicksian	3.82	3.82	3.82	3.82	4.08	4.08	4.05	4.05
High-income consumer: Mashallian	3.82	3.82	3.82	3.82	4.24	4.24	4.20	4.20
Low-income consumer: Hicksian	8.96	8.96	8.96	8.96	9.39	9.39	9.35	9.35
Low-income consumer: Marshallian	8.96	8.96	8.96	8.96	9.87	9.87	9.79	9.79
Trade								
Imports (Mt): Hicksian, LR	1.18	1.18	1.18	1.18	3.11	3.30	2.94	3.11
Imports (Mt): Hicksian, SR	1.18	1.18	1.18	1.18	2.62	2.00	2.49	1.93
Imports (Mt): Marshallian, LR	1.18	1.18	1.18	1.18	3.76	3.95	3.52	3.70
Imports (Mt): Marshallian, SR	1.18	1.18	1.18	1.18	3.27	2.65	3.08	2.52
Foreign exchange earnings (USD billion): Hicksian, LR	-1.62	-1.79	-1.62	-1.78	-0.96	-1.04	-1.01	-1.09

	Pre-RTL				Post-RTL			
	TP: Thailand		TP: Vietnam		TP: Thailand		TP: Vietnam	
	Case A	Case B	Case A	Case B	Case A	Case B	Case A	Case B
Foreign exchange earnings (USD billion): Hicksian, SR	-1.21	-0.70	-1.21	-0.69	-0.75	-0.45	-0.79	-0.47
Foreign exchange earnings (USD billion): Marshallian, LR	-2.17	-2.33	-2.16	-2.33	-1.35	-1.43	-1.42	-1.50
Foreign exchange earnings (USD billion): Marshallian, SR	-1.76	-1.24	-1.75	-1.24	-1.14	-0.84	-1.19	-0.87
Tariff Revenue (PhP billion): Hicksian, LR	8.02	8.02	8.38	8.38	21.23	22.54	20.95	22.19
Tariff Revenue (PhP billion): Hicksian, SR	8.02	8.02	8.38	8.38	17.89	13.67	17.78	13.76
Tariff Revenue (PhP billion): Marshallian, LR	8.02	8.02	8.38	8.38	25.63	26.94	25.14	26.38
Tariff Revenue (PhP billion): Marshallian, SR	8.02	8.02	8.38	8.38	22.29	18.08	21.96	17.95
Total Domestic Supply (Imports + Local Prod) (million metric tons)								
Hicksian, LR	12.79	12.79	12.79	12.79	13.50	13.50	13.49	13.43
Hicksian, SR	12.79	12.79	12.79	12.79	13.50	13.50	13.43	13.44
Marshallian, LR	12.79	12.79	12.79	12.79	14.15	14.15	14.07	14.02
Marshallian, SR	12.79	12.79	12.79	12.79	14.15	14.15	14.02	14.03
Welfare Effects (PhP billion)								
Consumption								
Total Expenditures: Hicksian	426.37	426.37	435.23	435.23	355.29	355.29	369.72	369.72
Total Expenditures: Marshallian	426.37	426.37	435.23	435.23	372.40	372.40	385.67	385.67
High-income consumer: Hicksian	127.34	127.34	129.99	129.99	107.38	107.38	111.41	111.41
High-income consumer: Marshallian	127.34	127.34	129.99	129.99	111.59	111.59	115.54	115.54
Low-income consumer: Hicksian	298.69	298.69	304.90	304.90	247.13	247.13	257.21	257.21
Low-income consumer: Marshallian	298.69	298.69	304.90	304.90	259.76	259.76	269.31	269.31
Change in consumer surplus: Hicksian	-169.57	-169.57	-162.32	-162.32	-88.95	-88.95	-92.53	-92.53
Change in consumer surplus: Marshallian	-160.50	-160.50	-154.24	-154.24	-90.20	-90.20	-93.47	-93.47
High-income consumer: Hicksian	- 50.17	- 50.17	-48.06	- 48.06	-26.68	-26.68	-27.73	-27.73
High-income consumer: Marshallian	- 47.87	- 47.87	-46.01	- 46.01	-26.99	-26.99	-27.96	-27.96
Low-income consumer: Hicksian	-119.96	-119.96	-114.76	-114.76	-62.18	- 62.18	-64.73	-64.73
Low-income consumer: Marshallian	-113.12	-113.12	-108.67	-108.67	-63.15	-63.15	-65.46	-65.46
Production								
Total Revenues: LR	387.03	387.03	395.08	395.08	273.45	268.44	290.22	283.89
Total Revenues: SR	387.03	387.03	395.08	395.08	286.34	302.66	300.94	316.62

	Pre-RTL				Post-RTL			
	TP: Thailand		TP: Vietnam		TP: Thailand		TP: Vietnam	
	Case A	Case B	Case A	Case B	Case A	Case B	Case A	Case B
Large-scale palay farmer	65.67	65.67	67.04	67.04	46.58	45.53	49.24	48.42
Small-scale palay farmer	321.36	321.36	328.04	328.04	237.65	251.07	249.78	262.71
Change in producer surplus: LR	145.90	143.21	140.20	137.80	66.29	64.36	70.02	68.11
Change in producer surplus: SR	152.77	161.46	146.32	154.06	71.34	77.96	75.01	81.56
Large-scale palay farmer	24.80	24.35	23.83	23.43	11.27	10.94	11.90	11.58
Small-scale palay farmer	126.80	134.01	121.45	127.87	59.21	64.71	62.26	67.70
Efficiency losses/gains								
Total: Hicksian, LR	-15.64	-18.34	-13.73	-16.13	-1.43	-2.05	-1.56	-2.23
Total: Hicksian, SR	- 8.77	-0.08	-7.61	0.13	0.28	2.69	0.26	2.79
Total: Marshallian, LR	- 6.58	-9.27	-5.66	-8.06	1.72	1.10	1.69	1.02
Total: Marshallian, SR	0.29	8.98	0.46	8.20	3.43	5.83	3.51	6.04
Production: LR	17.18	19.87	15.30	18.21	4.59	5.22	4.85	5.49
Production: SR	10.31	1.60	9.18	1.48	2.89	0.48	3.04	0.50
Consumption: Hicksian	-10.03	-10.03	-8.93	-9.32	-3.16	-3.16	-3.29	-3.32
Consumption: Marshallian	-19.09	-19.18	-17.00	-17.75	-6.31	-6.48	-6.54	-6.60

Notes:

- Own-price elasticities of supply used in Case A were as follows: LR= 0.5, SR = 0.3 (Hayami and Herdt 1978).
- Own-price elasticities of supply used in Case B were as follows: LR= 0.5785, SR= 0.0471 (Hinlo and Cruz 2013).
- Own-price elasticities of demand used in both cases were as follows: Market (Hicksian)= -0.2650, market (Marshallian)= -0.5046, high-income consumer (Hicksian)= -0.3126, high-income consumer (Marshallian)= -0.5159, low-income consumer (Hicksian)= -0.2235, low-income consumer (Marshallian)= -0.4814 (Lantican et al. 2013).
- Average exchange rate from 2010-2019 posted in Bangko Sentral ng Pilipinas is used in the analysis: 1 USD = 47 PhP.
- Tariff revenues pre-RTL period were computed based on the total volume of imports during that period valued at CIF price ATIGA rate
- TP refers to trading partner

Table 3. Effect of the policy shift from Quantitative Restrictions (Pre-RTL) to Ad Valorem tariffs (Post-RTL) in percent changes

	TP: Thailand		TP: Vietnam	
Supply	**Case A**	**Case B**	**Case A**	**Case B**
Market: Long-run	- 10.51	- 12.14	- 9.13	- 11.11
Market: Short-run	- 6.29	- 0.95	- 5.77	- 0.86
Large-scale palay farmer (> than 2 has)	- 0.15	- 12.18	- 9.14	- 10.66
Small-scale palay farmer (< or = 2 has)	- 6.33	- 1.04	- 5.81	- 0.93
Demand				
Market: Hicksian	5.55	5.55	5.08	5.08
Market: Marshallian	10.63	10.63	9.62	9.62
High-income consumer: Hicksian	6.81	6.81	6.02	6.02

	TP: Thailand		TP: Vietnam	
High-income consumer: Mashallian	10.99	10.99	9.95	9.95
Low-income consumer: Hicksian	4.80	4.80	4.35	4.35
Low-income consumer: Marshallian	10.16	10.16	9.26	9.26
Trade				
Imports: Hicksian, LR	163.56	179.66	149.15	163.56
Imports: Hicksian, SR	122.03	69.49	111.02	63.56
Imports: Marshallian, LR	218.64	234.75	198.31	213.56
Imports: Marshallian, SR	177.12	124.58	161.02	113.56
Foreign exchange earnings: Hicksian, LR	- 40.67	- 41.69	- 37.67	- 38.64
Foreign exchange earnings: Hicksian, SR	- 38.09	- 34.91	- 35.22	- 32.19
Foreign exchange earnings: Marshallian, LR	- 37.44	- 38.44	- 34.60	- 35.55
Foreign exchange earnings: Marshallian, SR	- 34.90	- 31.77	- 32.18	- 29.20
Tariff Revenue: Hicksian, LR	164.64	180.96	149.85	164.71
Tariff Revenue: Hicksian, SR	123.05	70.47	112.00	64.14
Tariff Revenue: Marshallian, LR	219.50	235.82	199.78	214.64
Tariff Revenue: Marshallian, SR	177.92	125.33	161.94	114.08

Welfare Effects	**TP: Thailand**		**TP: Vietnam**	
Consumption	**Case A**	**Case B**	**Case A**	**Case B**
Total Expenditures: Hicksian	- 16.67	- 16.67	- 15.05	- 15.05
Total Expenditures: Marshallian	- 12.66	- 12.66	- 11.39	- 11.39
High-income consumer: Hicksian	- 15.68	- 15.68	- 14.29	- 14.29
High-income consumer: Marshallian	- 12.37	- 12.37	- 11.12	- 11.12
Low-income consumer: Hicksian	- 17.26	- 17.26	- 15.64	- 15.64
Low-income consumer: Marshallian	- 13.03	- 13.03	- 11.67	- 11.67
Change in consumer surplus: Hicksian	- 47.54	- 47.54	- 42.99	- 42.99
Change in consumer surplus: Marshallian	- 43.80	- 43.80	- 39.40	- 39.40
High-income consumer: Hicksian	- 46.82	- 46.82	- 42.29	- 42.29
High-income consumer: Marshallian	- 43.62	- 43.62	- 39.23	- 39.23
Low-income consumer: Hicksian	- 48.17	- 48.17	- 43.59	- 43.59
Low-income consumer: Marshallian	- 44.17	- 44.17	- 39.76	- 39.76
Production				
Total Revenues: Long-run	- 29.35	- 30.64	- 26.54	- 28.14
Total Revenues : Short-run	- 26.02	- 21.80	- 23.83	- 19.86
Large-scale palay farmer	- 29.07	- 30.67	- 26.55	- 27.78
Small-scale palay farmer	- 26.05	- 21.87	- 23.86	- 19.92
Change in producer surplus: Long-run	- 54.56	- 55.06	- 50.06	- 50.57
Change in producer surplus: Short-run	- 53.31	- 51.72	- 48.73	- 47.06
Large-scale palay farmer	- 54.56	- 55.06	- 50.06	- 50.57
Small-scale palay farmer	- 53.31	- 51.72	- 48.73	- 47.06
Efficiency losses				
Total: Hicksian, LR	- 90.86	- 88.80	- 88.63	- 86.18
Total: Hicksian, SR	-103.17	-3,335.02	-103.37	2,075.14
Total: Marshallian, LR	-126.13	-111.81	-129.86	-112.66
Total: Marshallian, SR	1,067.44	- 35.04	656.27	- 26.35
Production: Long-run	- 73.25	- 73.75	- 68.28	- 69.88
Production: Short-run	- 71.99	- 69.75	- 66.94	- 66.43
Consumption: Hicksian	- 68.44	- 68.44	- 63.14	- 64.40
Consumption: Marshallian	- 66.93	- 66.20	- 61.53	- 62.85

Notes:

- Own-price elasticities of supply used in Case A were as follows: LR= 0.5, SR = 0.3 (Hayami and Herdt 1978).
- Own-price elasticities of supply used in Case B were as follows: LR= 0.5785, SR= 0.0471 (Hinlo and Cruz 2013).
- Own-price elasticities of demand used in both cases were as follows: Market (Hicksian)= -0.2650, market (Marshallian)= -0.5046, high-income consumer (Hicksian)= -0.3126, high-income consumer (Marshallian)= -0.5159, low-income consumer (Hicksian)= -0.2235, low-income consumer (Marshallian)= -0.4814 (Lantican et al. 2013).
- Average exchange rate from 2010-2019 posted in Bangko Sentral ng Pilipinas is used in the analysis: 1 USD = 47 PhP.
- Tariff revenues pre-RTL period were based on the total volume of imports during that period valued at CIF price ATIGA rate
- TP refers to trading partner
- Percent change was computed using the formula: (Post-RTL value – Pre-RTL value/Pre-RTL value)*100

In terms of farmer's revenue, this translates to a reduction of 20 to 26 percent in the short-run and 27 to 31 percent in the long-run from a maximum aggregate revenue of PhP395.08 billion pre-RTL period to as low as PhP286.34 billion in the short-run and PhP268.44 billion in the long-run post-RTL period. Given the estimated number of 2.4 million farmers in the Philippines, this is equivalent to a reduction of annual revenue per farmer from PhP164.62 thousand to PhP119.31 thousand in the short-run and PhP111.85 thousand in the long-run. There will still be gains in the producer surplus relative to zero intervention, but the gains will be reduced by 47 to 53 percent in the short-run and 50 to 55 percent in the long-run after the policy shift. However, this implies that the milled rice which can be bought at PhP33.62 to PhP33.73 per kg pre-RTL period under quantitative restrictions can now be bought by households at cheaper price of PhP26.32 to PhP27.51 per kg. In expenditure terms, this is equivalent to 11 to 17 percent reduction in their rice expenses (from PhP435.23 billion to PhP355.29 billion in aggregate terms or from PhP21.55 thousand to PhP17.59 thousand per household with a family of five). This will also reduce the welfare losses in consumer surplus from as high as PhP169.57 billion pre-RTL period to as low as PhP88.95 billion post-RTL period.

On the government side, the removal of quantitative restrictions will increase the government's revenue to a range of PhP13.67 billion to PhP22.29 billion in the short-run and to a range of PhP20.95 billion to PhP26.94 billion in the long-run—more than enough to cover the annual PhP10 billion appropriation for RCEF. This is because of the expected surge in rice imports by 64 to 177 percent in the short-run and by 149 to 235 percent in the long-run, that is, from 1.18 Mt pre-RTL period to a range of 1.93 to 3.27 Mt in the short-run and to a range of 2.94 to 3.95 Mt in the long-run post-RTL period, which will result to an increase in the total domestic rice supply from 12.79 Mt pre-RTL period to a range of 13.49 Mt to 14.15 Mt (equivalent to five percent to 11 percent increase) post-RTL period. The surge in the rice imports outweighs the reduction in the domestic rice production causing a net positive change in the total domestic rice supply. The increase in rice imports, however, increases the country's vulnerability to commodity price fluctuations in the international market and deteriorating terms of trade. Losses in foreign exchange will also be reduced due to the policy shift from a range of losses equivalent to USD 0.69 billion to USD 1.76 billion in the short-run to a range of losses equivalent to USD 0.45 billion to USD 1.19 billion attributed to the reduction in CIF rice prices which increases the availability of foreign exchange to the economy ignoring the cross-effects with other sources of inflow outflows for foreign exchange. In the long-run, losses in foreign exchange will be reduced from a range of USD 1.62 billion to USD 2.33 billion to a range of USD 0.96 billion to USD 1.50 billion.

Considering the net welfare impact of the policy shift to the society as whole, it was found that removing the quantitative restrictions improves the efficiency of the rice industry. In the pre-RTL period, there is a net efficiency loss ranging from PhP80 million to PhP8.77 billion in the short-run and PhP5.66 billion to PhP18.34 billion in the long-run. Although, there can be net efficiency gain in the short-run in the pre-RTL period if farmer's own-price elasticity of supply is less elastic (0.0471 instead of 0.3), as shown in case B using Marshallian's own-price elasticity of demand. In contrast, in the post-RTL period, there is a net efficiency gain of PhP260 million to PhP6.04 billion in the short-run and a net efficiency gain of up to PhP1.72 billion in the long-run. This is because although there is a reduction in gains in producer surplus, the improvement in consumption efficiency outweighs the losses in production. However, the net effect can be negative in the long-run if the real income effect is fixed, as shown by the estimation results using Hicksian's own-price elasticity of demand and the long-run own-price elasticity of supply.

Looking at the actual trends, there was a drop in the volume of domestic rice production immediately after the implementation of RTL (between 2018 and 2019) by 1.32 percent which can be explained by the three percent reduction in the area harvested in the same period. This is within the range of the author's estimate reduction of one to six percent in the domestic rice production in the short-run. This can be indicative of the fact that some farmers have shifted to other crops due to the reduction in paddy prices. PSA data showed that there was a huge drop in paddy prices from the annual average of PhP24.74 per kg in 2018 to PhP21.45 per kg in 2019. However, in the following years (2020 and 2021), rice production figures have improved considerably as shown in Table 4. This was despite the disruptions to farm operations caused by the COVID-19 pandemic, the series of strong typhoons, and lowered paddy prices. The efforts of the Department of Agriculture (DA) may have played a huge role on this with the full implementation of the RCEF in addition to their regular rice program and rice resiliency project, which is part of the stimulus program of the DA's "Plant, Plant, Plant" Program (otherwise known as *"Ahon Lahat Pagkaing Sapat (ALPAS) Kontra sa COVID-19 Program"*) launched in 2020 that provides fertilizer subsidy to the recipients of seed support and those who purchased or used their own seeds in a form of vouchers amounting to PhP2,000 per hectare for those using inbred seeds and PhP3,000 per hectare for those using hybrid seeds and which was specifically created to boost the domestic production in order to address the global rice supply situation affected by the temporary rice export suspension of Vietnam (Memorandum Order No. 52. 2020). Improved rice productivity is also noteworthy with the increasing trend in rice yields from 3.97 metric tons per hectare in 2018 to 4.15 metric tons per hectare in 2021 based on PSA data. This can be an indication of the improved efficiency in domestic rice production.

With regard to consumption, rice utilization accounts from PSA have shown that per capita consumption of rice has increased from 120 kgs per year in 2018 to 128 and 125 kgs per year in 2019 and 2020, respectively. The increased rice consumption might be explained by the increased affordability of rice and increased availability of rice in the local market. The Department of Finance (DOF) reported that the implementation of RTL in 2019 has decelerated inflation in the country as the entrants of rice imports has slashed prices by about PhP8 per kg on the average (Department of Finance 2020). PSA data also showed that between 2018 and 2019, total domestic rice supply increased by seven percent due to increased rice imports of about 56 percent. The increase in the total domestic rice supply between 2018 and 2019 is within the author's estimate which ranged from five to 11 percent in the short-run. The estimated increase in the rice imports, however, is higher (about 64 to 177 percent) compared to the actual.

Dela Peña (2014) noted that NFA's regulations allow the concentration of legally imported supplies in the hands of a few, making smuggling lucrative. Hence, the removal of NFA's powers and functions on rice importation and regulation could potentially reduce rice smuggling in the country. The positive implication of the policy shift on smuggling issue is also supported in theory by Tsakok (1990) noting that quantitative restrictions may tend to encourage smuggling if there is domestic scarcity of the

commodity and the price differentials between domestic and border prices are substantial, which is the case in the Philippines and will therefore enlarge parallel markets at the expense of official markets and collection of tariff revenues. Hence, the removal of the restrictions can also discourage this illegal practice. Rice smuggling has been a problem in the Philippines for so long as rice has been the top agricultural product being smuggled in the Philippines with around USD 1.96 billion worth of smuggled milled rice from 1986 to 2009 (Lantican and Ani 2020).

Table 4. Area harvested for rice, volume of rice production, and rice yield from 2010 to 2021, Philippines

Year	Area (hectares)	Volume (metric tons)	Yield (mt/ha)	Area (% change)	Volume (% change)	Yield (% change)
2010	4,354,161	15,772,319	3.62			
2011	4,536,642	16,684,062	3.68	4.19	5.78	1.53
2012	4,690,061	18,032,525	3.84	3.38	8.08	4.55
2013	4,746,091	18,439,420	3.89	1.19	2.26	1.05
2014	4,739,672	18,967,826	4.00	-0.14	2.87	3.00
2015	4,656,227	18,149,838	3.90	-1.76	-4.31	-2.60
2016	4,556,043	17,627,245	3.87	-2.15	-2.88	-0.74
2017	4,811,808	19,276,347	4.01	5.61	9.36	3.54
2018	4,800,406	19,066,094	3.97	-0.24	-1.09	-0.86
2019	4,651,490	18,814,827	4.04	-3.10	-1.32	1.84
2020	4,718,896	19,294,856	4.09	1.45	2.55	1.09
2021	4,805,077	19,960,170	4.15	1.83	3.45	1.59

Source of basic data: Philippine Statistics Authority

Disaggregating the welfare impact. Disaggregating the analysis, author's simulation found that the removal of quantitative restrictions causes small-scale farmers to reduce their rice output by one to six percent while large-scale farmers by nine to 12 percent. Larger percent reduction in output is expected with large-scale farmers since their own-price elasticity of supply is more elastic (0.5 to 0.5785) compared to small-scale farmers (0.0471 to 0.3) (Hayami and Herdt 1978; Hinlo and Cruz 2013). Expressed in quantity, this is a reduction from 9.64 Mt to a range of 9.03 to 9.55 Mt for small-scale farmers and a reduction from 1.97 Mt to a range of 1.73 to 1.79 Mt for large-scale farmers. In revenue terms, small-scale farmers' aggregate income will be reduced by 22 to 26 percent, that is, from an income range of PhP321.36 billion to PhP328.04 billion to an income range of PhP249.78 billion to PhP262.71 billion; whereas large-scale farmers' aggregate income will be reduced by 27 to 31 percent, that is, from an income range of PhP65.67 billion to PhP67.04 billion to an income range of PhP45.53 billion to PhP49.24 billion.

Gains in producer surplus of small-scale farmers will also be reduced by 47 to 53 percent, from a range of PhP121.45 billion to PhP134.01 billion to a range of PhP59.21 billion to PhP67.70 billion, while that of large-scale farmers will be reduced by 50 to 55 percent, from a range of PhP23.43 billion to PhP24.80 billion to a range of PhP10.94 billion to PhP11.90 billion. Hence, although both large-scale and small-scale farmers are negatively affected by the policy shift, larger economic loss is incurred by small-scale farmers because they contribute bulk of the total rice production, worsening the equity on the production side. While removal of quantitative restrictions increases efficiency, the displacement effects of the expected surge in rice imports will translate to larger negative income effects for household groups heavily relying on agriculture (especially palay production), worsening poverty situation and income inequality (Cororaton 2004).

However, the increase in income poverty comes in small increments and decreases over time. From the estimated loss of PhP2.84 billion in the first three years for the poor households to PhP2.10 billion in 2022 to 2024, to PhP500 million in 2025 to 2027, and further down to PhP134 million in 2028 to 2030, summing up to PhP14.9 billion for the six-year period, which is the minimum amount of cash transfers necessary to compensate the poor for any increase in their respective absolute poverty gaps (Briones 2021) This value of income loss suffered by the poor is way below the amount provided by the law (PhP60 billion) to mitigate its negative impact. It was recommended that targeted cash transfers be used to counter the poverty effect in the short-run and productivity improvement and infrastructure support provided, which are already accounted for in RCEF, to counter the long-term effects (Briones 2021; Cororaton 2004). This recommendation appears to be heard by the DA as it recently implemented the Rice Farmers Financial Assistance (RFFA) program, which gives direct cash transfers amounting to PhP5,000 to rice farmers with two hectares or less to till to compensate for the income losses they incurred due to drop of rice prices (Talavera 2022). The RFFA sources its fund from the annual excess of the PhP10 billion tariff revenues allocated for RCEF. According to DOF (2020), in only seven months after RTL's implementation, cash revenues of PhP12.3 billion were collected as of December 31, 2019, which was beyond the amount allocated yearly for RCEF. With the first two years of RTL's implementation, the DA was able to distribute cash assistance amounting to PhP7.6 billion in 2021 (Talavera 2022).

On the consumption side, removal of quantitative restriction increases rice consumption of low-income consumers by four to ten percent and of high-income consumers by six to 11 percent. Larger percent increase in demand is expected on high-income consumers since their own-price elasticity of demand (-0.3126 to -0.5159) is more elastic compared to low-income consumers (-0.2235 to -0.4814) (Lantican et al. 2013). Expressed in quantity, this is an increase from 8.96 Mt pre-RTL period to a range of 9.35 to 9.87 Mt for low-income consumers post-RTL period and an increase from 3.82 Mt pre-RTL period to a range of 4.05 to 4.24 Mt for high-income consumers post-RTL period. In expenditure terms, low-income consumers' aggregate expenditure will be reduced due to lower well-milled rice prices by 12 to 17 percent, that is, from an expenditure range of PhP298.69 billion to PhP304.90 billion to an expenditure range of PhP247.13 billion to PhP269.31 billion; whereas high-income consumers' aggregate expenditure will be reduced by 11 to 16 percent, that is from an expenditure range of PhP127.34 billion to PhP129.99 billion to an expenditure range of PhP107.38 billion to PhP111.41 billion. Consumer surplus of low-income consumers will also be improved from a loss ranging from PhP108.67 billion to PhP119.96 billion pre-RTL period to a loss ranging from PhP62.18 billion to PhP65.46 billion, while that of high-income consumers will be improved from a loss ranging from PhP46.01 billion to PhP50.17 billion to a loss ranging from PhP26.68 billion to PhP27.96 billion. This improves the equity in the consumption side since more savings are gained by low-income consumers than high-income consumers because the former spent larger share in rice expenditures than the latter. Rice affordability after the policy shift can improve food security in the country and may have an impact in addressing nutrition problems especially among the low-income group since reduced rice expenditures can increase their expenditures on high calorie foods such as meat and poultry products and can therefore, improve their diet diversity (Delos Reyes 2010). However, although the policy shift promises rice affordability, this may be constrained by the multi-layered supply chain of rice in the Philippines which increases marketing costs (Mataia et al. 2020), affecting rice retail prices. The continued efforts of the government in reducing the gap between the farmgate and retail prices of agricultural products including: (1) facilitation of efficient and seamless delivery of imported rice products from ports to markets; (2) effecting the immediate release of existing rice stocks in NFA warehouses; (3) watching closely the transport of rice from ports to NFA warehouses and retail outlets; (4) setting up of public markets where producers can sell their goods directly to consumers; and prioritizing the release of essential food items in the ports (DOF 2020), among others play a huge role in reaping the benefits of the policy reform.

CONCLUSIONS, RECOMMENDATIONS, AND POLICY IMPLICATIONS

Using partial equilibrium analysis, results showed that under post-RTL period, total domestic rice supply will increase substantially since the reduction in domestic rice production will be outweighed by the increase in the volume of rice importation. Household access to rice will also be improved especially among the low-income consumers, who aggregately spend more on rice than high-income consumers due to reduced rice expenditures. Inefficiency losses are reduced on the consumption side because the quantitative restrictions, which previously created a scarcity rent that blew up rice prices, were removed, and there are still gains in producer surplus relative to zero intervention, but the gains are reduced due to the reduced rice prices. Net effect to the society is positive mainly because the reduction of inefficiency losses on the consumer side outweighs the reduction of the welfare gains on the production side. In addition, more government revenues will be earned, and there will be some savings in foreign exchange. However, the increase in rice imports due to the removal of quantitative restrictions also increases the country's vulnerability to shocks in the international market, and while the policy shift may improve the country's food security due to more affordable rice prices, food self-sufficiency is threatened. Diversifying sources of imports may be explored for this purpose as also proposed by some experts since currently, the Philippines is importing rice primarily from Thailand and Vietnam, but Indian rice are also becoming cheaper.

On the other hand, the disaggregated simulations in this study showed that equity is improved on the consumer side because low-income consumers, who aggregately spend more on rice than high-income consumers, will save more on their rice expenditures. This may increase the expenditures of the households especially those belonging to the low-income group on high calorie foods such as meat and poultry products and can therefore, address nutrition problems in the country. However, it must also be noted that the policy shift must be coupled with cost-minimizing strategies that improve overall efficiency in the rice value chain to realize the gains of the policy reform. On the production side, equity is worsened because small-scale farmers contribute bulk of the local rice production and therefore, will be more aggregately affected by the lowered rice price and will suffer greater revenue losses. The continued targeted support to small-scale farmers which are currently being implemented through the RFFA program could help in offsetting the trade-offs between efficiency and equity in the domestic rice market.

Holistically, the implementation of RTL seems to have catalyzed the development of the rice sector. The pressures brought about by the policy shift to the sector have helped in increasing efficiencies and resiliency amidst shocks as evidenced by the improved rice productivity and its three percent growth in Gross Value Added (GVA) in 2020 and 2021 amidst the COVID-19 pandemic based on PSA data. In any case, this has also make the achievement of food security more sustainable.

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EFFECT OF DIETARY BLACK SOLDIER FLY, *Hermetia illucens* (Linnaeus) LARVAE MEAL AND POULTRY MEAL ON PRODUCTION PERFORMANCE, EGG QUALITY, AND NUTRIENT DIGESTIBILITY IN POST-PEAK CHICKEN LAYERS

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ABSTRACT

This study was conducted to determine the effects of feeding black soldier fly larvae meal (BSFLM) and poultry meal (PM) on production performance, acceptability of eggs through sensory evaluation, digestibility of dietary energy, fat, and protein in post-peak Babcock white layer chickens. A total of 192 54-week-old Babcock white layer hens were randomly allocated into one of four dietary treatments (T1 – control, T2 – 3% poultry meal (PM), T3 – 1.5% PM + 1.5% BSFLM, T4 – 3% BSFLM) with 16 replicates each, using randomized complete block design and with location of cages as blocking factor. There were no significant differences observed on hen-day egg production but the average egg weight was significantly greater ($P<0.05$) in birds fed with 3% BSFLM and control than T2 and T3. Significant improvements ($P<0.0001$) in albumen height, egg yolk color, and Haugh unit were observed with 3% BSFLM inclusion compared to T1 and T2. Inclusion of BSFLM in diets significantly increased the feed cost per bird but had no effect on the cost of feed per kilogram of eggs produced per bird. BSFLM supplementation at 3% did not have any significant effects on albumen texture, yolk color, yolk flavor, and overall acceptability of eggs. The results suggest that BSFLM can be included in the diet without any negative effect on overall acceptability at 3% inclusion. Inclusion of dietary BSFLM did not have a significant effect on the digestibility of dietary gross energy but significantly increased the digestibility of protein ($P<0.0114$) and fat ($P<0.0001$).

Key words: Stratiomyidae, Diptera, alternative protein source, supplemental animal protein, sensory evaluation

INTRODUCTION

Food insecurity is one of the fundamental challenges of today and, if left unaddressed, will continue to be a problem in the future. With the projection that the global human population will reach almost 10 billion by 2050, the demand for agricultural output is also expected to increase (Vos 2015). Such projections emphasize the need to consider the sustainability of various agricultural inputs. Poultry by-product meal or poultry meal (PM) is favored over fish meal due to the latter's effect on the taste of yolks and the problem in the overexploitation and the negative impact of the El Niño cycle led to a drastic decrease in the supply of fish meal from roughly eight million metric tons in the year 2000 to six million metric tons as of 2015 (Rabobank 2015). PM similar to Fish meal has a high crude protein

(CP) content which ranges from 58-68% on as fed basis, the relative abundance of amino acids, and high digestibility compared to soya and other sources. PM from poultry processing wastes provides a very sustainable source of protein for poultry; however, the price of PM is still high (Samli et al. 2006) but the cost is also high.

Insects are vastly present in any place on the globe, comprising as high as 80 million in diversity but with just roughly one million identified species (Stork et al. 2015). Its enormous biomass and ability to mass-produce biowaste with a high conversion rate make them a viable natural source of protein for animals, especially for poultry and fishes (Ravindran 2013). One of the most commonly mass-produced insects is the black soldier fly (*Hermetia illucens)* or BSF. The ability to efficiently convert various organic wastes, from kitchen wastes to human and animal manure, into large insect biomass in a short period of time makes BSF the most promising compared to other studied insects such as the common housefly, *M. domestica (*Pretorius 2011), meal worm, *T. molitor,* (Veldkamp et al. 2012), and locusts (Van Huis, et al. 2012). Approximately 42% crude protein and 29% crude fat render the BSF comparable to soya, and when defatted, the crude protein content can go as high as 60% crude protein which is close to the fish meal's protein content (Spranghers et al. 2017).

Insect meal can stabilize the demand for high-quality protein source which can result to much lower prices of raw materials and higher economic impact especially when reared by the farmers themselves (Onsongo et al. 2018). This lowers the input and maximizes the income of farmers, especially the smallholders. The overexploitation of fish for fish meal and fish oil production can also be minimized. Furthermore, the greenhouse gas emissions can also be reduced by shifting to insect production instead of converting rain forests into soya plantations. Black Soldier Fly farming GHG emission is 47% lower than of windrow composting (Mertenat et al. 2019.) Insects as decomposers can close the loop on the food chain by converting wastes into high quality protein source for animals, and possibly, for human consumption.

The study sought to determine the effect of the black soldier fly larvae meal (BSFLM) on production performance, egg quality, acceptability through sensory evaluation, cost of production, and nutrient digestibility of layer diets.

MATERIALS AND METHODS

Animals and experimental design. A total of 192 54-week-old Babcock white layers with an initial average weight of 1.58 kg were used in an 8-week production performance study. Layers were blocked by location and were randomly divided into four treatment groups. There were 48 birds per treatment, composed of 16 replicates of three birds per cage on a completely randomized block design. The layers were housed in A-type cages in an open-sided housing with a floor space of one square foot per hen. A total of 110 grams of feed per day were allocated to each bird with ad libitum access to water, under 16:8 (day:night) photoperiod. The study was carried out at the University Animal Farm of the University of the Philippines Los Baños located in Barangay Tuntungin-Putho, Los Baños, Laguna.

Experimental diets. Four experimental treatments were utilized in this study: 1) typical corn-soy based diet without BSFLM and poultry meal, 2) basal diet with 3% poultry meal (PM), 3) basal diet with 1.5% BSFLM and 1.5% PM, and 4) basal diet with 3% BSFLM. The BSFLM used was reared from a corn-coffee ground substrate, oven-dried and its particle size reduced with a mechanical grinder without any further defatting process. Poultry Meal (PM) used came from local supplier of poultry by-product meal. All dietary treatments were prepared in mashed form. The same diets were used in the digestibility study. The ingredient composition, calculated nutrient composition and analyzed nutrition composition of the experimental diets are shown in Tables 1, 2, and 3, respectively.

Table 1. Ingredient composition (as fed) of the experimental diets of post-peak chicken layers.

Ingredients %	Diet[1]			
	T1 Control	T2 3% (PM)	T3 1.5% PM + 1.5% BSFLM	T4 3% BSFLM Control
Yellow corn	48.4	52.2	51.1	48.7
US soya high protein	22.2	17.6	18.44	19.2
Rice bran D1	15.0	15.0	15.0	16.2
Palm oil	2.2	0.9	1.0	1.3
Monodicalcium phosphate	1.2	0.7	1.0	1.2
Limestone	10.0	9.7	9.5	9.4
Salt, iodized	0.2	0.2	0.2	0.3
DL-Methionine	0.2	0.2	0.2	0.2
L-Threonine	0.1	0.1	0.1	0.1
L-Lysine	0.1	0.02	0.03	0.03
Choline chloride, 60%	0.1	0.1	0.1	0.1
Vitamin Premix	0.1	0.1	0.1	0.1
Mineral Premix	0.1	0.1	0.1	0.10
Toxin binder	0.05	0.05	0.05	0.05
Antioxidant	0.05	0.05	0.05	0.05
Poultry meal	-	3	1.5	-
BSF larvae meal	-	-	1.5	3. 00
Total	**100**	**100**	**100**	**100**

[1]BSFLM – Black soldier fly larvae meal.
[2]PM- Poultry Meal

Table 2. Calculated nutrient composition (as fed) of the experimental diets.

Item	Diet			
	T1 Control	T2 3% (PM)	T3 1.5% PM + 1.5% BSFLM	T4 3% BSFLM
ME, kcal/kg	2700	2700	2753	2700
Crude protein, %,	16.5	16.5	16.5	16.5
Crude fiber, %,	3.4	3.37	3.4	3.5
Crude fat, %,	6.2	5.57	6.8	6.2
Linoleic acid, %,	1.7	1.71	1.7	1.7
Digestible Amino Acid, %				
Lysine	0.8	0.84	0.8	0.8
Threonine	0.7	0.7	0.7	0.7
Methionine	0.4	0.4	0.4	0.5
Methionine+Cystine	0.7	0.7	0.7	0.7
Tryptophan	0.2	0.2	0.3	0.2
Mineral composition, %				
Ca	4.0	4.0	4.0	4.0
P, available	0.4	0.4	0.4	0.4

Table 3. Analyzed nutrient composition (as fed) of the experimental diets.

Item	Diet			
	T1 Control	**T2 3% Poultry Meal (PM)**	**T3 1.5% PM + 1.5% BSFLM**	**T4 3% BSFLM Control**
Dry matter, %	90.5	90	90.5	91.1
Crude protein, %	16.1	16.3	16.2	16.0
Crude fat, %	3.3	4.4	3.2	4.2
Crude fiber, %	3.5	4.5	3.3	3.8
Ash, %	11.1	11.3	11.0	12.9
Gross energy, kcal/kg	3485.5	3464.4	3489.6	3466.19

Production performance. Eggs were harvested, counted, and weighed daily for evaluation of production performance and egg quality. Total feed intake and feed refusal were recorded on a weekly basis.

Hen-Day Egg Production (HDEP). Collected eggs were counted for each treatment to determine HDEP. HDEP was computed by dividing the number of eggs laid by the number of birds per treatment, then multiplying the result by 100.

$$HDEP = \frac{Total\ number\ of\ eggs\ laid}{Total\ number\ of\ birds}\ x\ 100$$

Average Egg Weight (AEW). All eggs upon collection were weighed and recorded. For each treatment, the total egg weight per cage was divided by the total number of eggs per cage to determine the AEW.

$$AEW = \frac{Total\ egg\ weight}{Total\ number\ of\ eggs\ weighed}$$

Economic parameter. The cost of feed consumed per bird for each treatment was calculated by multiplying the total feed intake (in kg) by the price per kg of feed.

$$Feed\ cost\ per\ layer\ chicken\ (PHP) = Total\ feed\ consumed\ x\ Price\ per\ kg\ of\ feed$$

For each treatment, feed cost per kg of eggs produced was computed by dividing the total feed cost per chicken layer by the weight (in kg) of the eggs laid.

$$Feed\ cost\ per\ kg\ of\ eggs\ produced\ (PHP) = \frac{Total\ feed\ cost}{Total\ kg\ of\ eggs\ produced}$$

Feed efficiency. Feed efficiency was determined by dividing the total amount of feed consumed per bird by the total weight of laid eggs multiplied by 100.

$$Feed\ Efficiency = \frac{kg\ of\ feed\ consumed}{kg\ of\ egg\ produced} x100$$

Egg quality. Egg weight was determined using a digital scale and a digital (Mitutoyo Digital Vernier Caliper 500-196-30/197-30/173 MM/Inch Electronic Micrometer Gauge 0-150/200/300mm/0.01mm) caliper for albumen height and eggshell thickness. Egg yolk color was determined using a color (DSM YolkFan™) fan.

Haugh unit. Haugh unit was computed using the following formula:

$$HU: 100\ log\ (H + 7.5 - 1.7W^{0.37})$$

Where:

H = albumen height
W = egg weight in grams

Eggshell thickness. Eggshell thickness was determined by getting the average thickness of the top, mid, and bottom parts of the eggshell upon removal of the eggshell membrane.

Sensory evaluation. A total of 11 trained panelists participated in the sensory evaluation of egg characteristics. The test was conducted at the Sensory Evaluation Laboratory of the Animal Products Science and Technology Division, Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines Los Baños, Laguna, Philippines. The panelists were placed in individual cubicles to avoid peer influence on the ratings. Evaluation was done in three separate sessions for three consecutive days. A total of 66 eggs were evaluated in terms of yolk color, albumen texture, yolk flavor, yolk off-flavor, and overall acceptability. Six eggs per treatment were randomly selected from the final week of the experimental period. Eggs were collected at the start of Week 8 at around 11:00 o'clock in the morning. All eggs were stored in the refrigerator to maintain freshness prior to cooking the following day. All egg samples were placed in a pan with boiling water (4L) for ten minutes. Boiled eggs were cooled with running tap water and shells were removed. Each egg was cut into halves and then quartered. Cut egg samples arranged in pre-coded tray using a 3-digit random numbers and served on a plate to the panelists in individual cubicles or stations for immediate evaluation. Samples from each treatment were served randomly to the panelists. Cold water was also served to the panelists for cleansing their palate in between tasting of samples. Yolk color, albumen texture, yolk flavor, yolk off-flavor, and overall acceptability were rated from zero to 100 using a 100-mm horizontal line sheet.

Digestibility of layer diets. A total of 96 layers were used for the digestibility study. Three birds per cage with eight replicates from four experimental diets were randomly selected for the trial using a completely randomized design. layers were housed in metabolic cages with installed waterers and feeders. Aluminum trays were placed directly under the cages for excreta collection. Marker-guided (using titanium dioxide) total collection method was used in the study.

Digestibility calculations. The apparent total tract digestibility (ATTD, %) of crude protein (CP), crude fat (CFat), and gross energy (GE) were calculated using the following equation:

$$ATTD\ of\ CP/GE/CFat, \% = \frac{(Feed\ intake\ x\ CP/GE/CFat\ feed) - (Excreta\ output\ x\ CP/GE/CFat\ excreta)}{(Feed\ intake\ x\ CP/GE/CFat\ feed)}\ x\ 100$$

Feeding and excreta collection. Titanium dioxide was mixed in all diets at 0.40% of the total diet to indicate the start and end of the five-day total collection period. Excreta samples were weighed, labelled, dried, homogenized (after drying), and stored at -20°C until analyses. Impurities such as feathers and broken eggs seen on the excreta were removed to avoid contamination and overestimation of CP, GE, and CFat contents.

Chemical analysis. BSFLM, Poultry Meal and dietary treatment samples were analyzed for moisture, CP, CFat, crude fiber (CF), ash, and nitrogen-free extract (NFE), as well as GE, following the guidelines and procedures established by AOAC (2007). Amino and fatty acid profile were analyzed using high-performance liquid chromatography (HPLC) at the Upscience Labs Solutions, Vietnam. The pooled and stored excreta samples were further oven-dried to constant weight at 70ºC. Dried samples were finely ground and analyzed for GE, CP, and CFat following the guidelines and protocols from AOAC (2007). All samples were analyzed in triplicates.

Statistical analysis. MIXED procedure (SAS Institute Inc., Cary, NC) of SAS was used in analyzing data with cage as experimental unit and diet and block as the fixed and random effects part of the model. Tukey-Kramer test was used as mean separation for the least square means for each independent variable. Orthogonal contrasts were used to compare the effect of each treatment on all production performance parameters and egg quality characteristics, sensory evaluation parameters, and digestibility of GE, CP, and CFAT. Significant level for each test was set at $P < 0.05$ to detect statistical significance.

RESULTS AND DISCUSSION

Production performance. Table 4 shows the analyzed nutrient composition of BSFLM and the locally sourced poultry meal. The CP content of BSFLM analyzed in this study (44.25 % CP) is similar to 44.2% CP for BSFLM reared on abattoir waste (Lalander 2019) and 42-44% CP reared on swine manure (St. Hilaire et al. 2007), but slightly higher than the 42.1% CP content published by Feedipedia.com.

Table 4. Nutrient composition of Black Soldier Fly Larvae Meal (BSFLM) and Poultry Meal (PM).

Component	Nutrient composition	
	BSFLM	**PM**
Dry matter, %	90.5	91.6
Crude protein, %	44.3	64.7
Crude fiber, %	7.8	1.8
Crude fat, %	38.8	14.9
Ash, %	6.4	14.8
Gross energy, kcal/kg	6328.3	4840.2

The observed CP content of BSFLM which was fed with a corn-coffee diet was different compared with previous studies. This may be attributed to nutrient composition of substrate used in mass producing the larvae (St. Hilaire et al. 2007). The high CP content corresponded to high quality amino acids present in BSFLM as shown in Table 5. The results of the amino acid profiling from this study and the high GE value can be attributed to the high CFat content of BSFLM at 38.82%, which agrees with the values reported by Barragan-Fonseca (2017); Choi (2013), Mutafela (2015), Mywaniki (2018), and St. Hilaire (2007.).

The Cfat,CP, and GE of both ingredients influenced the different levels of inclusion of corn and palm oil acrossdietary treatments to meet the layer requirement.

Table 5. Amino acid profile of Black Soldier Fly Larvae Meal (BSFLM)

AMINO ACID	Amount of AA(mg)/ 100g BSFLM
Alanine	3.1
Arginine	1.9
Aspartic acid	3.8
Cysteine	0.3
Glutamic acid	4.4
Glycine	2.2
Histidine	1.1
Isoleucine	1.8
Leucine	3.0
Lysine	2.4
Methionine	0.9
Phenylalanine	1.1
Proline	2.6
Serine	1.5
Threonine	1.6
Tryptophan	0.6
Tyrosine	2.1
Valine	2.4
Total Protein	36.8

Majority of the fatty acid present in BSFLM were the short chain fatty acid, lauric acid (Table 6). BSFLM is composed of 70% saturated fatty acid, 13.6% omega-9, and 13.4% omega-6 fatty acids. The variations of the nutrient composition of BSFLM and nature of its amino and fatty acid profile showed the ability of BSFL to adopt the nutrient profile of substrates used as feed (Shumo et al. 2019.) The higher the protein and fat content of the substrates are, the more likely to produce BSFLM with high CP and CFat content (Choi et al. 2013, Mutafella et al. 2015, and Mywaniki et al., 2018). The corn used in the study to feed the larvae had CP content of 8% but due to its high energy content, the rearing period of the BSF larvae ranged from 15-16 days but with a small to medium size larvae. The BSF larvae reared on pure coffee yielded a very small size of larvae over a longer rearing period of 30-40 days. The combination of coffee and corn increased the rearing duration to 21-25 days that allowed the larvae to accumulate more protein that translated to a higher CP content and fat (Tschirner and Simon 2015).

Table 6. Fatty acid profile of Black Soldier Fly Larvae Meal (BSFLM).

Fatty acid (FA)		% Relativity	Amount of FA (mg) /100g BSFLM
Total Saturated Fatty Acid		71.0	26554.2
C10:0	Capric	1.0	376.4
C12:0	Lauric	43.8	16621.0
C14:0	Myristic	7.6	2817.7
C15:0	Pentadecanoic	0.1	40.5

Fatty acid (FA)		% Relativity	Amount of FA (mg) /100g BSFLM
C16:0	Palmitic acid	15.4	5609.4
C17:0	Margaric	0.7	238.9
C18:0	Stearic acid	2.0	730.1
C20:0	Arachidic acid	0.2	60.5
C22:0	Behenic acid	0.1	28.2
C24:0	Lignoceric acid	0.1	31.5
Total Mono Unsaturated Fatty Acid			
C14:1 n-5	Myristoleic acid	0.1	40.5
C16:1 n-7	Palmitoleic acid	1.1	387.2
C17:1 n-8	9-cis-heptadecenoic acid	0.0	14.4
C18:1	Oleic acid	13.8	4921.2
C20:1 n-12	Cis-8-eicosenoic acid	0.1	38.9
Total PolyUnsaturated Fatty Acid			
C16:2	Hexadecadienoic acid	0.0	14.4
C18:2 n-6	Linoleic acid	13.5	4769.3
C18:3 n-3	alpha-linoleic acid	0.4	144.3
C18:4	Octadecatetraenoic acid	0.1	45.4
C20:5 n-3	Cis-5, 8,11,14,17-eicosapentaenoic acid (EPA)	0.0	13.8
SUMMARY			
Sum of omega-3	n-3	0.5	158.1
Sum of omega-6	n-6	13.4	4740.9
Sum of omega-9	n-9	13.6	4853.3
Sum of Trans fat		0.1	28.6
Saturated fatty acid		70.9	26569.5
Monounsaturated fatty acids (MUFA)		15.1	5383.7
Polyunsaturated fatty acids (PUFA)		14.1	4987.2
Total fatty acids		127.7	36940.4

Characteristics such as egg weight, albumen height, Haugh unit, egg yolk color, and eggshell thickness were measured to determine the quality of eggs produced by birds fed with experimental diets. Table 7 shows the hen-day egg production (HDEP) of birds fed with experimental diets formulated in this trial. It can be noted that no significant effect was observed for all experimental diets on the HDEP of birds. This is contrary to the result published by Al-Qazzaz et al. (2016) of significantly higher HDEP and house-hen egg production (HHEP) at 5% inclusion of BSFLM in the experimental diet. The increase in HDEP and HHEP from the above report was not realized in this study perhaps due to the lower inclusion of BSFLM in the diet at only 3%. The isocaloric and isonitrogenous formulated diets met the nutrient requirements for egg production and the inclusion of 3% BSFLM was, perhaps, inadequate to increase HDEP of layers used in this study.

It was generally observed that T1- and T4-fed birds expressed significantly higher AEW compared to T2- and T3-fed birds, with T2-fed birds having the lowest AEW. The increased egg weight observed in T4-fed birds was contrary to the results reported by Al-Qazzaz et al. (2016) and Mwaniki et al. (2018) where the lowest egg weight was observed from birds fed with diets including 5% BSFLM.

The score for egg yolk color in this study was higher (*P <0.0001*) in T4 with 3% BSFLM compared to the rest of the treatments (Table 7). High score on egg yolk color is important due to the consumer preference towards eggs with golden yellow to orange yolk colors. Yolk color improvement in treatments with BSFLM can be explained by the elevated carotenoid concentration found in the BSFLM (Secci et al. 2018).

The highest albumen height was at T4 with 10.24 mm (P<0.0001). Albumen height was observed to be higher in T4 (P<0.0001) with 3% BSFLM, followed by T3 (P=0.0001) with 1.5% BSFLM and 1.5% PM (Table 7). Average albumen height from the study ranged from 9.57 - 9.99 mm, which is higher than the results from Kawasaki et al. (2019) at 7.16 - 8.08 mm and from Ruhnke et al. (2018) at 8.91 - 9.18 mm.

None of the experimental diets in this study yielded significant effect on eggshell thickness. The average thickness of eggshell ranged from 0.32 - 0.33 mm, lower than the results from Ruhnke et al., (2018) at 0.44 - 0.46 mm, and within the range of the results from Park et al. (2017) at 0.33 – 44 mm. The thinner eggshell was due to the age difference of the birds since older birds produce bigger eggs with thinner shells.

Table 7. Effects of Black Soldier Fly Larvae Meal (BSFLM) and poultry meal on production performance of laying hens.

Item	Diet				SEM	*P*-value
	T1	T2	T3	T4		
	Control	3% PM	1.5% PM + 1.5% BSFLM	3% BSFLM		
Hen-day egg production, eggs	19.3	19.7	19.5	19.6	0.210	0.482
Average egg weight, g	64.1_{a}	61.7_{b}	62.9_{ab}	63.9_{a}	0.528	0.008
Albumen height, mm	9.6_{bc}	9.4_{c}	9.7_{b}	10.0_{a}	0.063	<0.0001
Haugh Unit	97.3_{bc}	96.5_{c}	97.7_{b}	99.0_{a}	0.296	<0.0001
Egg yolk color	5.6_{b}	5.3_{b}	6.6_{a}	7.1_{a}	0.216	<0.0001
Eggshell thickness, mm	0.3	0.3	0.3	0.3	0.005	0.749

[1] Least square means of 16 replicates per treatment with 3 birds per replicate

Haugh unit (HU) is dependent on the albumen height and egg weight and used as an indicator of egg freshness. It can be noted that the highest average significant HU value recorded was from T4 with 3% BSFLM at 99.3 (P=0.0004) on week 8 of the trial. The HU in the study ranged from 96.5 (T1) to 99.03 (T4) at p-value of <0.0001. Higher albumen height corresponds to superior egg quality (Selim

et al. 2018). Results from this trial on HU are within the range set by USDA standards. Categories set by USDA on the quality of eggs are as follows: "AA" for HU value of > 72, "A" for HU values 60-72, "B" for HU values 31-60, and "C" for values < 31. Therefore, eggs from this study fell under AA category, indicating better quality eggs.

There were significant differences on the average feed cost per bird among the dietary treatments (Table 8). Feed costs per bird on diets containing BSFLM (T3 and T4) were significantly greater than in T1 and T2, with T4 having the highest feed cost per bird. This study showed that increasing the level of BSFLM in the diet will result in higher average feed cost per bird due to the present high cost of production of the BSFLM. However, this is compensated by the higher AEW resulting in T1 and T4 (P=0.008)

Table 8. Economic analysis on the inclusion of Black Soldier Fly Larvae Meal (BSFLM) and poultry meal in the experimental diets.

Item	**Diet**				**SEM**	***P*-value**
	T1	**T2**	**T3**	**T4**		
	Control	**3% PM**	**1.5% PM + 1.5% BSFLM**	**3% BSFLM**		
Feed cost, Php per Kg	25.1	25.3	25.4	25.6		
Average total feed intake, Kg (56 days)	6.1	6.2	6.1	6.1	0.010	0.240
Average total egg weight per bird, Kg	3.3	3.2	3.3	3.32	0.041	0.369
Average feed cost per layer, Php	154.3_c	155.4_b	155.9_b	165.8_a	0.264	<0.0001
Average feed cost per kilogram of eggs (Php/kg)	47.0	48.3	48.2	47.3	0.633	0.384

[1] Least square means of 16 replicates per treatment with 3 birds per replicate

[2] Cost of BSFLM/kg used in the study is Php55.00

[3] Cost of PM/kg used in the study is Php54.00

Sensory evaluation. Albumen texture, yolk color, yolk flavor, yolk off-flavor, and overall acceptance were not significantly affected by experimental diets with BSFLM as shown in Table 9. The result of the study was not consistent with Al-Qazzaz et al. (2018) who reported that increasing levels of BSFLM in the diet improved the appearance, texture, taste, and overall acceptance of the eggs. The level of glutamic acid content in eggs has shown interaction with human perception of taste (Yoshida et al. 1998). The enhancement on the flavor was seen to come from eggs treated with increasing level of BSFLM. Though BSFLM in this study has high levels of glutamic acid, the inclusion of 3% in the diet was probably inadequate to improve the taste of the eggs.

Table 9. Sensory evaluation of egg quality fed with Black Soldier Fly Larvae Meal (BSFLM) and poultry meal.

Item	Diet				SEM	*P*-value
	T1	T2	T3	T4		
	Control	3% PM[2]	1.5% PM + 1.5% BSFLM	3% BSFLM[1]		
Albumen texture	46.8	46.8	45.7	40.5	3.266	0.252
Yolk color	49.6	52.0	47.5	49.3	2.863	0.496
Yolk flavor	68.2	66.0	67.4	68.3	0.512	0.548
Yolk off-flavor	0.8	1.6	1.1	0.9	0.566	0.512
Overall Acceptability	71.7	72.7	72.1	70.6	2.223	0.704

[1]BSFLM – Black soldier fly larvae meal.
[2]PM- Poultry Meal

Digestibility of layer diets. Inclusion of BSFLM did not have a significant effect on the ATTD of GE (Table 10). Reports for ATTD of GE for layers fed with BSFLM are limited, hence, comparison with the result of the present study could not be made. However, ATTD of GE for broilers fed with BSFLM was reported to be lower than that of this study at 64% for *Tenebrio molitor* and 69% for BSFLM (De Marco et al. 2015). The difference in ATTD values can be explained by the differences in utilization of energy due to the development of gastrointestinal tract in broilers in contrast to layers. Significantly higher *(P<0.0001)* ATTD of CFat was observed in diets containing BSFLM than in the control. There was also a significant (*P<0.0114)* difference on ATTD of CP. Treatments supplemented with BSFLM had higher ATTD of CP than the control diet. This suggests that up to 3% BSFLM's improved dietary protein and fat utilization without negatively affecting laying performance.

Table 10. Apparent total tract digestibility of crude fat, crude protein, and gross energy.

Item	Diet				SEM	*P*-value
	T1	T2	T3	T4		
	Control	3% Poultry Meal (PM)	1.5% PM + 1.5% BSFLM	3% BSFLM		
Crude fat, %	83.8c	89.1a	87.2ab	85.3bc	0.57	<0.0001
Crude protein, %	55.7b	61.6a	62.5a	61.6a	1.614	<0.0114
Gross Energy unit, %	79.8a	79.9a	81.6a	79.3a	0.808	0.1633

[1] Least square means of 8 replicates per treatment with 3 birds per replicate

This was supported by the results published by Marono et al. (2015) where chitin lowered the digestibility of protein *in vitro.* This is contrary to the study by De Marco et al. (2015) which reported that chitin from the BSFL exoskeleton had negative influence on the ATTD of nutrients in broiler chickens, with 25% inclusion of BSFLM in the diet.

SUMMARY AND CONCLUSIONS

Inclusion of 1.5% and 3% BSFLM and PM in the diets did not have a negative effect on hen-day egg production. The weight and size of eggs produced by BSFLM-fed birds were superior to those produced by PM-fed birds and comparable to the control. The inclusion of combined 1.5% BSFLM with 1.5% PM and 3% BSFLM significantly improved the egg quality in terms of albumen height, Haugh unit, and yolk color. The cost of feed per bird was higher with increasing level of BSFLM in the diet but the cost of feed per kilogram of egg produced was not affected due to the higher number and bigger sizes of eggs produced. BSFLM supplementation at 1.5% and 3% did not have a negative effect on albumen texture, yolk color, and yolk flavor. The overall acceptability of eggs produced by BSFLM-fed birds was similar to those produced by birds given other dietary treatments. The inclusion of 1.5% and 3% BSFLM in the diet increased the ATTD of crude protein and fat but did not have a negative effect on the ATTD of energy.

Therefore, the black soldier fly larvae meal can be used as an alternative protein source in the diet without affecting negatively production performance, egg quality in terms of higher average egg weight, albumen height, Haugh unit, egg yolk color, and digestibility of fat, protein, and energy in post-peak chicken layers.

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WHY GOVERNMENT SUPPORT IS NOT SO EFFECTIVE TO BOOST PERFORMANCE OF COOPERATIVES: A CASE STUDY OF SONLA PROVINCE, VIETNAM

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ABSTRACT

Cooperative play an important role in agricultural and rural development, especially in developing countries including Vietnam. To develop this model, numerous support programs have been carried out by central and local governments. In order to understand the effect of government support, this study examines the case of Sonla Province, which had explosive growth in the number of this model recently. The results shown that the cooperatives in Sonla have grown fast in terms of quantity but slowly in terms of performance. The study found major reasons for the (partial) failure of some supports, including: (1) the barrier created by resource-based requirements from the support programs; (2) inadequate training programs, which have not met the needs of cooperative; and (3) some support activities that created a money-incentives driver for setting up cooperative and distorted the mission and vision of cooperatives. Some implications are recommended for policymakers and local governments in developing and implementing more appropriate and effective supports, which will be based on principle compliance-based requirements; be tailored to each stage of the cooperative's development; and be carried out on the basis of upholding the principles of this model, towards to the sustainable development of the cooperative in the coming time.

Key words: economic collective, intervention, agriculture, developing country

INTRODUCTION

In Vietnam, the cooperative model was formed in the 1950s but had to go through many ups and downs. Since 1955 to 1986, cooperative model was identified as one of the two official economic forms in Vietnam (besides state-owned enterprises), which promoted collective strength to bring benefits for farmers. However, since 1986, this traditional cooperative model no longer played a key role as it was not suitable with the transformation economy context. In early 2010s, when the Cooperatives Law 2012 was issued which reflected innovative thinking about a new cooperative model and contributed to improve production relations following market orientation, the development of cooperatives has entered a new stage. Based on advantages of economies of scale and scope, cooperative production is generally seen as a the way to group of small-holder capacity to improve product quality, as well as capital investments and management skills (Coles and Mitchell 2013; Segismundo and Ralleta-Navarro 2009). Purchasing in the form of bulk sales helps the cooperative member to reduce transaction costs (Blandon et al. 2009; Hellin et al. 2009; Holloway et al. 2000; Markelova et al. 2009; Valentinov 2007). Simultaneously, collective power helps improve the standing of farmers when they are negotiating in the market to gain more competitive prices for both inputs and outputs (Deng et al. 2010; Padilla-Fernandez and Nuthall 2012). Furthermore, the research of Altman (2015a) has shown that production in cooperative would also expect higher levels of effort inputs (x-efficiency) than that of corporate employment or large investor-owned firm, given cooperative governance. More importantly, via cooperatives, farmers and small businesses cooperate with each other to strengthen their cooperative efforts to cope with difficulties and avoid high risk of losses. For the purpose of fair trading, cooperative also support peasants by reducing information asymmetry between them (the poor farmers) and the external market (Bijman and Hendrikse 2003). Finally, cooperatives can facilitate the support programs of governments by establishing extension networks that can provide better information on technology and information services to their members (Fock and Zachernuk 2006). The attractiveness of the new cooperative model is evidenced by the rapid growth in the number of cooperatives, as well as the voluntary participation of the members in this model. By the end of 2019, Vietnam had 24,618 cooperatives and 85 cooperative unions with more than 7 million members (Vietnam Cooperative Alliance 2020).

Sonla is a northwest mountainous province of Vietnam with many potential advantages in agricultural production. Like many other agriculture-based provinces, cooperatives are considered as the core model to organize the implementation of the association to overcome the shortcomings of current agricultural production in Sonla, such as risk production, quality management, market connection and so on (Vietnam Ministry of Planning and Investment 2020). Actual activities of the cooperatives in Sonla over the past time shown that the models of cooperatives have partially contributed to the process of economic structure transformation and created benefits for the farmers. One of the important factors affecting the development of the new cooperative model in Vietnam in general and in Sonla Province in particular is the support programs of the government from the central to local levels, in various forms including start-up support, resource (financial, land, technology) support and enhancing management capacity support programs. However, in reality there are still gaps between the intended (i.e. to effectively boost the performance of the cooperatives in Sonla province) and the actual effects of these supporting programs (i.e. the average level of the performance of cooperative in Sonla is lower than national average level). The cooperatives in SonLa received support from government only, nothing from non-government organizations and private firms.

This above paradox raised the question why government support is not so effective to boost performance of cooperatives in this province. To answer the question, this study sought to review the performance and the obstacles in the development of the cooperatives in Sonla Province since 2012, review the cooperative support programs of government from central to local levels since 2012, and find the reason why these support systems are not so effective in reality, then draw lessons learned from the case study to improve the effects of those activities on the performance of cooperatives.

METHODOLOGY

Analytical framework. Critical contribution to the growth of the cooperative is the government's role with a set of rules, regulations and policy support(Altman 2015; Deng et al. 2010). In many countries, it is necessary that governments be involved to support the development of cooperatives in one way or another (Ribašauskienė et al. 2019). For instance, some government agencies in the US supply the extension service to support the work of cooperatives. In Japan, the government sets up a special ministry level cooperative commission for the purpose of aiding this type of organization (Fulton 2005).

Currently, in China, policy supports not only account for most of the growth of the farmer professional cooperative but also for promoting and fostering those organizations (Deng et al. 2010). However, due to the problems arising in the implementation, the purpose of those support policies are not always achieved. It could be the issue of eligibility of cooperative in accessing public policy support programs which may lead to situation of inappropriate beneficiaries (Iliopoulos 2013).

During the implementation process, the policies have not been consistently implemented across agencies or at different administrative levels (Cox and Le 2014); or the government over-intervention could negatively affect the self-autonomy of these cooperatives (Garnevska et al. 2011). The reality in many developing countries even show the phenomenon that the direct government intervention with cooperatives has led to cooperatives becoming political instruments and/or cooperatives being inefficient due to incompetent managers (Hussi et al. 1993). Therefore, it can be seen that the support/intervention of government have both positive and negative impacts on the development of cooperative, despite its original purposes.

Specifically, in term of training support programs, the research of Ford & Hoyt (2017) has proved that those programs can be effective only if the training designed to meet the cooperative boards' specific needs in different development stages. The research developed a three-stage model (Creation/ Start up; Direction/ Establishment; and Delegation/Institution) that describes many characteristics of cooperative boards at each stage, including board culture and composition, director attributes, board focus and decision making style. Research has shown that cooperatives experience many changes as they grow from start up to maturity. Therefore, it will be ineffective if government supports the same training programs for all cooperatives.

The analytical framework designed for this study is presented in Figure 1. Following this framework, cooperatives selected in samples have been divided into different groups resulting from the implementation of support programs, including eligible/non-eligible cooperatives; groups of cooperatives that do not operate after receiving support; cooperatives with positive impacts/no impacts from government supports. Lessons learned are drawn from the reasons which affected the proportion of cooperatives in each group, as well as if the training support program match the actual needs of cooperatives in the different growth stages.

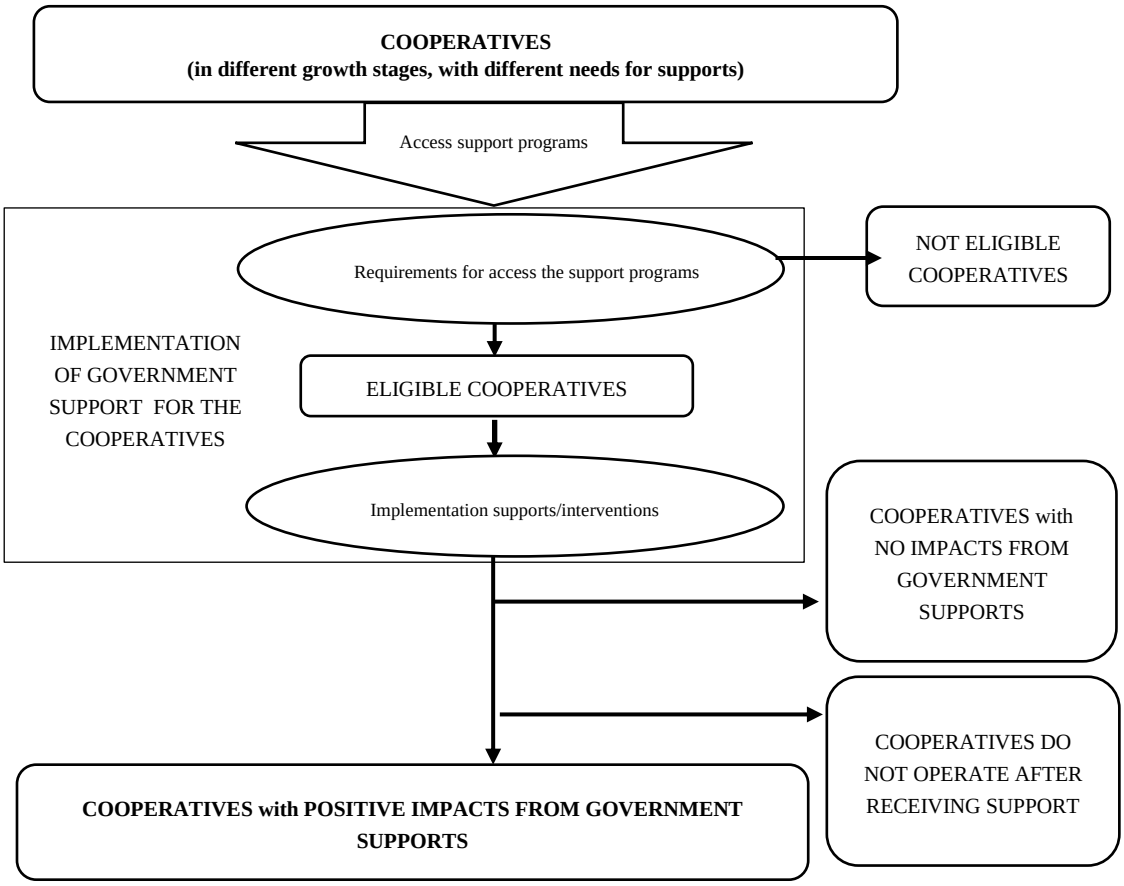

Fig. 1. Research analytical framework

Research site. Sonla is a province located in the center of the Northern mountainous areas of Vietnam. According to Sonla Department of Statistics, total Gross Regional Domesstic Product (GRDP) of Sonla in 2020 reached $ 1.432 mil. The economic growth rate in 2020 was 6.23% compared to 2019; in which agriculture, forestry and fishery increased 4.26%; industry - construction increased 9.18%; the service sector increased 5.34%. The service sector accounted for a high proportion (39.1%) and continues to contribute the most to the economy; followed by industry – construction sector, which accounted for 30.3%; agriculture, forestry and fishery sector accounted for 23.6% (Diep 2020). The cooperative model started in the 1970s, with a small number of cooperatives for a long time. However, since 2012, the development of cooperatives in Sonla has changed dramatically with 661 cooperatives in 2020. Sonla had the highest growth rate of the number of cooperatives in Vietnam in the period 2015-2019 (Vietnam Ministry of Planning and Investment 2020). This is also the reason why Sonla was choosen as sample site.

As the survey started in 2019, the sample included 105 cooperatives selected randomly from 555 cooperatives in Sonla province in 2018. The sample size of cooperatives for the survey was calculated using the formula below (Slovin 1984):

$$\boldsymbol{n} = \frac{\boldsymbol{N}}{\boldsymbol{1+N*e^2}} \qquad \textit{Equation (1)}$$

where “n” is the sample size), “N” is the total number of cooperatives in the research site in 2018) = 555, and “e” is design margin of errors = 0.1 (with a 90% confidence level). From the calculation, the result of n is 87. The actual sample size then needed to be increased from 85 to 105 in order to have a sufficient number of cooperatives which have existed for long time, because in the period 2017-2019, nearly 300 cooperatives were established.

The key information about the cooperatives in the sample is presented in Table 1. In these samples, agriculture cooperatives mainly operate in the following activities: collective purchasing of agricultural inputs (seed, fertilize, feed..) to reduce input cost; carrying production in the same standard to get uniform agricultural products; bulk selling agricultural product to reduce transaction cost and strengthening in bargaining power. Meanwhile, non-agriculture cooperatives mainly involve in transporting or providing services of community-based tourism or non-agricultural services such as electricity or gasoline, etc.

Table 1. Basic information on selected cooperatives

Type of cooperative	Number of sample unit (coop.)	Average number of operation years (year)	Average number of members (person)	Average of business capital ($)
Agriculture cooperative	82	3.92	15	36,341.92
Non-agriculture cooperative	23	6.21	10	72,370.81

(Source: Cooperative Survey in Sonla 2019-2020)

Data collection. Fieldwork was carried out in the study site from August 2019 to August 2020. Secondary data regarding support programs for cooperatives in Sonla was gathered from different local government offices and published papers/reports. Primary data was collected by using different research tools. Data on basic information, performance and difficulties of cooperatives were collected, and combined to identify the development phase of the cooperatives and the impacts of support programs.

The two research tools used for field research were:

Key Informants' Interviews (KIIs): Several key persons from local governments at three administrative levels: province, district, and commune, were interviewed in order to obtain information about the support programs of government enforcement related to those programs.

Survey in cooperatives: 105 managers (on behalf of 105 cooperatives in sample) were involved in interview with assistant of questionnaire about the performance of his/her cooperative, the obstacles/difficulties in running cooperative, as well as their evaluation of the impact of the support programs of government on the cooperative. The small sample size coupled with the unsuccessful contacting of cooperatives in difficult mountainous areas are limitations of this study. Due to sampling bias, it has some limitations when generalizing the results to larger groups.

Data analysis. In this study, a comparative analysis was applied to evaluate the match between the implementation support programs and the actual needs of cooperatives. Actual needs of cooperative was found based on the model three stages of development following the instruction of US Overseas Cooperative Development Council – OCDC (Ford and Hoyt 2017), several questions about *(1) Product and service of Cooperatives; (2) Board focus; (3) Decision making process; (4) Organizational culture; (5) Board composition; (6) Director attributes; (7) Committee structure; (8) Planning; (9) Organization growth issues; (10) Board growth issues; (11) Organizational systems; (12) Resources (Financial and non – financial); (13) Top leadership; and (14) Staffing* were given, each with a different score (1, 2, 3). Upon answering all of the questions, the total calculated score provided a numerical identification of a cooperative's governance development stage based on the following ranking.

Attributes of cooperate governance was used to evaluate the actual needs for training support of cooperatives.

- Score 20-32 = Development Stage 1: Creativity/Start up
- Score 33-46 =Development Stage 2: Direction/Establishment
- Score 47-59 =Development Stage 3: Delegation/Institution

In addition, cause-effect analysis was used to identify the reasons which impacts on the effectiveness of the support programs of government for the cooperatives in Sonla.

RESULTS AND DISCUSSIONS

Performance of cooperatives. Cooperative model has existed in Sonla since 1970s. In 2012, after two decades, as the result of the "top-down approach" in establishing cooperative, there were 126 cooperatives in this province (Lai 2008). However, since 2013, the issuance of 2012 cooperative law together with many policies/interventions of government from state to the provincial level had created a rapid growth in the number of cooperatives in Sonla, which was 3.33 times higher than the national average growth level (Vietnam Ministry of Planning and Investment 2020), with 661 cooperatives in 2020 (Sonla Provincial Cooperative Alliance 2021) (Fig. 2). Contrary to the first

period, these cooperatives were established by the "bottom-up approach" -mainly based on the willingness of the cooperative members. Following 2012 Cooperative law, those new model cooperatives' sizes (7 members to 70 members) are smaller than previous ones (over 100 members). The reason was the conventional cooperative was set up for the whole commune whereas the new model consists only a group of members in the small village/community who have the common interest. In 2020, one commune in Sonla may have more than one cooperative, some even have 12 to 17 cooperatives.

Given the natural and social condition of this mountainous province, agriculture cooperatives account for 86% of total cooperatives of Sonla, while non-agriculture cooperatives account for 14%. From 2012 up to present, the positive contribution of the cooperatives to the economic and social development of Sonla in general as well as of the cooperative members' household has been stated in many previous studies (Lai et al. 2019; Le et al. 2020; Nguyen 2020).

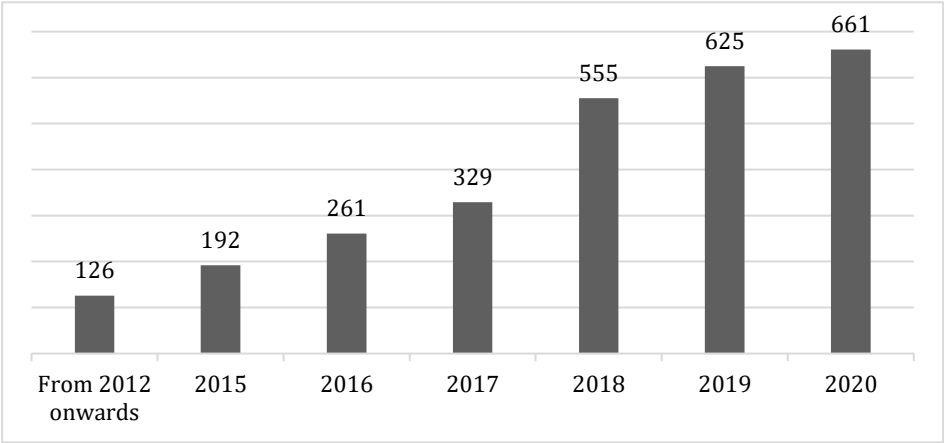

Fig. 2. Number of cooperatives in Sơnla in period 2012-2020

(Source: Sonla PCA, 2020)

Despite the fact that Sonla has been ranked 11th among 63 provinces of Vietnam in terms of the number of cooperatives, the performance of this model in this province was below the national average. According to the survey of Vietnam Ministry of Planning and Investment (Vietnam MPI) and the report of Vietnam Cooperative Alliance, the percentages of cooperatives in Sonla which earned profit in 2017, 2018 and 2019 were 19%, 6% and 15% lower than those of national average, respectively (Fig. 3). Similarly, the average monthly labor income in cooperatives in Sonla were $30-40 lower than national level (Fig. 4). According to the evaluation measures framework of Vietnam MPI, in 2019 only 30% of cooperative in Sonla performed effectively (Sonla Provincial Cooperative Alliance 2021; Vietnam Cooperative Alliance 2020; Vietnam Ministry of Planning and Investment 2020).

Following the guideline of US Overseas Cooperative Development Council – OCDC (Ford and Hoyt 2017), using the data collected from the survey of this study, 90 cooperatives which were established more than one year have been classified into 3 stages based one the criteria about cooperative governance. The analysis based on the model of OCDC shown that 35% of cooperatives were in stage 1 – stage of creativity/start up; 57% of them were in stage 2 – stage of direction/ establishment; and the minority, only 8% of them, were in stage 3 – stage of delegation/institution (Table 2). This result indicated the fact that majority of the cooperatives was still in start-up and setting up direction phase of the development process, after nearly ten years of explosive growth of cooperatives in Sonla. In addition, against the general trend of the world which was analyzed in the report of Gotz (2017): "*The analysis of the highest ranking businesses per sector in the World Cooperative Monitor database proves not only that cooperatives can succeed even as large businesses, but that for the most part the economic leaders of the cooperative sector remained strong throughout the years of the global economic crisis.*", the proportion of cooperatives dissolved over the years 2018, 2019 and 2020 in Sonla was 10%; 12%; and 7%, respectively.

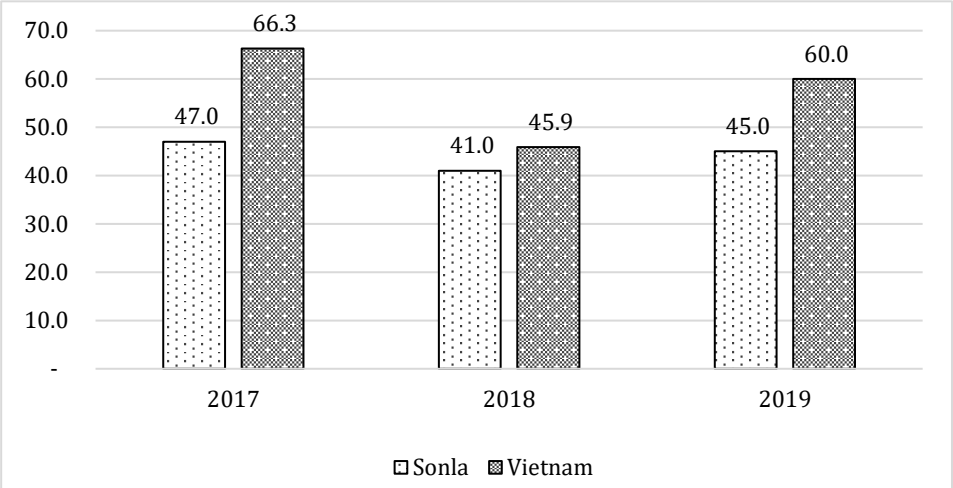

Fig. 3. Proportion of cooperatives earned profit (2017-2019) (Unit: %)

(Source: Vietnam MPI,2020)

(Source: Vietnam MPI,2020)

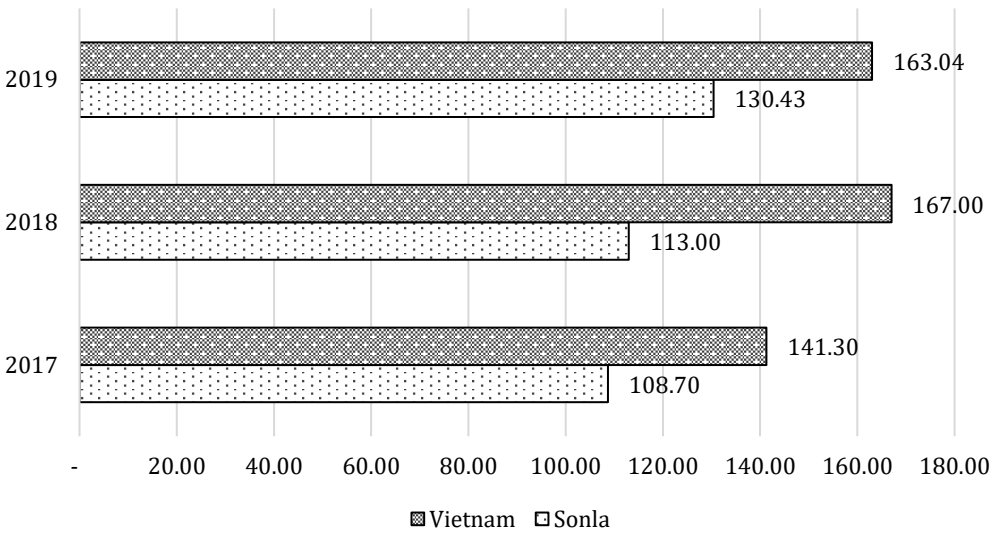

Fig. 4. Average monthly income of labor in cooperative (Unit: $/month)

For a deeper view, a question about the difficulties challenging the cooperative has been included in the survey. Similar to the opinion of Simmons & Birchall (2008), the data collected from our survey indicated numerous obstacles existing in the running process of this kind of entity, including difficulties in the start-up activities; the lack of resources for business activities (*i.e, land, capital, technology*); the lack of access to the market beyond their locality ; and the most serious one being the poor management capacity. Remarkably, the weakness of management capacity appeared differently in each phase of development (Table 2) while the other issues may happen in any phase.

In general, the overview picture about the performance of cooperatives in Sonla presented not only the speedy growth in the number of cooperatives in the period 2012-2020 but also the imbalance between the escalation in quantity and the increase in quality of this model. During the running process, several obstacles and difficulties have occurred and affected the performance as well as the role of cooperatives in rural area, which definitely need the supports/interventions from government for those entities to cope with them and develop sustainably.

Table 2. Management capacity weakness of cooperatives in Sonla province

Stage 1: Creativity /Start-up (*accounted for 35% cooperatives*)	**Stage 2: Direction/Establishment (*accounted for 57% cooperatives*)**	**Stage 3: Delegation/Institution (*accounted for 8% cooperatives*)**
• Lack of the knowledge about nature and principles of cooperatives • Lack of the business management skills. • Lack of business resources (capital, land…)	• Lack of business planning and resource monitoring skill. • Inadequate marketing and financial accounting experts • Weak internal control system • Weak skill to attract the loyalty of members	• Lack of long-term planning skill, monitoring, evaluating and responding to risks. • Lack of knowledge about opportunities for fair trade

(Source: Cooperative Survey in Sonla 2019-2020)

Government support for cooperatives in Sonla Province, Vietnam. With the purpose to help the cooperative to overcome the obstacles in business process, several support programs for the cooperatives in Sonla were launched by governments from central to provincial level since 2015, three years from the establishment of the new model. Although the programs were designed to address most the difficult issues, only a few cooperatives received those supports. Except for the programs focused on start-up supports and enhancing management capacity, the remaining ones received by less than 30% of surveyed cooperatives (Table 3).

Besides, the assessment of the beneficiaries also revealed that the impact of those programs was not rated highly by them (Table 3). For example, in evaluating the management capacity supports programs, 28 percent of interviewers said "*this was ineffective*" and 54 percent said "*it had impact but lower than expected*"', whereas only 18 percent considered "*it created positive impact*". More specifically, they found that some training courses were not necessary, some other courses lasted too long, or the contents of the course were so theoretical while they needed to learn more practical experiences. Another example concerned technology support. Eleven percent of respondents valued the impact of the programs, while 89 percent of them said that "*having impact but not as expected*" due to problems arising after receiving technology, for example, limited capacity and financial capacity to maintain these technologies. Obviously, although all the programs targeted to resolve the current issues of cooperatives, they seemed to be far from reaching their purposes. The next part discusses why and whether there was the gap between the hard effort of the government at different levels and resources focused to support the cooperatives and the low benefits received by the beneficiaries.

Lessons learned from the case study of Sonla Province, Vietnam

***Lesson 1*:** Resource-based requirements from support programs created barriers for targeted cooperatives to access

The first lesson was learned from the fact that technology, land and capital supports of the programs were received by very few cooperatives (Table 3) although capital, land and technology are the big problems for the majority of Sonla cooperatives (Le et al. 2020). Like other firms, these three resources are necessary and desirable for co-operatives to conduct business, grow, and meet the demands of key stakeholders, especially for the agriculture ones (Sultana et al. 2020). Meanwhile, it is generally argued in economic theory and proved by many researches that cooperatives have more difficulties raising capital than other types of firms, then consequently have obstacles in land and technology issues (Poungchompu and Chantanop 2017; Ramanauskas et al. 2017; Theron 2010; Warlow and Kasabov 2014)

Table 3. Support programs for the cooperatives in Sonla Province since 2012

Name & starting point of the support programs	Proportion of cooperatives that received supports	Proportion of cooperatives received positive impacts from government
Management capacity supports programs (*provides training courses in production technology, market access and cooperative management skills*)	100%	18%
Technology support programs (*partly financial supports for hi-tech application*)	15%	11%
Market access support programs (*support in connecting to markets, trade-mark registration, trade, traceability stamp printing …*)	29%	25%
Land support programs (*provide land for building office of cooperative*)	2%	2%
Start-up support programs (*administration and cash supports for new established cooperative*)	72%	60%
Financial support programs (*provide loans at low interest rate*)	11%	10%
Vietnam 2012 Cooperative Law		
2012 **2015** **2016** **2017** **2020**		

(Source: Cooperative Survey in Sonla 2020)

The requirements for the cooperatives to receive the support of government are the key explanation for the above paradox. Resource-based requirements created high barriers for the cooperative to be given and then fully benefit from theses support programs (Table 4). That is, in order to receive the technology sponsorship program, the beneficiary entity needs to pay money in advance for the suppliers, then will be refunded later when they submit the full proven records. Together with the problems in administrative procedure, the majority of agriculture cooperatives cannot benefit from this aid because they are not able to raise enough capital for the advance payment or have reserve financial resource during the time waiting for refund. Next, in terms of financial support, several programs offer loans to these entities at low interest rate. However, to be entitled to these offers, cooperatives need to own a collateral, such as land or other valuable assets. Ironically, having land as a cooperative-owned asset is also another obstacle of cooperative. To deal with this issue, provincial government supports in the procedure of transfer land use right so that it becomes the asset owned by cooperatives, but again, with the conditions about the contribution land from the members and the cooperative's own approved business plan. Given the fact that most members of agriculture cooperatives are farmers, it is really difficult for them to satisfy the requirements. As a consequence, due to the lack of collateral, most cooperatives, especially agriculture ones, cannot access the supports if the programs are attached with the resource-based requirements. In other words, if the target of the programs is to aid the cooperative to develop, the conditions should be principle compliance-based requirements (*i.e, for the purpose to confirm this organization is truthfully cooperative in nature*), rather than resource-based requirements. Indeed, if they have enough resources as the requirements, they might do not need the support anymore.

Table 4: Obstacles encumbering cooperatives in accessing support programs

Support Programs	**Resource – based Requirement**	**Actual situation of cooperatives before the support programs have been carried out**			
Technology support programs	*Financial resource for investment payment in advance*	**Limited financial resources**		Year 2017	2018
		Proportion of small size cooperatives (<10 staff)		66%	63%
		Average capital level of small size cooperatives (USD)		15,909	31,455
Financial support programs	*Land or other valuable assets as colleteral*	**Limited land resources**			
		Proportion of cooperatives owing land for office	36%	Proportion of cooperatives owing land for office	39%
		Average area for office per one cooperative (m2/cooperative)	153	Average area for production activities per one cooperative (m2/cooperative)	19,886
Land support programs	*Detail feasible business plan*	**Limited resource for forming business plan** • Lack of technology • Lack of financial resources • Lack of skill in making feasible business plan			

Source (Cooperative Survey ; KPIs; Cooperative White book 2020, Sonla Provincal Documents)

This lesson is similar to the experience in cooperative development in Central Asia, which suggests that grants and subsidies should be made available to healthy, fundamentally viable cooperatives, which ultimately will have sufficient own resources to finance their business activity, although they may require temporary assistance to achieve sustainability (Lerman 2013). In the other words, like experiences from public policy support for agricultural cooperatives in developed countries namely USA, Australia, Canada, France, Germany and the Netherlands, the requirement for cooperative to ensure the basic principle "user-owned, -controlled, and -benefited" should be considered for the eligibility to participate any government support programs (Iliopoulos 2013).

***Lesson 2*:** Abundantly of training support programs but were inadequate because they have not met the needs of the trainees

In the period 2017-2020, the Sonla provincial government invest more than $ 200,000 expenditures in training support program, with the trainees from all cooperatives. The same training supports was applied for all, even though they met different issues in management, different training demand as they were in different phases (Table 5).

Table 5: Responsiveness of training programs compared to training demand of cooperatives in Sonla province.

Training demand of cooperative in each stage		**Topics of actual training courses** Trainees: Manager/ Accountant from all cooperatives
Stage 1 (***accounted for 35% cooperative***)	Nature and principles of cooperatives*	
	Production Management	Production Techniques
	Marketing skills	Marketing skills
	Basic skill for business management	General skills for Business Management
Stage 2 (***accounted for 57% cooperative***)	Short-term business planning	Short-term business planning
	Marketing skills	
	Accounting for accountant	Update accounting knowledge for accountant
	Accounting for management*	Training course for "One Community One Product" Program
	Internal control system*	
Stage 3 (***accounted for 8% cooperative***)	Long - term planning skills*	
	Monitoring & risk management skills*	
	Opportunities for fair trade	

Source (Cooperative survey 2020, KPIs)

*Note:(*): The training demand has not been addressed by any training course*

For that reason, the second lesson learned from the case is the training support programs should be tailored to each stage of cooperative's development. Cooperatives should be classified into different development phases before designing the training courses. By doing so, the training contents will help group of managements in each specific stages of development to learn knowledge and skills which can address the current problems of their own cooperatives. Furthermore, this activity will create a network of managers who have same interests, same difficulties so that they will share practical experiences to each other, effectively.

***Lesson 3*:** Some support activities created the money-incentives driver for the cooperative in operation, which distorted the mission and vision of cooperatives

To illustrate, the first experience was the start-up support program of Sonla Provincial Government which finance around $ 250 to a newly established cooperative. Starting in 2015, this policy had kicked off a boom in the number of cooperatives in the period 2015-2018 (*see figure 2*). When asked about the reason for establishing a cooperative, 40% of the respondents mentioned that this allowance was the main motivation. This reason shows insufficient awareness of the members about the fundamentals codes of this model. Meanwhile, the "lack" of training on nature as well as the principles of cooperatives (*see table 5*). Even though the purpose of the policies was to help the cooperatives in the setting up phase, it also created the money-incentive for the farmers to establish cooperatives without understanding the basic benefit of this model. To some extent, it also seemed to have bred a class of "false cooperatives", i.e., established only for the purpose of gaining access to the aids, without any regard for true

cooperative principles. Moreover, it could also affect the sustainability of cooperatives, as cooperatives established based on government programs seldom survive, and only cooperatives created based on user initiative in rural areas are more likely to be sustainable. The world experience about similar cases has been noted in the research of Sedik and Lerman (2015). Another experience is about the impact of technology support activities and autonomy principle. Fifteen percent of cooperatives in Sonla received the finance aid from the government for setting the high-tech agricultural system. However, the finance aid was only enough to cover setting up and running cost for some first years. As the result, 50 percent of the systems had stopped as those cooperatives could not afford to maintain it, in terms of finance and management capacity. Government programs, when they provide support, focused on subsidies, rather than training the farmers on how to autonomously exploit those resources in the long-term and sustainable way. Principally, cooperatives are autonomous, self-help organizations controlled by their members (International Co-operative Alliance 2017). Obviously, when the cooperatives rely on external supports for their business, they cannot be regarded as autonomous and independent entity anymore. Furthermore, maintaining the operation of the principle of the cooperative model is also creating an opportunity to exploit X-efficiency gained from economics of scale and scope as well in transaction costs that can be captured by the cooperatives (Altman 2015). Therefore, support activities on the ground of implementation and practice of cooperative principles is vital to the success of the cooperative, especially in agriculture sector.

Generally speaking, the legislative frameworks and support programs are considered as the "sine qua non"[1] of cooperative development process in developing countries (Theron 2010). As a special economic entity with its own characteristics, core values and principles, the cooperative receives supports from the government and donor organizations to improve operational efficiency and create value for community. Therefore, if these support activities are not carried out on the basis of maintaining the principles, after receiving the support, these organizations will no longer be the cooperative model in nature. For that reason, governments need to develop a support/intervention program for helping cooperative sectors on the basis of upholding the nature the principles of the cooperative.

CONCLUSION AND IMPLICATIONS

The cooperative model in Sonla Province has undergone an explosive growth in the period from 2012 to 2020. The formation and operation of cooperatives in Sonla has made noteworthy contributions to the socio-economic development of the community as well as stabilized the livelihoods of farmers in this area. In order to help the cooperatives to overcome these difficulties, the government from the central to provincial level has many policies and programs to support the development of new models of cooperatives. Nevertheless, in general, the performance of cooperatives in Sonla was still lower than national average level even under the various support efforts of government. The percentage of cooperatives receiving positive impacts from these support programs is still a minority and these programs still have many shortcomings in the implementation process.

Obviously, there is thus a need to review intervention/support programs on an ongoing basis, in the light of experience. In the scope of this research, a number of lessons are given to overcome the shortcomings and help policies reach more cooperatives and create higher efficiency, including: (1) Resource-based requirements from the support programs for the cooperative should be replaced by principle compliance-based requirements; (2) Training support programs should be tailored to each stage of cooperative's development; and (3) Support activities should be carried out on the basis of upholding the nature the principles of the cooperative. However, to be able to do so, it is necessary to have accurate assessments; thereby adding support policies and programs that better suit the characteristics of the cooperatives in their corresponding phase of development, as well as focus on innovating and developing the cooperative model in depth, with many diversified models emphasizing operational efficiency and bringing practical benefits to members as well as to the wider community.

ACKNOWLEDGMENTS

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[1] The "sine qua non": a necessary condition without which something is not possible

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FACTORS INFLUENCING POTENTIAL CONSUMPTION OF BIOSAFETY PORK IN URBAN AREAS OF THE NORTHERN DELTA, VIETNAM

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ABSTRACT

The paper examined factors influencing the potential consumption of pork produced using biosafety breeding practices (BBP) in urban areas of the Northern Delta region, Vietnam. The primary data were collected from a sample of 402 consumers in 2020 by the stratified convenient method in 11 provinces belonging to the region using structured questionnaires. Two kinds of models were applied e.g., (i) binary logit was employed to estimate the factors affecting the consumers purchase intention for BBP pork; (ii) ordered logit was developed to estimate the factors affecting the willingness to pay (WTP) for BBP pork. The factors affecting purchase intention for BBP pork consisted of consumer's knowledge, household characteristics, and income. Regarding WTP for BBP, three principles of BBP i.e., waste treatment, infectious disease control, and input material management had positive effects on WTP. Moreover, household income, availability, origin, and certification of the BBP pork were factors playing important roles for supporting the acceptance probability of the higher price premium. Meanwhile, the pork consumption quantity and purchase intention negatively influenced WTP. Based on the findings, the study proposed some appropriate solutions and policy implication to improve biosafety pork consumption as well as expand the BBP pork distribution in Vietnam.

Key words: binary choice, consumer behavior, ordered logit, purchase intention, willingness to pay

INTRODUCTION

The Northern Delta is a historical region of Vietnam with over four thousand years of history and is the origin of Vietnamese culture. The region has a high population density, sharing 22% of the total population but only 5% of total land area of the country. In the region, the urban population occupies for 34% of the total population. This is a dynamic economic region, accounting for 35.8% of the country's GDP. The average monthly expenditure per capita in the Northern Delta is about 3,296 million VND[1], and for income, the figure is approximately 5,085 million VND (VGSO 2020), ranking in the top two regions among the seven economic regions of the country. In Vietnam, the share of food expenditures in a household is about 53.3% of the total expenditures of a household, which is approximately 1.3 million VND per person per month, this figure in urban area is about 1.5 million VND, accounting for 48.2% of the total expenditures of a household (Vu 2009). In recent years, the monthly average expenditure per capita for foods in the Northern Delta urban areas has increased rapidly due to the performance of remarkable economic growth. Such a large potential consumption in this area can make it become an important market for BBP pork.

Pig farming is one of the most important agricultural sectors in the Northern Delta, Vietnam. At the beginning of 2020, the number of pigs in the Northern Delta was 3,497 thousand heads,

[1] 1USD ~ 23.000 VND

accounting for about 20% of the total number of pigs of the whole country (VGSO 2020). However, in this area, agricultural production that does not take biosafety into account has led to the threat of disease transmission as well as food safety. Some issues related to inorganic waste treatment in livestock, sales, and butchery have been increasing in parallel with the growth of the pig breeding scale in the Northern Delta (Pho et al. 2018). Biological hazards are the source of many foodborne illnesses nationally. Thus, the epidemic has recently become more and more complicated, and has caused serious consequences for pig farming in Vietnam (Cook and Phuc 2019). As such, biosafety breeding practices (BBP) should be developed to ensure that production prevents the entry of diseases and as tools to limit the effects of infectious diseases (Jaffee and Henson 2005). Utilizing the BBPs in agriculture production could create a potential market for agricultural products and make a difference in the value chain of agricultural products, including clear identification of the responsibility specifications of producers in the food value chain (Elbakidze 2003).

Recently, the Government of Vietnam issued the National Technical Regulation QCVN 01-14: 2010/BNNPTNT about BBP principles in pig farms in order to control the problems related to bio-security in pig farming and biosafety in pork (MARD 2010). In which, three main principles outline the management of inputs, the control of infectious diseases, and the procedures for waste treatment in pig farms. The BBPs will be able to model consumption trends because consumers pay more attention to products that are healthy and environmentally friendly. Many consumers have an intention to pay more for environment-friendly products (Laroche et al. 2001) because this consumption behavior not only shows personal responsibility for one's health, but it also displays the role of individualism for social development and environment protection (Briceno and Stagl 2006). In addition, issues such as environmental protection, health consciousness and infectious disease control can be listed as some main goals of BBP livestock in Vietnam (VNA 2010). The middle-income class in urban areas started becoming concerned about consumption for health-beneficial purposes and environmental protection (De Koning et al. 2015). Urban consumers have better knowledge about safe foods than rural consumers. From this point of view, the probability of WTP for BBP food of urban residents tends to be higher than that of rural residents.

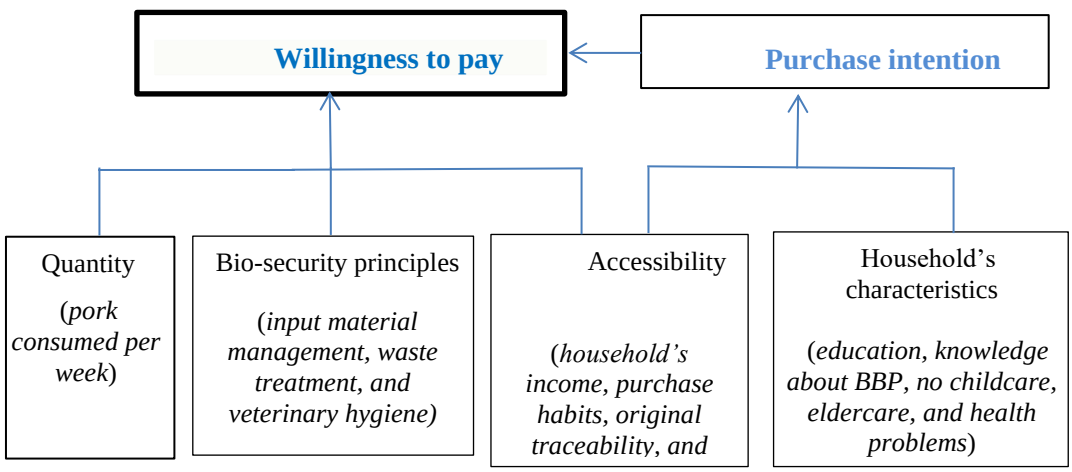
The linkage between the BBP principles and consumer attitudes for food include management of flock, control of incoming animals, control of inputs and outgoing materials, and control of other animals (Aila and Oima 2013). Bio-security objectives were bio-exclusion or external bio-security; bio-management or internal bio-security; and bio-containment (CSHB 2010). In a social cost-benefit analysis, the BBP principles can bring out the different quantities for the product but those also take extra cost, which should be smaller than the estimated benefits (Trewin 2001). Thus, the BBP principles should be conducted to reach the consumer intention, the WTP premium price for BBP pork can bring more return for manufacturers to cover their production costs, which can lead to their survival. Complicating the issue is the fact that the majority of Vietnamese pig farms are small in size (from 1 to 9 pigs per farm) and the large size farms (over 300 pigs) only account for a small proportion (VGSO 2020). Since the investments of large facilities and techniques in pig farms for ensuring the biosafety standards lead to a rise in the product cost with high investment and tech. The level BBP application of pig farm stayed in low, and BBP pork have not yet met the consumer demand, especially small-scale pig farms (Cuc et al. 2020). Despite the increase of demand for bio-safe pork, small-scale pig farms have a disadvantage in finding buyers because the pork quality and biosafety have not yet met consumer expectation. Thus, improving consumer intention to purchase BBP pork was suggested to improve BBP pork consumption.

Under these circumstances, it is necessary to ascertain the answers to the following questions: Do consumers have an intention to buy BBP pork? How much are they willing to pay for BBP pork? What are the main factors affecting these consumer's intention and willingness to pay for BBP pork? By answering these questions, we can propose reasonable solutions to improve BBP pork accessibility to target consumers. From these points of view, the study sought to examine the potential consumption

habits of consumers through their intention to buy BBP pork and the prices they are WTP for BBP pork in the urban areas of the Northern Delta, Vietnam.

MATERIALS AND METHODS

Analytical framework. An analytical framework was designed to examine the potential consumption of households for BBP pork in urban areas in the Northern Delta of Vietnam and to estimate the factors affecting the consumers' decisions of WTP (Fig. 1). Potential consumption for BBP pork was approached on the evaluation of purchase intention and willingness to pay. The influences of factors on purchase intention, including consumer's knowledge, household characteristics, the origin, availability and certification, and purchase habit were examined. Then, the factors affecting willingness to pay, including household income, consumer's knowledge, household characteristics quantity of pork consumed, bio-security principles, the origin, availability and certification, purchase habit were also further evaluated.



Source: Diagram developed by the authors 2020

Fig 1. Analytical framework of the research

Site selection. The data were collected from the Northern Delta of Vietnam which is an alluvial area made up of two rivers, the Red River and the Thai Binh River, located in Northern Vietnam. Based on the convenient sampling method, the survey was carried out in urban areas of four provinces, namely Hanoi, Vinh Phuc, Hai Duong, and Quang Ninh in the Northern Delta. The selected provinces are located in the key economic zone in the Northern Vietnam with high speed of urbanization. The customer surveys for BBP pork were conducted at supermarkets and grocery stores in the selected sites. The respondents were housewives who were responsible for the food purchases in their households. Housewives are responsible mainly for food consumption decision-making and food purchasing behaviors and cooking for all family members. The survey was carried out from September to November in 2020. The allocation of the sample was based on the urban population proportion of each selected province in the total urban population in the Northern Delta region. In our study, 429 urban customers were selected as respondents for the survey (Table 1). After refining the surveyed data, 402 respondents of the customer survey were used for analysis.

Table 1. Distribution of respondents in the four provinces of the Northern Delta region, Vietnam

No.	Province	Urban areas population		Number of samples
		Population (1000 people)	Proportion (%)	
1	Hanoi	4,000.3	69.65	299
2	Vinh Phuc	295.2	5.14	22
3	Hai Duong	594.2	10.35	44
4	Quang Ninh	853.7	14.86	64
	Total	**5,743.4**	**100**	**429**

Source: Survey (2020)

Sample selection. For multivariate regression analysis, the minimum sample size needed was calculated using the formula: n = 50 + 8×m; *where*: n is the number of samples to be investigated, and m is the number of independent factors. The study intended to include 20 variables in the regression analysis model; therefore, the minimum number of survey samples was 210 (Tabachnick et al. 2007). According to Scheaffer et al. (2006), the size of the sample is defined by following function:

$$n = \frac{N}{(N-1)\,\delta^2+1},$$

where N is the urban population count in the Northern Delta and δ is the chosen error. In the economic field, the generally accepted level of confidence is 95% corresponding to an accepted error of 0.05. At an accepted error of 0.05, the minimum number of survey samples was n = 399.

The choice experience methods. In the study, choice experience method was employed to design the questionnaire to investigate the consumers' purchase intention of BBP pork. Consumers were introduced to BBP pork, alternative choice of characteristics of BBP product were presented as well as premium prices. They were asked for their optimal choice. The questionnaire included three main parts: (i) Information about the respondents' socio-economic characteristics and household characteristics; (ii) The purchase intention for BBP pork, i.e., the frequency of purchasing and the purchasing place; and (iii) The willingness to pay. Respondents were asked to find the highest price premium they would be willing to pay for BBP pork instead of conventional ones. Respondents were presented the highest premium price, if they accept that price, the WTP would be decided, if not, they were continuously presented lower premium price, then the WTP would be decided when they said "yes". The third part was designed to discover information about the independent variables. The respondents were introduced to the BBP principles and then asked how they agreed with a set of given statements. The answers demonstrated how respondents believed in BBP principles such as what they expected and how they evaluated the traceability, availability, and certification of BBP pork.

Data analysis. The study applied basic methods of data analysis such as descriptive statistics and comparison, and also used quantitative method to analyze data on independent variables presented as categories. The ordinal regression model was employed to explain the effects of factors on the potential consumption. Factors affecting purchase intention and willingness to pay for BBP pork i.e., household income, consumer's knowledge, household characteristics, quantity of pork, bio-security principles, the origin, availability and certification, and purchase habit were included in the models.

Binary logistic model. First, the binary logistic model was utilized to estimate the explanation variables for consumer purchase intention. The dependent variable, purchase intention, was measured

by a dummy variable, taking the value of 1 if the households intended to buy often and zero if not. The binary logistic regression model is specified as follows:

$$g(\pi) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \ldots + \beta_n x_n.$$
$$g(\pi) = \log\left[\frac{\pi}{1-\pi}\right]$$

in which, π is the probability that consumers choose between [0, 1], while x_1 ... x_n are explanation variables influencing the probability of the purchase intention for BBP pork.

The ordinal logit regression model. This model was selected to evaluate the price premium that consumers were WTP for BBP pork. WTP was measured by the percentage of increase in price that consumers were WTP higher than the original price (the original price was the price for conventional pork). The WTP was measured by using ordinal variables to ask the respondents about what price they were willing to pay for BBP pork as compared to conventional pork (Ghorbani and Hamraz 2009 and Haghjou et al. 2013).

The estimation functions of WTP (y) can be specified as follows:

$$y* = \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_n x_n + \varepsilon = x\beta + \varepsilon$$
$y*$ is the estimation of the probability of given price premium for BBP pork.
$$y* = \log\left[\frac{y}{1-y}\right]$$

The constant (cut-off) was the threshold, which was estimated in the model. The probability that the consumer is WTP at given price premium was defined by:

$$P(y=1|x) = P(y* \leq \alpha_1|\, x) = P(\alpha_1 - \beta' x < \varepsilon\, |x)$$
$$P(y=2|x) = P(\alpha_1 < y* \leq \alpha_2|\, x) = P(\alpha_1 - \beta' x < \varepsilon \leq \alpha_2 - \beta' x|x)$$
$$\ldots$$
$$P(y=n|x) = P(\alpha_{n-1} < y*|\, x) = P(\, \varepsilon \leq \alpha_{n-1} - \beta' x|x)$$

The dependent variables. Based on the developed analytical framework and the review of relevant research literature, the study used a number of explanation variables to examine the impacts of the variables on the purchase intentions and the WTP for BBP pork. The dependent variable representing purchase intension (PI) was employed in form of the binary logit model, while the willingness to pay (WTP) was applied in the form of ordinal logit model. The 5-point Likert scale from 1 for totally disagree to 5 for totally agree was applied to represent some independent variables i.e., *Inp, Waste, Inf, Org, Lab, Cer and Habit* (Table 2).

Table 2. Descriptions of the variables in the potential consumption model

Variable name	Code	Description	Expected sign
		The dependent variables	
Purchase intention	*PI*	Does the customer intend to buy BBP pork? (Dummy variable, taking value of 1 if the customers intend to buy and zero if not)	n/a
Willing to pay	*WTP*	Which level of price is the consumer willing to pay for BBP pork? (0) the same as conventional pork (1) 10% higher than conventional pork	n/a

Variable name	Code	Description	Expected sign
		(2) 20% higher than conventional pork (3) 30% higher than conventional pork (4) 40% higher than conventional pork	
		The independent variables	
Education	*Edu*	What is the level of the consumer's education? (1) Under high school (2) High school (3) University or college (4) Post graduate	+
Knowledge about BBP	*Kno*	How much does the consumer know about BBP? (0) Never heard (1) Sometimes hear about (2) Quite clear	+
No childcare	*Child*	Does the consumer's household have any children? (0) No (1) Yes	+
Eldercare	*Old*	Does the consumer's household have any elderly persons over 60 years old? (0) No (1) Yes	+
Household's income	*Inco*	Which is the level of the customer's household monthly income per person (MI)? (3) MI ≥ 10 million VND (2) 5 ≤ MI < 10 million VND (1) 3 ≤ MI < 5 million VND (0) MI < 3 million VND	+
Quantity	*Qua*	What is the amount of conventional pork that the customer's household consumes per week (kg)?	-
Input material management	*Inp*	Were the respondents presented the "Input material management" principles of BBP and how did they agree that those principles can efficiently reduce contaminants, banned weight gain hormone and antibiotics in pork?	+
Waste treatment	*Waste*	Were the respondents presented the "Waste treatment" principles of BBP and how did they agree that those principles can efficiently protect the environment from pig farm wastes?	+
Veterinary hygiene	*Inf*	Were the respondents presented the "Veterinary hygiene" principles of BBP and how did they agree that those principles can efficiently reduce infectious disease in pork?	+
Original traceability	*Org*	Has it packaging information about the manufacturer to easily trace the origin?	+
Availability	*Lab*	Is it easy to find and to buy BBP pork in the market?	+

Variable name	Code	Description	Expected sign
Certification	*Cer*	Is it very safe and reliable because it is given the certification of BBP?	+
Purchase habits	*Habit*	How often does the consumer purchase BBP pork at a supermarket or safe-food store? (1) Never (2) 1-2 times/week (3) 3-4 times/week (4) 5-6 times/week (5) Over 6 times/week	+

RESULTS AND DICUSSIONS

Consumer household profile. Table 3 shows some characteristics of the surveyed consumer households for BBP pork. About 38% of the surveyed households had children under 10 years of age, 6.5% of the total households had members with health problem, and 26.1% of the households had elders. The education level of the consumers was quite high, where 73.9% of the respondents had a university or college background, a great majority of the respondents (82.6%) heard about BBP, but only 8% of the respondents had a clear understanding about BBP, indicating low level knew clearly about BBP. Majority of the income of the consumer's households ranged from 3 to 10 million VND/person/month (67.9%), in which, 31.3% of the households had an income of 3-5 million VND/person/month and 36.8% of households had an income of 5-10 million VND/person/month.

Table 3. Consumer household profile

Variable name	Situation	Frequency	Percent
Childcare	No	249	61.9
	Yes	153	38.1
Health problems	No	376	93.5
	Yes	26	6.5
Eldercare	No	297	73.9
	Yes	105	26.1
Education of consumer	Under high school	16	4.0
	High school	44	10.9
	University or college	297	73.9
	Post graduate	45	11.2
Knowledge about BBP	Never heard about BBP	38	9.5
	Sometimes heard about BBP	332	82.6
	Clearly understand BBP	32	8.0
Household income	≥10 million VND	70	17.4
	From 5 to < 10 million VND	148	36.8
	From 3 to < 5 million VND	125	31.1
	<3 million VND	59	14.7
Total		402	100

Source: Survey (2020)

The consumers in the Northern Delta urban areas purchased pork mainly from wet markets. The frequency of purchasing pork at wet markets are from five to six times per week which accounted for 45.5% of the total respondents. The majority of the consumers in the study areas had a tendency to frequently purchase at wet markets. On the other hand, under the development of retail markets in urban areas in the Northern Delta, a small number of consumers had a tendency to purchase in supermarkets. However, they still purchased pork in supermarkets less frequently than at wet markets, as nearly 22.6% of the respondents purchased pork in supermarkets or safe–food stores at the rate of 3-4 times per week (Table 4).

Purchasing at wet markets was the consumption habit and it is believed that they could get the expected bargain price there, which they could not get in the supermarkets. Otherwise, the supermarkets often offer higher prices than wet markets. In addition, wet markets are usually located nearer to the consumer's houses than supermarkets, so it is more convenient for consumers to buy pork from wet markets.

Table 4. Consumer's behavior regarding place and frequency of purchasing pork

Frequency	**Place of purchase**			
	Wet market		**Supermarket/ Safe-food store**	
	Number	**%**	**Number**	**%**
Less than 1 time/ week	21	5.22	41	10.2
1-2 times/week	19	4.73	188	46.8
3-4 times/week	113	28.11	91	22.6
5-6 times/week	183	45.52	55	13.7
Over 6 times/week	66	16.42	27	6.7
Total	**402**	**100**	**402**	**100**

Source: Survey (2020)

Table 5 shows the descriptive statistics on some of the explanation variables, i.e., the principles of BBP on the consumer's expectations and evaluation about the product's origin, availability, and certification of BBP. The variables of input material management, waste treatment, and veterinary hygiene reflected the expectation levels of how consumers perceived the BBP principles, i.e., reducing contaminants; banning weight gain hormones compounds and antibiotics in pork; protecting the environment; and limiting infectious diseases in pork product. The score for the principles of input material management (Inp) was highest with a score of 3.63 out of 5, while the score for environment (Envi) was lowest at 3.42 out of 5.

The evaluations of consumers about the traceability/origin, availability, and certification of BBP were from 2.89 to 3.47. In the surveyed market, a large amount of pork was sold with a brand name, with no information of the livestock farm, manufacture, or awarded certifications, even if it was sold in supermarkets and safe-food stores. The consumers' trust about certification was not very high and BBP pork is often found in supermarkets or safe-food stores, which are sometimes quite far from the consumers' residence.

Table 5. Consumer **e**valuation of BBP principles, the origin, availability, and certification

Variable code	Variable name	Min.	Max.	Total (n=402)		Purchase intention (n=303)	
				Mean	Standard deviation	Mean	Standard deviation
Inp	Input material management	1.00	5.00	3.63	0.988	3.71	0.923
Waste	Waste treatment	1.00	5.00	3.42	1.129	3.56	1.128
Inf	Veterinary hygiene	1.00	5.00	3.44	0.974	3.59	0.937
Cer	Certification	1.00	5.00	3.42	1.083	3.46	1.114
Org	Original traceability	1.00	5.00	2.89	1.038	2.90	1.073
Ava	Availability	1.00	5.00	3.47	0.888	3.48	0.916

Source: Survey 2020

Intention to consume BBP pork. The average quantity of pork consumption was about 1.85 kg/week/household (Table 6). This is lower than the average quantity of pork consumption in Vietnam (nearly 2 kg/week/household) (VGSO 2020). The average price of conventional pork in the retail market was about 117 thousand VND/kg. The purchase intention for BBP pork accounted for 75% of the total respondents. A majority of the households (61.4% of the total observations) were WTP for BBP pork at the premium price of 20% higher than that of conventional pork, and at the price of 30% higher than that of conventional pork, it was about 24.6% of the total observations.

Table 6. Consumption of quality pork and purchase intention for BBP pork

Variable code	Variable name	Minimum	Maximum	Mean	Std. deviation
Qua	Quantity of pork	0.12	10.08	1.85	1.39
PI	Purchase intention	0.00	1.00	0.75	0.431
WTP	Willing to pay	0.00	4.00	2.22	0.80
Pla	Habit	1.00	5.00	2.59	1.06

Source: Survey 2020

The influence of the factors on the intention to purchase BBP pork. The estimates of the factors affecting the intention to buy BBP pork in the Northern Delta urban areas are shown in Table 7. The likelihood ratio (LRχ^2=276.86) had a significance level of 1%, which meant that the whole the model was fit, the R square was 53.4%, which was suitable for the estimation of the binary logistic model (Gracia and Magistris 2008), and the chi-square ratio was 178.734 at the significance level of 1%. Education, knowledge, childcare, eldercare, and household income were significant factors influencing the customer's purchase intention for BBP pork. The correct prediction rate was quite high at 88.1%.

Table 7. The binary logit regression estimation of factors affecting purchase intention for BBP pork

Variable codes	Variables	Coefficient estimation	Standard error	Significance	Marginal effect
Edu	Education	***0.973	0.274	0.000	2.647
Kno	Knowledge about BBP	***3.594	0.579	0.000	36.368
Child	Childcare	***1.313	0.380	0.001	3.719
Ill	Health problem	1.382	1.024	0.177	3.982
Old	Eldercare	**0.965	0.410	0.019	2.625
Inco	Household income	***1.025	0.190	0.000	2.786
Cer	Certification	-0.107	0.162	0.511	0.899
Org	Original traceability	-0.204	0.167	0.221	0.815
Ava	Availability	0.030	0.187	0.874	1.030
Habit	Purchase habits	**0.401	0.164	0.015	1.493
Constant		***-6.941	1.287	0.000	0.001
Model fit	Likelihood ratio = ***270.058				
	The correct prediction rate = 88.10%				
	Nagelkerke R Square = 0.534				
	Chi-square= *** 178.734				

*Note: *, **, *** are significant levels at 10%, 5% and 1%, respectively.*
Source: Data collection and analysis (2020)

Consumer knowledge is a significant determinant factor for purchase intention (Haghjou et al. 2013). Results of the logistics regression would show which of the characteristics of the consumers in terms of their knowledge whether the information is quite clear for them, sometimes heard about it or never heard. If knowledge about BBP change from never heard to sometimes heard, the probability of purchase intention would increase 36 times. The estimates also indicated that education was one of the most important factors affecting the purchase intention with a marginal effect of 2.647, meaning that, on average, if the education level of the customers change from high school to university or college, it could motivate the probability of purchase intention, or an increase of 2.647 times. This finding is consistent with earlier studies that revealed education plays an important role in food purchase determination (Trung et al. 2014).

Households with children had the probability of purchase intention 3.7 times higher than households without children. For households with elders, the probability of purchase intention was 2.6 times that of household without elders. Children and elderly persons are commonly sensitive to contaminated foods, so customers with children and elders in their households usually cared about safe food consumption. This was the reason why households with childcare and eldercare had higher health consciousness (Prakash et al. 2018), and higher tendencies of purchase intention for BBP. The results stayed in line with previous researches that showed children can increase the safe food demand (Thompson and Kidwell 1998) and households that had seniors had stronger intention for purchase safe food (Haghjou et al. 2013).

As showed in Table 7, the household income also played an important role in purchase intention. The estimate of the household income effect was 0.1025 at the 1% level of significance, indicating that if the income of the household increase to over 3 million VND, 5 million VND and 10 million VND, respectively, the probability of their purchase intention for BBP pork tended to be 2.786 times in increase. Findings in the study were consistent with those in previous studies, e.g., high-income households preferred to purchase safe pork with full information about certifications, traceability, and no additive labels, and consumers' understanding about the certifications and food standards were able to create the purchase intention (Wang et al. 2018). The estimated coefficient of purchase habit was 0.401 at the significance level of 5%, meaning that when the consumer had a purchase habit at supermarkets and safe-food stores increase each 2 times per week, the probability of the purchase intention for BBP was 1,49 times in increase. These results agree with prior findings that revealed buying habits in super markets can determine the consumer's intention for buying safe food (Fleșeriu et al. 2020).

Factors influencing WTP for BBP pork. The model fitness test results proved the appropriateness of the estimated model, as shown in the scaled R-square of 47.9% and the likelihood ratio ($LR\chi^2$=637.185) at the 1% level of statistical significance (Table 8). The estimated coefficient of income was significant at the 1% level, which showed that income was one of the key explanations for WTP for BBP. The household's income drove an important impact on the acceptance of higher prices, and if the household income was range from 5 million VND to 10 million VND or over 10 million VND, the probability of acceptance of the higher price is greater than that of lower income household. Our results were consistent with the reported highest positive determinant of WTP for safe pork that was monthly household income (Hao et al. 2019; Kataria et al. 2019) and income can positively effect on WTP because increasing income mean increase household's economic power for purchasing BBP (Oni et al. 2005). Meanwhile, the quantity of pork consumption and purchase intention were displayed as having a negative impact on WTP. If the household consumed higher quantities of pork, their WTP was reduced. Furthermore, the opposite relationship between purchase intention and WTP was found with the coefficient estimated of -1.024 at the significance level of 1%.

The estimated coefficients of input material management, waste treatment, and veterinary hygiene were all significant at 5%, 10% and 5%, respectively. Thus, the expectation and perception of

consumers about the influences of BBP could positively impact WTP. These findings were consistent with earlier report that consumer's expectation and perception on BBP principles can affect the preference for agriculture products (Aila and Oima 2013). Production principles aimed at satisfying consumers' concerns with food safety, disease safety and environmental protection could be preferred by consumers (Koklic et al. 2019; Zhang et al. 2019).

Table 8. Ordinal logit regression estimates of price WTP for BBP pork

	Variable codes	Variable names	Coefficient estimation	Standard error	Significance
Dependent variable	[WTP = 0.00]		***3.934	0.793	0.000
	[WTP = 1.00]		***5.022	0.775	0.000
	[WTP = 2.00]		***9.814	0.894	0.000
	[WTP = 3.00]		***12.523	1.007	0.000
Independent variable	*Inco*	Income	**0.259	0.125	0.038
	Qua	Quantity	***-0.526	0.057	0.000
	Inp	Input material management	**0.253	0.123	0.040
	Waste	Waste treatment	*0.189	0.106	0.074
	Inf	Veterinary hygiene	**0.299	0.140	0.033
	Cer	Certification	***0.505	0.112	0.000
	Org	Original traceability	***0.369	0.117	0.002
	Ava	Availability	***0.911	0.152	0.000
	Pla	Purchase habit	***0.520	0.115	0.000
	PI	Purchase intention	***-1.024	0.297	0.001
Model fit	Likelihood ratio = ***637.185				
	Chi-square = *** 220.582				
	R square = 0.479				

Note: *, **, *** are significant levels at 10%, 5% and 1%, respectively.
Source: Data collection and analysis (2020)

The estimated coefficient of the certification, origin, and availability were significant at the 1% level, which indicated the significantly positive relationships between the certification, origin, and availability and WTP for BBP pork. It revealed that the perception of consumer about packaging with full information about manufactures and certifications can increase the WTP for BBP pork. Additionally, the availability of BBP presented a positive impact on the WTP for BBP. Certification was one of the promotors of WTP for BBP pork because it can raise the trust of consumers on livestock farmers. Practically, consumers do not know how to assess pork safety; pork is purchased with the expectation that livestock farmers are honest, keep their promises, and follow government regulations (Yee et al. 2005). The perception of consumers about certifications and traceability of origin are one of the most important types of information for ensuring the biosafety of products.

CONCLUSION

The factors influencing potential consumption of BBP pork in urban areas of the Northern Delta in Vietnam can be modeled using two kinds of model i.e., purchase intention and willingness to pay. In the study area, there is significant potential consumption for BBP pork through high proportion of consumers who intend to purchase BBP pork and high level of price premium that consumers are

willing to pay for BBP. Empirical evidence suggested that the increase in the consumer's knowledge, the household characteristics, and income could lead to the increase in the purchase intention for BBP pork. Purchasing habit in super markets could support for WTP. Similarly, the increase in income, bio-security principles preference and production identification played an important role for WTP improvement. However, the higher quantity of consumption for BBP pork could lead to the lower WTP.

The following policy recommendations are proposed to develop big production oriented biosafety practices: (1) motivate and expand the production and distribution of BBP pork in order to meet the potential consumption demands for BBP pork; (2) continue to improve the BBP principles to meet the consumers' expectations for some objectives such as environment protection, people's health, and infectious disease control; (3) improve the distribution of BBP, and establish a close linkage between farms and final consumers in order to reduce the price; and (4) provide detailed information about the availability, origin and certification related to BBP pork to ensure necessary information is on the packaging, which can increase the reliability of BBP pork in terms of consumers.

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A PRELIMINARY SCREENING OF PHILIPPINE MANGROVE SOIL BACTERIA EXHIBIT SUPPRESSION OF *RALSTONIA SOLANACEARUM* (SMITH) YABUUCHI ET AL. CAUSING MOKO DISEASE OF BANANA (*MUSA ACUMINATA* CAVENDISH SUBGROUP) UNDER LABORATORY CONDITIONS

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ABSTRACT

The study was conducted in 2021 in Los Baños, Laguna, Philippines to determine the potential of the mangrove soil bacteria isolated from Palawan, the Philippines to inhibit local *Ralstonia solanacearum* (Smith) Yabuuchi et al. strains from causing moko disease in Cavendish banana. Out of 55 isolates screened, three bacteria designated as S31 (*Bacillus altitudinis*), S37 (*B. subtilis*) and S38 (*B. thuringiensis*) exhibited antagonism against the four *R. solanacearum* strains based on the cylinder plate assay, with zones of inhibition (ZOIs) ranging from 9.50 ± 0.5 mm to 28.2 ± 0.3 mm. The best isolate, S37, possessed a significant activity and had a minimum inhibitory concentration (MIC) of as low as 78.1 ± 0.0 µg/ml using the broth microdilution method with resazurin as the indicator. Results of the screenhouse assay revealed the ability of S37 to control wilting of the Cavendish banana plants after injection into the pseudostem. Whole genome analysis using antiSMASH indicated the presence of six secondary metabolites in S37 with putative antimicrobial activity. The data showed that the mangrove bacteria may be possible candidates for production of a new biological agent containing non-pathogenic and fast-growing bacterial species to control the said disease.

Key words: antagonism, zones of inhibition, antimicrobial activity, whole genome, secondary metabolites

INTRODUCTION

Banana (*Musa* spp.) and plantains are perennial crops that have been considered as the top traded fruit worldwide and the eighth most important crop in the world in terms of production volume and trade (DA 2018; Dadrasnia et al. 2020; FAOSTAT 2014). The Philippines is one of the major banana exporters and was ranked number 2 next to Ecuador (DA 2018). Area planted to banana was 440,000 ha in 2016, increased to 450,000 ha in 2017 and remained at this level until 2020 (PSA 2016-2020). Banana production has also been threatened by different diseases and thereby posing a risk to global food security. The resulting production losses often cause a major impact on the banana value chain and the socio-economic livelihood as well.

The moko, bugtok and blood diseases are the three main banana bacterial diseases occurring in the tropics. These diseases are closely-related but their distribution is quite distinct. The moko disease, also known as bacterial wilt, is a major systemic disease affecting bananas of the Cavendish cultivar (Blomme et al. 2017). This disease has been recorded in Central and South America and the Caribbean (CABI 2014). In the Philippines, it is rampant in the southern Mindanao region (Raymundo 2001). This disease causes wilting of leaves and necrosis of the vascular tissues. Moko disease can be transmitted through contaminated tools and irrigation water, infested soil, and by contact with roots or vector insects (Alvarez et al. 2015). The bugtok disease is limited to the Philippines and infects the Cardaba and the Saba cultivars (Molina 2006). The symptoms of the bugtok disease include discoloration of the vascular tissue of the peduncle and the fruit pulp of the banana plant. Unlike the moko disease, plants infected with bugtok do not wilt and the suckers remain unaffected. Bugtok disease is commonly transmitted through insects (Molina 1999). Another banana disease transmitted by insects is the blood disease which is caused by *Ralstonia syzygi* subsp. *celebensis*. It was reported to originate from an island off the coast of Sulawesi in Indonesia in the 1900s and affected the dessert bananas (Thwaites et al. 2000). Symptoms of the blood disease include discoloration and shriveling of the male flower bud and peduncle, reddish dry rot of the fruit pulp and reddish discoloration of the vascular tissue throughout the plant. (Blomme et al. 2017).

The causative organism of both the moko disease and the bugtok disease, *Ralstonia solanacearum* (Smith) Yabuuchi et al., is a Gram-negative aerobic bacterium that naturally inhabits the soil and is present in all continents (Blomme et al. 2017). This species is genetically diverse in that it has been classified into biovars based on acid production from certain sugars and oxidation of hexose alcohols (Buddenhagen and Kelman 1964). These biovars differ in geographic distribution, epidemiology and physiology. They are also grouped into races based on their host range. Moko disease, in particular, is caused by *R. solanacearum* race 2 biovar 1 (Blomme et al. 2017; Hayward 1994). Several strategies for managing and mitigating moko disease have been practiced. These include biological, physical, chemical and cultural means, among others (Yuliar et al. 2015). In Colombia, clean and thermotherapy-treated seeds are used as part of primary preventive management. Likewise, tools, footwear and vehicle tires are disinfected. Diseased plants are eradicated from the plantation to prevent further spread (Alvarez et al. 2015). To help contain the progression of the disease, soils can be drenched with copper oxychloride (2000 ppm) or antibiotics such as streptomycin or streptocycline (500 ppm) (Thiribhuvanamala et al. 2018). In the Philippines, formalin was used to drench the soil around the infected banana plant to reduce *R. solanacearum* count and the insecticide and nematicide Furadan® was also tested (Pava et al. 2003). The use of chlorine dioxide to control the disease has also been explored (Ramirez et al. 2015). However, there is still no direct chemical treatment that has been identified to treat moko disease. Chemical treatments are also not recommended for long-term use as these could cause harm to humans and the environment, and may increase the risk of antibiotic resistance. Currently, most of the Cavendish banana plantations in Mindanao Island in the Philippines just disinfect the tools, and cut and burn infected plants (personal communication). Thus, there is a need for a safer, more effective and more environment-friendly means to address to such problem.

The main goal of this study is to determine the potential and suitability of the mangrove soil bacteria to be developed into a new biocontrol agent against the moko disease of banana. Specifically, the study aimed to screen for antimicrobial activity of the mangrove bacteria against four local moko disease-causing *R. solanacearum* strains; to identify the three best-performing bacteria through phenotypic and genotypic methods; to determine the antimicrobial secondary metabolites of the most promising isolate through whole genome sequencing (WGS); and to determine the effectiveness of the most promising bacterium to control the disease under screenhouse conditions.

To the best of our knowledge, this is the first published report on mangrove soil bacteria isolated in Palawan, Philippines exhibiting significant activity against four different local strains of pathogenic *R. solanacearum* causing moko disease of Cavendish banana. Through this study, local isolates inherent

to the Philippine soil possessing promising antimicrobial activity against the bacterial wilt-causing pathogen can be made readily available for stakeholders in the country as these have been deposited at the Philippine National Collection of Microorganisms (PNCM) for long-term preservation and distribution.

MATERIALS AND METHODS

Mangrove bacterial isolates. The 55 bacteria from Palawan, Philippines mangrove soil were previously isolated by the lead author and obtained from the Philippine National Collection of Microorganisms (PNCM) for this study. The isolates were grown on Marine Agar (MA) and incubated at 30°C for 24 h.

***Ralstonia solanacearum* test organisms.** The four *R. solanacearum* local isolates were obtained from four different Cavendish banana farms in Davao, Philippines provided by Lapanday Banana Plantation. These were designated as M1, M2, M3 and M4. The test strains were grown on Kelman's Agar (KA) medium supplemented with 0.1% (w/v) 2,3,5-triphenyl tetrazolium chloride (TZC) and incubated at 30°C for 24 h (Kelman, 1954). The cultural, morphological and some of the biochemical and physiological characteristics of the *R. solanacearum* strains were determined.

Pathogenicity testing. The *R. solanacearum* cell suspensions (9.0 x 10^8 CFU/ml) were prepared in sterile distilled water from 24-hr old cultures. Five milliliters of each bacterial suspension were injected into the lower pseudostem of four two-month-old tissue-cultured Cavendish banana plants. The control set-ups were injected with uninoculated sterile distilled water. The inoculated banana plants were monitored every day up to 30 days for symptoms of bacterial wilt. The *R. solanacearum* strains were re-isolated from the wilted plants and were used in the succeeding tests. After the experiment, the banana plant set-ups were decontaminated in an autoclave for 30 mins at 15 psi.

Extraction of antimicrobial bioactive compounds. The antimicrobial compounds of the 55 mangrove soil bacterial isolates were extracted following the method of Hayashida-Soiza and group (2008) with modifications, active metabolites from a marine bacterium broth culture were extracted using ethyl acetate. Each bacterial isolate was cultivated in 1L Marine Broth (MB) (Pronadisa, Spain) and incubated at 30°C for 48 h with shaking (150 rpm). After incubation, the culture broth was centrifuged at 8000 *g* for 30 mins. An equal volume of ethyl acetate was added to the cell-free supernatant and shaken gently (120 rpm) overnight. The resulting top organic layer was collected and evaporated at 40°C using the rotary evaporator. The concentrated crude extract was suspended in technical-grade methanol (10,000 µg/ml final concentration) and was immediately used for the antimicrobial assay. Unused extracts were sealed and stored in the laboratory refrigerator (4°C).

Antimicrobial Assay (*in vitro*). The antimicrobial analysis was performed on the 55 bacteria by the cylinder plate assay following the modified Kirby-Bauer Disk Diffusion Method (Hudzicki 2009) which is a standard procedure to determine the susceptibility of the test microorganisms to an antimicrobial agent. The 24-hour old cells of each *R. solanacearum* strain were suspended in 5-ml 0.85% NaCl solution (1.5 x 10^8 CFU/ml). Twenty milliliters of sterile molten basal Kelman's Agar medium (KA) were poured onto glass petri plates and allowed to solidify. Five milliliters of seeded KA (0.05% v/v inoculum) top agar were overlaid onto each basal agar and allowed to solidify. Sterile stainless cylinder cups were carefully positioned on top of the agar. One hundred microliters of the crude extract were dispensed into each cylinder cup. A negative control (100% AR-grade methanol) and a positive control (10,000 µg/ml streptomycin) were also included. The set-ups were incubated at 30°C for up to 24 h. The presence of zone of inhibition (ZOI) was observed and recorded. The experiment was performed in triplicates.

Minimum inhibitory concentration (MIC) of the crude extracts. The MICs of the crude extracts from the three best-performing mangrove bacteria were determined by the broth microdilution method

using resazurin as the bacterial growth indicator, with slight modifications (Bouhdid et al. 2010). The selected method makes use of several two-fold dilutions of the antimicrobial agents in a broth medium placed in a disposable microtiter plate, and visual examination of the plates for inhibition of bacterial growth. For this study, serial two-fold dilutions were prepared in Kelman's broth (KB) in a 96-well microtiter plate. Each sample dilution (50 µl) was inoculated with 50 µl of *R. solanacearum* suspension (1.5 x 10^5 CFU/ml). Final concentrations of the samples were 39.06 to 5,000 µg/ml. Sterile KB was used as the negative control. The experiment was done in triplicates. After incubation of the microtiter plates at 37°C for 18 h, 10 µl of filter-sterilized resazurin solution (0.015% w/v) was added to all wells. The set-ups were incubated for additional two h. The color change was assessed visually. Color changes from blue to pink or colorless indicate growth of the test organism. The lowest concentration that inhibited the growth (no color change) was taken as the MIC value.

Phenotypic characterization of the three best bacteria. The cultural characteristics (color, form, opacity, elevation, margin of colony, presence/absence of soluble pigment) of the bacteria on MA were recorded. The bacterial cells were Gram-stained and the cellular morphology (Gram reaction, shape, size and arrangement of cells) was observed under the oil immersion objective (1,000X) using Olympus BX50 light microscope. Through the VITEK 2 Compact (bioMérieux, France) automated identification system, the substrate utilization, enzyme production and antibiotic resistance profiles of the promising bacteria were determined.

Whole genome sequencing of the three best bacteria. The genomic DNAs of the three best-performing bacteria were isolated using the Quick-DNA™ Fungal/Bacterial Miniprep kit (Zymo Research, USA). The DNA samples were submitted to Macrogen Korea for *de novo* whole genome sequencing on HiSeq X Ten platform with 150 bp paired-end setting and 1G throughput. The sequencing library was prepared through the TruSeq Nano DNA kit (Illumina, USA). Prokka v.1.12 was used for functional annotation (Seeman 2014). The annotated genome was analyzed using the Antibiotics and Secondary Metabolite Analysis Shell (antiSMASH) v.5.0. (Blin et al. 2019). To ascertain the identity of the bacteria, digital DNA-DNA (dDDH) hybridization was performed through the Type Strain Genome Server (Meier-Kolthoff and Göker 2019). Average Nucleotide Identity (ANI) was estimated using the Kostas Lab ANI calculator (Rodriguez and Konstantinidis 2016).

Screenhouse assay for biocontrol potential. The antimicrobial activity of the most promising bacterial isolate against the *R. solanacearum* M1 strain was confirmed using actual banana plants in a screenhouse set-up. The treatments were: 1 – Positive control (M1 strain cell suspension), 2 – Negative control (sterile distilled water), 3 – S37 crude extract only (10,000 µg/ml), 4 – S37 whole cell suspension, 5 – M1 combined with S37 crude extract (1:1), and 6 – M1 combined with S37 whole cells (1:1). For the whole cell treatments, the cultures were centrifuged at 8000 *g* to collect the cells, washed with sterile distilled water and the concentration adjusted to 9.0 x 10^8 CFU/ml. Each plant was injected with a 5 ml sample (suspended in sterile water) on the lower part of the pseudostem as described by Ramirez and group (2015) with modifications for treatments 5 and 6. For the 5 and 6 set-ups, 5 ml of the mixture (M1 with S37 extract/cells) were injected for two consecutive days. Each sample was tested on five two-month-old Cavendish banana plants. Injections for all treatments were repeated on the 7th, 14th and 21st days. Symptoms of moko disease were monitored for up to 30 days.

Statistical analysis. The one-way Analysis of Variance (ANOVA) using the Tukey post-hoc test was performed to analyze results for the antimicrobial assay and MIC determination by SPSS Statistics v.23.0 (IBM Corp., USA).

RESULTS AND DISCUSSION

Morphological, biochemical and physiological characteristics. The results of the morphological, biochemical and physiological tests performed on the four locally isolated *R. solanacearum* strains are

presented in Table 1. The tests include Gram reaction, oxidase, catalase, utilization of carbohydrates and optimum growth temperature. The *R. solanacearum* strains M1, M2, M3 and M4 were Gram-negative, rod-shaped and oxidase- and catalase-positive which are important characteristics of the genus *Ralstonia*. All four strains were not capable of utilizing disaccharides (cellobiose, lactose and maltose) and hexose alcohols (dulcitol, mannitol and sorbitol). Their sugar utilization profiles suggest that they belong to biovar 1 which causes moko disease. The strains grow only at 30° to 37°C, and are thus mesophilic. This indicates that they can proliferate in tropical regions.

Table 1. Morphological, biochemical and physiological characteristics of *R. solanacearum* M1, M2, M3 and M4 strains.

TESTS	M1	M2	M3	M4
Gram-reaction	Gram-negative	Gram-negative	Gram-negative	Gram-negative
Cell shape	Rods	rods	rods	rods
Oxidase	+	+	+	+
Catalase	+	+	+	+
Cellobiose	-	-	-	-
Lactose	-	-	-	-
Maltose	-	-	-	-
Dulcitol	-	-	-	-
Mannitol	-	-	-	-
Sorbitol	-	-	-	-
Growth at 4°C	-	-	-	-
Growth at 30°C	+	+	+	+
Growth at 37°C	+	+	+	+
Growth at 45°C	-	-	-	-

+ = positive reaction; - = negative reaction

Pathogenicity of the *R. solanacearum* strains. The four test strains (M1, M2, M3 and M4) produced pinkish mucoidal colonies on KA supplemented with TZC with varying colony sizes and consistencies. The KA medium is particularly useful in distinguishing virulent wild-type *R. solanacearum* strains from avirulent mutant ones. Colonies of virulent strains on KA are white with pink centers and fluidal whereas those of the avirulent strains are smaller, rounder, a darker red with a drier consistency. The cultural characteristics of the four test strains indicate virulence (Figure 1). This has been confirmed by the pathogenicity test using Cavendish banana plant as the host (Figure 2). The leaves of the test plants started to exhibit yellowing two weeks after inoculation and eventually wilted by the 30th day. In addition, their pseudostems turned soft and wet due to the oozing of bacterial growth. The most pronounced symptoms of bacterial wilt were observed for the M1 strain which implied that it was the most pathogenic strain of the four. M1 was followed by M2, M3 then M4 in decreasing virulence. On the other hand, the uninoculated control set-up remained green and healthy after incubation.

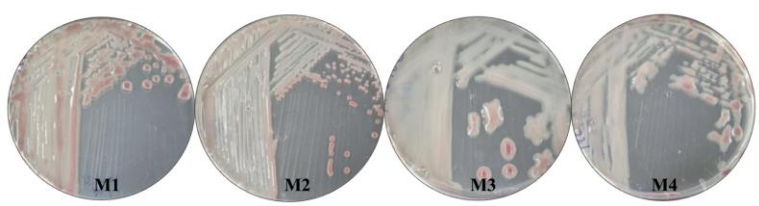

Fig. 1. Colonies of *R. solanacearum* M1, M2, M3 and M4 on KA with TZC after incubation at 30°C for 24 h.

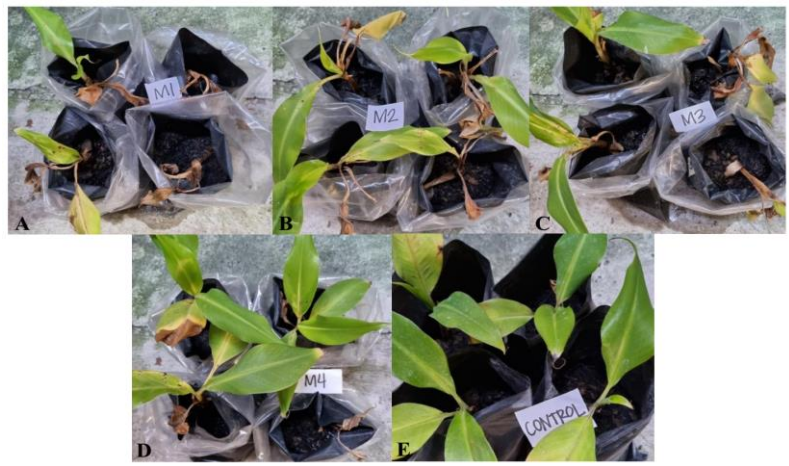

Fig. 2. Wilting of Cavendish banana plants inoculated with *R. solanacearum* M1, M2, M3 and M4 (A,B,C and D) after 30 days. A healthy banana plant in the negative control set-up (E).

Antimicrobial activity of the mangrove bacterial isolates (*in vitro*). Out of 55 bacterial extracts tested, only three exhibited antagonistic activity against the four pathogenic *R. solanacearum* strains. The three mangrove isolates designated as S31, S37 and S38 produced ZOIs against *R. solanacearum* M1, M2, M3 and M4 ranging from 9.50 ± 0.5 to 28.2 ± 0.3 mm in diameter (Table 2). Isolate S37 exhibited the highest ZOI among the three mangrove bacteria which is significantly higher than S31 and S38 against all four *R. solanacearum* strains. The most susceptible *R. solanacearum* strain was M3 where the ZOIs of the extract measured up to 28.2 ± 0.3 mm in diameter. This ZOI is about three times higher than the report of Hasinu et al. (2021) in his study on the antimicrobial activity of his *Bacillus subtilis* strains against *R. solanacearum* bacterial wilt of banana.

Table 2. Antimicrobial activity of S31, S37 and S38 extracts against *Ralstonia solanacearum* M1, M2, M3 and M4 determined by the cylinder plate assay.

SAMPLE	Diameter of zone of inhibition (mm)			
	M1	**M2**	**M3**	**M4**
S31	9.50 ± 0.5b	10.3 ± 0.6c	11.2 ± 0.3c	10.2 ± 0.3c
S37	16.8 ± 1.0a	21.0 ± 1.0a	28.2 ± 0.3a	25.3 ± 0.6a
S38	10.5 ± 0.5b	14.0 ± 0.0b	15.5 ± 0.5b	11.5 ± 0.5b
Positive Control	20.2 ± 0.8	25.3 ± 0.6	30.3 ± 0.6	27.7 ± 0.6
Negative Control	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0	0.00 ± 0.0

Values that are not significantly different are marked with the same letter ($\alpha = 0.05$, Tukey test).

Minimum Inhibitory Concentration (MIC). Results of the microtiter plate broth dilution assay confirmed that the S37 extract significantly had the best activity among the three bacteria against the four pathogenic *R. solanacearum* strains. Its MIC ranged from 78.12 ± 0.0 to 312.5 ± 0.0 µg/ml. As shown in Table 3, the MIC of S37 against M3 was significantly low and very promising compared to S31 and S38 when tested against all four *R. solanacearum* strains. It is at least 16 times more potent than the other two extracts when tested against the most virulent strain of *R. solanacearum* (M1) having an MIC of only 312.5 ± 0.0 µg/ml. Its MIC against M3 is only 78.12 ± 0.0 µg/ml. Isolate S31 had the highest MIC (≥ 5,000 ± 0.0 µg/ml) and thus the weakest antimicrobial activity. The MIC of S38 ranged from 2,500 ± 0.0 to 5,000 ± 0.0 µg/ml.

Table 3. Minimum Inhibitory Concentration (MIC) of S31, S37, S38 against *Ralstonia solanacearum* M1, M2, M3 and M4 using the broth micro-dilution method.

SAMPLE	MIC (µg/ml)			
	M1	M2	M3	M4
S31	> 5000 ± 0.0c	> 5000 ± 0.0c	5000 ± 0.0c	> 5000 ± 0.0c
S37	312.5 ± 0.0a	156.2 ± 0.0a	78.12 ± 0.0a	156.2 ± 0.0a
S38	5000 ± 0.0b	5000 ± 0.0b	2500 ± 0.0b	2500 ± 0.0b
Positive Control	≤ 39.06 ± 0.0	≤ 39.06 ± 0.0	≤ 39.06 ± 0.0	≤ 39.06 ± 0.0
Negative Control	NA	NA	NA	NA

NA = No antimicrobial activity

Characteristics and identities of the three best mangrove bacteria

Isolate S31. S31 is a Gram-positive rod-shaped spore-forming bacterium. It produces cream to beige small, circular, raised, opaque colonies with entire margin on MA. Based on the results of VITEK 2 Compact identification using BCL card (Table 4), it was able to produce arylamidases (five types), beta-xylosidase, beta-galactosidase, alpha-mannosidase and beta-glucosidase. It utilizes D-mannose, D-tagatose, D-trehalose, D-glucose and D-ribose. S31 can grow at 6.5% NaCl and can also hydrolyze esculin. The VITEK 2 system identified S31 as *Bacillus pumilus* (96% similarity) based on phenotypic characteristics. The pairwise dDDH (85.8%) and ANI (98.4%) analyses identify S31 as *B. altitudinis*. The generally accepted species boundary for dDDH and ANI values are 70.0% and 95.0%, respectively (Chun et al. 2018; Goris et al. 2007). Results in comparison with the *B. pumilus* type strain were just 36.1% for dDDH and 87.7% for ANI. These results strongly suggest that S31 belongs to *B. altitudinis* and not to *B. pumilus.*

Isolate S37. S37 is also a Gram-positive rod-shaped bacterium capable of producing endospores. Its colonies on MA appear off-white to cream, circular to irregular in form, raised, opaque with undulating margin. This isolate produces alpha-galactosidase, beta-galactosidase, beta-glucosidase and six types of arylamidases. It can utilize D-glucose, D-ribose, inositol, glycogene, maltotriose, D-mannose, palatinose and inulin. It also acidifies methyl-A-D-pyranoside, hydrolyzes esculin and grows at 6.5% NaCl (Table 4). S37 was identified as *Bacillus subtilis* based on phenotypic characteristics (94% similarity). The pairwise dDDH and ANI analyses results between the genomes of S37 and the *B. subtilis* type strain were 89.1% and 98.7%, respectively. Both phenotypic and genotypic methods indicate that S37 belongs to *B. subtilis*.

Isolate S38. This is a spore-forming Gram-positive rod-shaped bacterium, produces off-white to cream, circular, raised, opaque colonies with entire to undulating margin on MA. Its enzymes include beta-N-acetyl-glucominidase, alpha-glucosidase and six types of arylamidases. This bacterium assimilates glycogen, maltotriose, N-acetyl-D-glucosamine, D-trehalose, D-glucose, D-ribose. It likewise hydrolyzes esculin and grows at 6.5% NaCl. S38 is the only bacterial isolate that has resistance to kanamycin, oleandomycin and polymyxin B (Table 4). S38 was identified as *Bacillus thuringiensis* based on phenotypic characteristics (95% similarity). The pairwise dDDH and ANI values between the whole genomes of S38 and the *B. thuringiensis* type strain were 84.7% and 98.6%, respectively. Phenotypic and genotypic analyses agree that S38 belongs to the said species.

As members of the *Bacillus* group, the promising bacteria grow fast and very easy to cultivate in large quantities with very minimal nutritional requirements. This group is known to harbor biosynthetic gene clusters that have been reported to have antimicrobial activities against a wide range of microorganisms. These promising bacteria produce endospores which render them relatively resistant to harsh environmental conditions, and thus will have comparably better survival when

applied in the field. Isolates S31, S37 and S38 were found to belong to non-pathogenic *Bacillus* species and can be safely utilized by humans.

Table 4. Substrate assimilation, enzyme production, antibiotic resistance profile and identity of S31, S37 and S38 determined by the VITEK 2 Compact Identification System (BCL card).

TESTS	S31	S37	S38
Beta- Xylosidase	+	+	-
L-Lysine-Arylamidase	-	-	-
L-Aspartate Arylamidase	(-)	+	+
Leucine Arylamidase	+	+	+
Phenylalanine Arylamidase	+	+	+
L-Proline Arylamidase	-	-	-
Beta-Galactosidase	+	(+)	-
L-Pyrrolidonyl-Arylamidase	-	+	+
Alpha-Galactosidase	-	+	-
Alanine Arylamidase	+	-	+
Tyrosine Arylamidase	+	+	+
Beta-N-Acetyl-Glucosaminidase	-	-	+
Ala-Phe-Pro Arylamidase	-	-	-
Cyclodextrine	-	-	-
D-Galactose	-	-	-
Glycogene	-	+	+
myo-Inositol	-	+	-
Methyl-A-D-Glucopyranoside	-	+	-
Acidification			
Ellman	+	-	+
Methyl-D-Xylosidase	-	-	-
Alpha-Mannosidase	+	-	-
Maltotriose	-	(+)	+
Glycine Arylamidase	+	+	-
D-Mannitol	-	-	-
D-Mannose	+	+	-
D-Melezitiose	-	-	-
N-Acetyl-D-Glucosamine	-	-	+
Palatinose	-	+	-
L-Rhamnose	-	-	-
Beta-Glucosidase	+	+	-
Beta-Mannosidase	-	-	-
Phosphoryl Choline	-	-	-
Pyruvate	+	-	+
Alpha-Glucosidase	-	-	+
D-Tagatose	+	-	-
D-Trehalose	+	-	+
Inulin	-	+	-
D-Glucose	+	+	+
D-Ribose	+	+	+
Putrescine Assimilation	-	-	-
Growth in 6.5% NaCl	+	+	+
Kanamycin Resistance	(-)	-	+
Oleandomycin Resistance	-	-	+
Esculin Hydrolysis	+	+	+
Tetrazolium Red	+	+	(-)
Polymyxin B Resistance	-	-	+
IDENTITY	*Bacillus pumilus* (96%)	*B. subtilis* (94%)	*B. thuringiensis* (95%)

+ = positive reaction; - = negative reaction; (+) = weak positive, reaction slightly below detection threshold; (-) = weak negative, reaction slightly above detection threshold

Secondary metabolites produced by the most promising bacteria. The functional annotation indicated that the genes in S37 encoding biosynthesis of secondary metabolites account for only 1.2% of the genome. About 20% of the genes are responsible for general functions, while around 23% correspond to genes with unknown functions. The rest of the genes of the promising bacteria are involved in vital cellular processes such as transcription, translation, replication, cell wall-, membrane- and envelope biogenesis, motility, energy production, and carbohydrate and amino acid transport.

The antiSMASH analysis predicted a total of nine secondary metabolite biosynthetic gene clusters (smBGCs) from the S37 genome (Table 5). However, only six have been found to encode specific compounds. These include bacillaene, fengycin, bacillibactin, subtilosin A, bacilysin and surfactin.

Table 5. Secondary metabolites detected from the S37 genome by antiSMASH (v.5.0).

Location (Region)	Most similar known cluster	Percent Similarity
1.1	terpene-type	-
1.2	bacillaene	100
1.3	fengycin	93
1.4	terpene-type	-
1.5	T3PKS-type	-
2.1	bacillibactin	100
2.2	subtilosin A	100
2.3	bacilysin	100
3.1	surfactin	78

- = Not determined; T3PKS = Type III Polyketide Synthase

Bacillaene is a polyketide that has been found to exhibit antimicrobial activity against *Staphylococcus aureus* and *Fusarium* spp. (Patel et al. 1995; Um et al. 2013). Fengycin is an NRP lipopeptide which has been reported to have antifungal activity against the plant pathogen *Colletotrichum gloeosporioides* (Kim et al. 2010) and antibacterial activity against the human pathogen *Listeria monocytogenes* (Lin et al. 2020). It has also been reported to inhibit the growth of other pathogenic bacteria such as *Aeromonas hydrophila*, *Xanthomonas axonopidis pv. vesicatoria* and *Pseudomonas aeruginosa* PA01 by producing alterations on the cell surface of the target bacteria (Medeot et al. 2020). Bacillibactin is a siderophore type of NRP that exhibited antimicrobial activity against the plant pathogen *Pseudomonas syringae* pv. *tomato*, and human pathogens *Staphylococcus aureus, Enterococcus faecalis, P. aeruginosa* and *Klebsiella pneumoniae* (Chakraborty et al. 2022; Dimopoulou et al. 2021). Subtilosin A, an antibiotic peptide produced by *B. subtilis*, had antagonistic activity against Gram-positive bacteria such as *S. aureus* and *Streptococcus faecium* (Babasaki et al. 1985). Bacilysin, a type of non-thiotemplate NRP, has been reported to have antagonistic activities against the pathogenic bacteria *Erwinia amylovora* and *Microcystis aeruginosa*, and fungi *Aspergillus fumigatus* and *Candida albicans* (Chen et al. 2009; Milewski et al 1986; Wu et al. 2014). Surfactin is a cyclic lipopeptide that acts as a potent biosurfactant (Santos et al. 2018). It has been explored in the development of animal feed additives as it possesses antibacterial activity against *Clostridium perfringens* and *Brachyspira hyodysenteriae* (Horng et al. 2019). One or more of the six mentioned compounds may be responsible for the antagonistic activity of S37 against the pathogenic *R. solanacearum* strains.

Biological activity of the most promising bacteria (*in planta*). The biological activity of the most promising bacterial isolate, S37, was tested in a screenhouse assay. On Day 0, all Cavendish banana plants from all treatments were healthy (Fig.3 - 1A to 6A). On the 2nd week of incubation, plants

inoculated with the pathogen M1 cell suspension (T1) started to exhibit yellowing and wilting. At 30 days after treatment (DAT), all the plants were completely wilted, whereas the negative control (T2), S37 crude extract only (T3) and S37 cell suspension only (T4) remained alive and healthy (Fig. 3 - 1B to 4B). Three out of five plants inoculated with M1 combined with the S37 crude extract (T5) stayed alive 30 DAT (Fig. 3 – 5B). The two plants exhibited wilting only on the 4th week. Two out of five plants inoculated with M1 and S37 whole cell suspension (T6) remained unwilted at 30 DAT though the plants did not remain as healthy (Fig.3 – 6B). The three plants wilted only on the 4th week. This preliminary study showed the potential of S37 as a possible biocontrol agent against moko disease of banana as shown in the results from treatments 5 and 6.

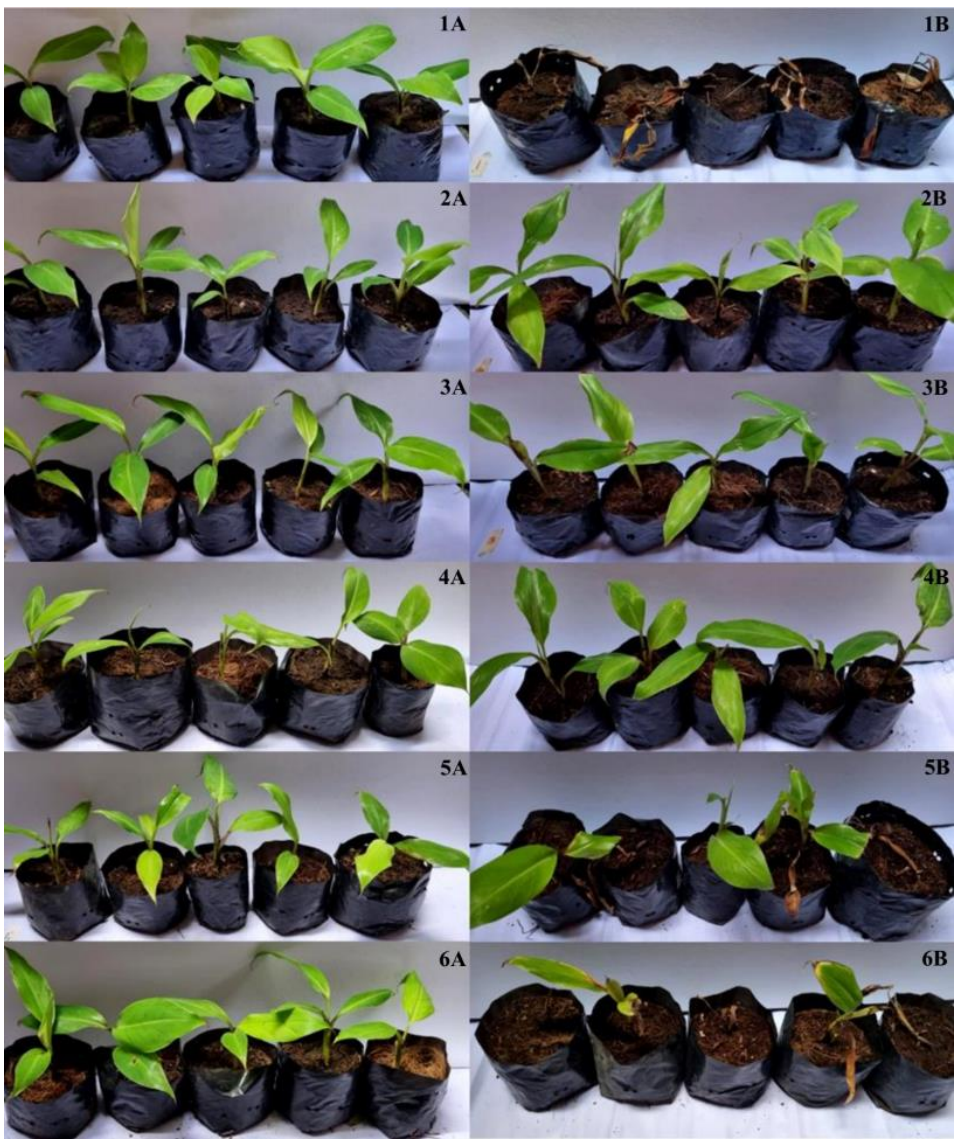

Fig. 3. Effect of the six screenhouse treatments on the Cavendish banana test plants: (1A to 6A) appearance of plants at Day 0 and (1B to 6B) condition of the same plants 30 DAT.

CONCLUSION

The mangrove bacterial isolates, designated as S31 (*Bacillus altitudinis*), S37 (*B. subtilis*) and S38 (*B. thuringiensis*), may be considered potential biological agents to control moko disease-causing *R. solanacearum* due to their non-pathogenicity, short generation times, non-fastidious nature, spore-forming ability thus more hardy compared to non-sporeformers, and capacity to produce antimicrobial metabolites. The bacterium with the strongest activity against the pathogenic *R. solanacearum* strains, S37, may be harnessed to develop a new microbial-based product that can help suppress moko disease in banana. Its biocontrol potential, however, will have to be tested in the field. The proper concentration/formulation and time of application will have to be studied. Having six antimicrobial secondary metabolites, the wider application of isolate S37 against other important plant disease-causing microorganisms, especially *Fusarium oxysporum* TR 4 which is also a big problem in the banana industry not only in the Philippines, will be explored in future studies. These isolates will be available for stakeholders in the country to acquire as they have been deposited at the PNCM culture collection.

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ISSAAS PHILIPPINES

NATIONAL CONGRESS 2021

EXTENDED ABSTRACTS

SWEET ABULUG POMELO (SAP): The Lucky Fruit of Cagayan Valley, Philippines

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ABSTRACT

Sweet Abulug Pomelo brought good luck to farmers amidst pandemic. Mother trees are maintained in Foundation Grove of Department of Agriculture - Northern Cagayan Experiment Station, Maquire, Lucban, Abulug, Cagayan and was certified by Bureau of Plant Industry last 2008 as Siamese Abulug Pomelo as NSIC 2008 Pm 04 now popularly known as the Sweet Abulug Pomelo (SAP). After 3-5 years from planting, it can bear as many as100-300 fruits per tree. The sweetness reaches from 10.9 to 12.5 percent Brix. However, SAP is susceptible to Citrus Rind Borer (CRB) causing ugly appearance although the taste remains sweet. A verification trial on the different control of Citrus Rind Borer (CRB) was conducted and resulted to 90% CRB free damaged coupled with recommended cultural management. After 13 years, SAP Field Day was conducted last August 10, 2021 to showcase and promote SAP and attended by 114 participants and ten (10) partner Government agencies. Hence, the promotion of SAP through field day resulted in generating a net income of Php.1, 017,270.00 / ha / year.

Key words: sweet abulug pomelo, citrus rind borer, pandemic, brix

INTRODUCTION

Obtaining sustainable food system in this time of new normal is a challenge to everyone. But in Cagayan Valley, amidst this threat of pandemic, we see an opportunity. The Sweet Siamese Pomelo now popularly known as Sweet Abulug Pomelo (SAP) was registered as NSIC 2008 Pm 04 by Bureau of Plant Industry (BPI) under the administration of Celerina T. Miranda last 2008. Although this high value commodity is already existing long before this pandemic and it seems low promotion with limited market penetration, but this time, it is making its own identity. The demand for its fruit is very high specifically this is a very good source of Vitamin C which everyone needs in boosting immune system to fight Covid19. Likewise, its juiciness and sweetness is comparable to Davao pomelo in spite of its unattractive appearance because it remains green even when ripe.

MATERIALS AND METHOD

Verification on Insect Pest Management was conducted last January 2021 in a 7 years old Fruit Bearing SAP at the NCES Foundation Grove following the Package of Technology for SAP except for the treatments such as:

1.) Fruit Bagging – Plastic net with 15 inches' x 45 inches' bag size was used for fruit bagging

2.) Chemical Spraying - Parker Neem insecticide was used against Citrus Rind Borer (CRB) and other pest attacking pomelo.
Frequency of Spraying: From Flushing to Ball size: 2times a month (every 14 days interval)

3.) Insect Trapping –super net insect attractant sticker was sprayed to an empty plastic bottle and hanged near the tree using bamboo pole was installed for insect trapping

4.) Biological Control Agent – The three Entomopathogenic Fungi (EPF), *Metarhizium spp., Isaria, Beauveriabassania*, were applied to 10 sample trees following the recommended application.

Verification trial on 1-year-old SAP intercropped with various Cash crops with an area of 2,500 sq.m , 39 trees was conducted following the Package of Technology for SAP and cashcrops. Crop used were Corn (IES Glut 4), Upland Rice (Calatrava), Peanut (3 seeded), Sweet Potato (Swerte), Cassava (Golden Yellow), Bush Sitao (Los Banos white) and Pineapple.

Hosting the Field Day with 150 participants

-Farm tour was conducted in the Foundation Grove where the bearing and non-bearing pomelo were maintained. Likewise, the result of the trials on the different technologies was showcased. -The growth and performance of SAP model tree was showcased. -Testimonies from successful SAP growers inspired the participants to venture in SAP production. Packaging and Labelling of the SAP was developed to encourage potential buyers to purchase the product. Awarding and distribution of 6,000 seedlings to FCA was done during the field day

- Online advertising like social media platform (Facebook, Instagram), Direct Marketing, Product giveaways and samples, and branded promotional gifts.

RESULTS AND DISCUSSION

Result shows that the net income of different Pest Management Practices in controlling the insect pest of SAP especially Citrus Rind Borer (CRB) the gross income derived from the 5 to 7 year-old SAP was Php. 1,248,000.00. Chemical Spraying practice gave the highest net income with 909,570.00 followed by insect trapping with Php. 899,170.00 . The treatments are not different among each other in terms of ROI. Therefore, any of the treatments can be recommended to SAP growers. Cash crops are a viable source of additional income to pomelo growers. Intercropping is a practice to maximize utilization of land use in SAP plantation. Result shows that the top three cash crops used with the highest net income were the Pineapple, Sweet Potato and bush sitao with Php.155,543.00, Php. 81,779.00, and Php. 80,250.00, respectively. The Field day conducted brought a significant impact to the pomelo growers. Farmer's option on Pest Management Practices for SAP was established aside from Chemical Pest Control. The different Pest Management Practices employed against Citrus Rind Borer improved the fruit quality of SAP up to 90% CRB damage free. Various crops were identified that can be grown in between non-bearing pomelo. There was an increase in demand of SAP fruit as source of Vitamin C during the pandemic, as well as planting materials. 6,000 SAP seedlings to Farmer Cooperative/Associations and 1,000 to Municipal Wide Backyard Tree planting in Abulug, Cagayan were distributed to recognize Abulug as the Home of SAP.
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CONCLUSION

Opportunity comes when least expected and even at the midst of hard times. It was in this pandemic, the health crisis brought by COVID-19 that each one struggle to boost one's immune system to combat the virus. The closure of many borders limits the entry of fruits and vegetables as part of essential hence, the supply decreased and the demand is high resulting to increase in price. Now, the hidden treasure of Abulug sparks and shines due to the call of the moment. The DA initiatives with other partner agencies made SAP a lucky fruit of Cagayan Valley, Philippines. The field day conducted at DA-NCES and NVES was carried out successfully in promoting SAP and now adopted as one of the priority crops to consider. This high value crop is now an integrated part as tourist attraction of Cagayan Valley. Government support system played significantly in the promotion of SAP that enable farmers to earn a million of pesos from the sale of fruits. SAP farming is a productive endeavour that keeps farmers alive in the new normal as indicated by the demand of seedlings as well as fruits. Growing fruit trees is environment friendly and minimizes soil erosion.

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ADAPTATION STRATEGIES AMID THE PANDEMIC AMONG AGRI-TOURISM ENTERPRISES IN LA UNION, PHILIPPINES

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ABSTRACT

Prior to the pandemic, agri-tourism was on its rise in the Philippines. It is known as a fusion of Agriculture and tourism, linked to other sectors, including hospitality and transportation. However, travel bans and other restrictions amid the COVID-19 pandemic have adversely affected tourism. This study aimed to describe the pandemic effects on agri-tourism enterprises in the province of La Union, Philippines, and their adaptation strategies. A survey of 16 enterprises was conducted in May 2021. Descriptive and content analysis was employed to analyze the data and information gathered. The pandemic was found to have significantly affected the enterprises' marketing function, resulting in financial losses and adjustments to their business models. Their adaptation strategies were categorized as either survival or sustaining. Survival strategies were the farms' initial coping mechanisms like shifting to a new market, lowered prices, and switching to online marketing. On the other hand, sustaining strategies were also known as long-term solutions like diversification and expansion. The enterprises that adopted both strategies had wider offerings and possessed more than one accreditation. It is recommended that agri-tourism enterprises continue strategizing as the pandemic remains a threat. They should consider employing sustaining strategies for post-pandemic.

INTRODUCTION

The tourism industry of the Philippines has been continuously rising for the past years (Cabiladas, 2020). On the other hand, Agri-tourism's popularity continues to escalate with the passage of Republic Act No. 10816 or the Farm Tourism Development Act of 2016. The sector's development was disrupted when the entire island of Luzon in the Philippines was put under Enhanced Community Quarantine (ECQ) in mid-March 2020. The pandemic negatively affected businesses from various industries that also caused labor disruptions to millions of Filipinos (International Labor Organization, 2020). The United Nations (UN) recognizes the COVID-19 pandemic as a health and humanitarian crisis that threatens the food security and nutrition of the worldwide population (United Nations, 2020). Food security has been the reason for retaining farming. An argument follows that if agri-tourism can make farms more economically feasible and sustainable, it contributes positively to public goals (Schilling et al., 2012). It was fortunate for the agri-tourism enterprises to operate during the pandemic despite travel restrictions. The Inter-Agency Task Force on Emerging Infectious Disease (IATF-EID) approved the unrestricted movement of agriculture workers despite ECQ to ensure food security during the pandemic.

This study sought : 1) to identify the status of the agri-tourism enterprises in La Union, Philippines. 2) To assess the effect of the pandemic on the enterprises' business functions amid the pandemic, and 3) To analyze their adaptation strategies in mitigating the effects of the pandemic.

MATERIALS AND METHODS

The descriptive research design was used to describe the background of the identified agri-tourism sites in La Union. Primary Data and Secondary data were used for this study. A semi-structured questionnaire collected primary data from the sixteen (16) identified respondents, and data collection was from May 1 to 29, 2021. A complete enumeration was employed in selecting the respondents due to their minimal number. The secondary data included government records, existing legislation, related research, and articles from journals. Descriptive statistics were used in presenting the frequency distribution of the results, and a content analysis was used to present and evaluate the relevant parameters from the responses. Non-parametric statistical tools were performed to see the degree of relationship or association among variables—the results of the quantitative analysis and other observations were the basis of the qualitative analysis.

RESULTS AND DISCUSSION

Travel restrictions and suspension of face-to-face activities pushed the farms to stop some of their offerings while some adjusted the prices. Consistent with Liguori & Pittz's (2020) study, the agri-tourism farms in La Union also altered their business model. Regardless of the farm's size and the number of offerings, they did not escape from the pandemic effects. All enterprises experienced a huge drop in their visitor count that greatly affected their operations during the pandemic—employees in the training and food service needed to be in alternative working arrangements sometime. The most affected business function was the enterprises' marketing and logistics functions that had financial implications, too. The total minimum estimated loss due to the pandemic amounted to Php 6.7 million. The pandemic made the owners realize the importance of maintaining liquidity to remain resilient.

This study categorized strategies as survival strategies and sustaining strategies to assess the farms' approaches. These strategies differ in purpose. Survival strategy can be the same as emergency responses described by the International Trade Center (ITC) as the immediate actions taken to withstand a crisis. Meanwhile, some strategies provided long-term economic growth. These strategies were the sustaining strategies or those that consider post-pandemic operations. Survival strategies employed were shifting to a new market, utilizing digital platforms, and cutting expenses. Sustaining strategies included diversification and expansion of operations. A significant association between the level of agri-tourism enterprises and their strategies' classification. It shows that enterprises falling on the higher classification level could most likely implement survival and sustaining strategies. Moreover, it justifies that an enterprise with more offerings could highly survive a pandemic and prepare for post-pandemic. While it is crucial to surviving the pandemic, the enterprises should be prepared when things get back to normal.

CONCLUSION

In general, the pandemic brought negative effects to the agri-tourism enterprises in La Union, Philippines. The strategies imposed by the agri-tourism enterprises also depended on their offerings' uniqueness. Despite measures to lessen the pandemic risks, the pandemic remains a threat to the agri-tourism sector. 4) The agri-tourism farms in La Union are surviving the pandemic and should continue innovating their strategies to remain resilient.

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PUNGENCY LEVELS OF GARLIC *(ALLIUM SATIVUM)* GROWN FROM VARIOUS AREAS IN THE PHILIPPINES

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ABSTRACT

Garlic is a very important spice in the tropics. However, it can only be grown during the dry months of the year. Hence, it is important to make it available the whole year round. This study was done to compare garlic bulbs sourced from various production areas in the country and to determine how long their pungency levels can be maintained. Garlic bulbs taken from various areas in the country were sampled during its harvest season (February–March, 2019) and stored for 6-8 months at ambient conditions (~30C). The total soluble solids (TSS) content and its pungency in terms of pyruvate content were analysed. Bulbs taken from Ilocos Norte and Nueva Vizcaya initially had higher pyruvate content (128-130 ug/g FW) than those from Mindoro (99 ug/g FW) or Batanes (89 ug/g FW). After about 6-8 months under ambient conditions, pyruvate levels generally increased by 7-27 ug/g FW in most samples. Comparison between bulb sizes showed that the bigger ones had usually lower pyruvate content. The average TSS values were about 31°Brix from all samples with slight increment (33°Brix) after storage. Both pyruvate content and TSS values of Philippine garlic were comparable or even higher than those reported by another country.
Key words: pyruvic acid, TSS, ambient storage, various garlic sizes

INTRODUCTION

In the Philippines, garlic is a popular spice being used as ingredient in many food preparations. Perception of garlic flavour is closely related to its pungency levels. Garlic pungency is developed when sulphur precursors after tissue disruption, react with the enzyme allinase, liberating numerous volatile sulphur compounds. Pyruvic acid is a secondary product of this reaction and is used as an indirect measure of pungency (Natale and Camargo 2005). Argentina, the second garlic exporter in the world has garlic with 64-97 umol/g pyruvic acid among the following cvs. INTA: Castano, Nieve, Morado, Fuego (Natale and Camargo, 2005). A local maturity study on garlic (Nuevo 1996) showed that when harvested 90 days after planting (immature bulbs), weight loss was high, softening was faster and there was low total soluble solids (TSS) and pyruvate content. Hence, these bulbs must be harvested at least 105 days after planting. Bulbs were also found to be more pungent than its corresponding scapes, which is used in some food preparations (Gonzales et al., 2012). Heat treatments of peeled and unpeeled garlic cloves could control its sprout and root growth also, which was as effective as controlled atmosphere storage (Cantwell et al. 2003). Low temperature storage (4-10C, 60-90% RH) for 120 days resulted in increased allicin content of garlic (Sukkaew and Tira-umphon, 2012). Maximum allicin level (36.5 mM/g DW) was found in bulbs stored at 4-6C, 80-90%RH after 60 days. Only 4-11% weight loss was observed under the said conditions. Locally, there is no study yet on the comparison of garlic pungency. Hence, this study was conducted: 1) to compare the pungency levels of garlic from various growing areas in the county, and 2) to determine the changes in its pyruvate content after prolonged storage under ambient condition.

MATERIALS AND METHODS

Garlic grown from various areas in the country was collected at the following places: Itbayat in Batanes, Pasuquin in Ilocos Norte, Bambang in Nueva Vizcaya and Lubang Island in Occidental Mindoro during the harvest season (February –March 2019). Various sizes of garlic in each area were collected (extra-large, large, medium, small, extra-small). Samples were further dried at ambient condition in the laboratory for a month before analyses were done. Total soluble solids (TSS) content and pungency levels were measured initially and after about 6-8 months at ambient conditions (29-33C). Pungency was determined according to Schwimmer and Weston (1961) with some modifications (Gonzalez et. al., 2012). Ten garlic bulbs of each size served as replicates for the chemical analyses. Results were subjected to analysis of variance and Tukey's test at .5% level of significance.

RESULTS AND DISCUSSION

Initial TSS levels of garlic ranged from 28 to 34^0Brix. Samples from Ilocos Norte had the lowest initial TSS levels (28^0Brix), followed by Nueva Vizcaya (31^0Brix), Batanes (33^0Brix) and then Mindoro (34^0Brix). Those from Batanes, Ilocos Norte and Nueva Vizcaya all had decreasing TSS levels as the bulb size increased, unlike those from Occidental Mindoro which showed the opposite pattern. However, after 6-8 months of storage, a slight increase in TSS levels (about 30-35^0Brix) was observed in all samples. With respect to sizes, almost same

pattern as the initial observation was noted after storage. Moreover, the TSS values obtained here were slightly higher than those reported in garlic from Spain (25-29^{0}Brix) by Pardo et.al., 2007.

The initial pyruvate levels were observed to be highest in samples from Ilocos Norte (128 ug/g FW) and Nueva Vizcaya (130 ug/g FW), while the lowest were in bulbs from Batanes (89 ug/g FW). With respect to bulb sizes, there is increasing pyruvate content (5 to 23 ug/g FW) in general, as the size of bulbs increase from extra-small to extra-large.

After 6-8 months storage at ambient conditions, there was an increase in pyruvate content in most samples except those from Batanes which showed a slight decline (8 ug/g FW). The increase in pyruvate levels in garlic were as follows: from Ilocos (8 ug/g FW), Nueva Vizcaya (7 ug/g FW) and Occidental Mindoro (27 ug/g FW). Both Ilocos Norte and Nueva Vizcaya samples had nearly constant levels compared with those of Occidental Mindoro. It is possible that samples from Mindoro were not properly dried as the other samples were, hence the big difference in pyruvate content over time. However, the pyruvate levels obtained from the local garlic were higher than those previously reported in Argentina having only about 60-97 umol/g (Natale and Camargo, 2005).

The effect of bulb size on pyruvate content cannot be conclusive since garlic from various areas showed either decreasing or increasing trend with respect to garlic size.

CONCLUSION

The study shows that pungency levels of garlic grown from different areas in the country did not show dramatic decline even after 6 to 8 months under ambient condition. Those from Ilocos Norte and Nueva Vizcaya consistently had similar high pungency levels (~136 ug/g FW). This is followed by those from Occidental Mindoro (126 ug/g FW). In contrast, samples from Batanes not only had the lowest pyruvate levels (89 ug/g FW) but were also the only ones that showed slight decline with time (81ug/g FW). Moreover, changes in pyruvate levels with respect to bulb sizes were not consistent in all sources. Except for bulbs from Ilocos and Mindoro, others have increasing pyruvate levels with increase in bulb size. The pyruvate levels of garlic from the Philippines are quite comparable with those from other countries like Argentina. Hence, it may be exported to other countries especially in the sub-temperate regions like Japan and Korea.

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SHOOT AND FRUIT DEVELOPMENT OF CALAMANSI (*Citrus* x *microcarpa* Bunge*)* AS INFLUENCED BY FERTILIZER AND VESICULAR-ARBUSCULAR MYCORRHIZA (VAM) TREATMENTS

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ABSTRACT

The effect of inorganic fertilization and VAM application on shoot and fruit development of calamansi (*Citrus* x *microcarpa* Bunge) was studied from October 2020 to May 2021. Two-year old potted calamansi trees with calamandarin as rootstock were used. Treatments observed were: (a) unfertilized, uninoculated; (b) fertilized at 100% rate; (c) VAM-inoculated; (d) fertilized at 50% rate, VAM-inoculated, and (e) fertilized at 100% rate, VAM-inoculated. VAM inoculation combined with half the amount of recommended fertilizer application enhanced shoot and fruit development compared with the other trees. Improved shoot growth was due to increased shoot length and number of leaves per shoot, higher change in plant height, wider stem diameter, and greener leaf color. Fruit development was due to optimum NPK uptake in mature leaves and VAM association in roots which resulted to higher number of harvestable fruits. A standard for harvesting calamansi fruits based on their use – as fresh fruit or as processed juice was also established. Fresh market fruit use is recommended to be harvested between Stages 79 (fruits are about 90% of their final size) and 81 (mature green) while processed juice use should be harvested before Stage 83 (breaker) manisfest.

Keywords: biofertilizer, calamansi, citrus, mycorrhiza, VAM

INTRODUCTION

Citrus is the most economically important fruit crop in the world. *Citrus* x *microcarpa* Bunge, locally known in the Philippines as calamansi, is considered a major crop in the Philippines. As of 2019, the Philippine Statistics Authority has recorded about 8.33 million bearing calamansi trees planted in 19.62 thousand hectares of land and produced a volume of 125.97 thousand metric tons of fruits. Amidst this volume of produced fruits, however, calamansi production decreases at an average annual rate of 5.1 percent from 2015 to 2019. The area planted and the number of bearing trees decreased annually at an average rate of 0.5 and 0.6 percent, respectively. There is a need to revitalize the calamansi industry by improving its production and fruit quality by utilizing proven technologies and management practices. The association of VAM has been observed to be beneficial in fruit crops production including citrus in enhancing growth and ion uptake, improving tolerance to drought and salt stress, and enriching fruit quality. The study sought to determine the effect of fertilizer treatments on shoot growth and fruit yield and quality and determine the effect of VAM biofertilizer inoculation on the indigenous VAM root association.

MATERIALS AND METHODS

The study was conducted at 14.1804375, 121.1800625 in Brgy. Pansol, Calamba City, Laguna from October 2020 to May 2021. The area is classified to have Type 3 Climate. Sixty (60) healthy and uniform, already fruiting, budded calamansi trees, about 2 years of age, with Calamandarin (*Citrus reticulata Blanco x C. madurensis lour.)* as rootstock, were used in the study. Trees were transferred to larger pots and were acclimatized for 7 weeks. Five (5) treatments with four (4) trees per treatment replicated thrice were done. The treatments were (T1) unfertilized and uninoculated; (T2) fertilized at 100% rate; (T3) VAM-inoculated; (T4) fertilized at 50% rate and VAM-inoculated; and (T5) fertilized at 100% rate and VAM-inoculated. Fertilizers used were urea and complete. Urea was applied at a rate of 7g every 3 weeks for 6 weeks, then 10g every 3 weeks for another 6 weeks. Complete fertilizer was applied at a rate of 15g and repeated every 3 weeks for another 6 weeks. Whereas, for VAM-inoculation, the biofertilizer used is Mykovam™. Other management practices such as pesticide application and weeding were done on a per-need basis. The study was laid out employing the Completely Randomized Design (CRD).

Effect of fertilizer and VAM inoculation on shoot growth and fruiting of calamansi. The following data were collected and recorded: l*ength of shoot and number of leaves, plant height and stem diameter, leaf color.* At the end of the study, four (4) fully expanded leaves from each tree were used to determine the leaf color using the Leaf Color Chart. Other effects evaluated were: nutrient soil content and leaf uptake, mycorrhizal colonization and spore count, and fruits harvested.

RESULTS AND DISCUSSION

Plant growth parameters. For all parameters, T4 is significantly different from the other treatments with an average of 16.23 cm length of shoot, 11 number of leaves per tagged shoot, 18.32% increase in plant height and 20.19% increase in stem diameter. T4 also has the greenest leaves among the treatments.

NPK soil content and plant uptake. N content of soil samples did not differ among treatments. All of them however showed an increase from the initial soil N content of 0.02%. For soil P content, T5 has the highest amount with an average of 126 ppm. For soil K content, T2 and T5 have the highest amount with 532 ppm and 557.33 ppm respectively. The N in lemon leaf is 1.9–2.2% (Plessis and Koen 1992). In comparison, T1 and T2 have low N, T3 and T5 have excessive, while T4 has high. Citrus needs more N than any other nutrients as it is a vital part of proteins & chlorophyll. When there is excess N, there is vigorous vegetative growth as observed in T2 and T5 trees. The optimum requirement for P in lemon is 0.11–0.15% (Plessis and Koen 1992) and only T4 attained the norm. Fertilized T2 and T5 were deficient while unfertilized T1 and T3 had excessive levels. Based on Plessis and Koen (1992), K contents of T4 fits the norm (1.1–1.4%). T2 and T5 falls below the norm while T1 and T3 exceeds it.

Mycorrhizal colonization and spore count. Root samples have initial VAM colonization of 0-3%. Two months after treatment application, laboratory analysis showed that T1 (unfertilized, uninoculated) has the highest average colonization of 68%. The said treatment is not statistically different with VAM-inoculated T3 (62%) and with fully fertilized T2 (61%). T4 has 57% while T5 has 28%. Five months after inoculation, root colonization generally increased with T3 having the highest rate of 70%, followed by T1 with 67% and T2 with 65%. T1, T2 and T3 almost have the same colonization. T3's result is expected since the trees are unfertilized and inoculated with VAM. The VAM present in uninoculated T1 and T2 may have been due to the endogenous VAM present in the medium since trees were transplanted in unsterilized soil. Mycorrhizal fungi are naturally occurring and as long as there are compatible hosts, they infect the roots. For T4 and T5, both showed colonization as well but is smaller than the other 3 treatments. The presence of fertilizer may have contradicted the colonization of exogenous VAM. Root colonization is highest on inoculated treatment with no N and P applied while lowest in treatment applied with both N and P (Youpensuk, et. al. 2003).

Fertilized T2, T4 and T5 had lower P uptake while unfertilized T1 and T3 had high. There is no statistical difference among treatments in terms of spore count. The application of VAM may have not influenced the population of spores present in the soil and this may be due to AM fungi species, root age, root density, soil moisture, and position of mycorrhizal inoculum.

Fruits harvested.. T4 had the highest number of fruits (42) among the treatments, followed by T2 (25 pcs; 59.75 g) and T5 (12 pcs; 38.25 g). Both T1 and T3 have no harvest as the majority of plants were still in the shoot development stage.

CONCLUSION

Inoculation of VAM biofertilizers to trees fertilized at minimal amount (50% rate) indeed enhanced the growth and development of shoots and fruits. T4. Though VAM seemed to have contradicting effects when trees are fully fertilized (as seen in T5), fertilizer is still needed as VAM only enhances the nutrient uptake and cannot provide the nutrients needed by trees as observed in unfertilized trees.

RECOMMENDATIONS

Various fertilizer levels should be studied to identify the optimum amount needed to elicit the best flowering and fruiting response. For the VAM association, the response of calamansi trees to mycorrhiza in other soil and weather conditions should also be done to fully evaluate the potentials of mycorrhizal associations. Moreover, additional parameters should also be done to support claims on VAM effects. Such includes but are not limited to chemical and physical soil properties, root architecture, dry matter partitioning, and fruit quality. Variations on the age and quality of trees should also be considered.

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EFFECT OF HARVESTING CASSAVA LEAVES AT DIFFERENT PLANT AGE AND FREQUENCY ON CASSAVA LEAF PRODUCTION AND TUBER YIELD

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ABSTRACT

Cassava leaves are rich in protein and other essential nutrients, thus, it has the potential to be an alternative source of protein for human and animal consumption. This study investigated the effect of harvesting cassava leaves at 4, 6, 8, and 10 months after planting (MAP) on the leaf production and tuber yield. Leaf numbers 7-15 were harvested from Binulak, Lakan 1, and Sultan 6 (Year 1) while all the leaves except leaf numbers 1-6 were harvested from Rayong 72, KU 50, and Golden Yellow (Year 2). The results showed that harvesting of cassava leaves starting at 4 MAP had no significant effect on the fresh weight of the harvested leaves as well as on the tuber yield 33for all the six cassava varieties. Therefore, deleafing every two months did not reduce the production of cassava leaves and tubers. Sultan 6 and Rayong 72 had the highest tuber yield but Binulak and Golden Yellow obtained the highest total fresh weight of harvested leaves. Thus, it can be recommended that the harvest of cassava leaves can be done starting at 4 MAP and every two months thereafter without compromising the tuber yield.

Key words: *Manihot esculenta* Crantz, Binulak, Lakan, Sultan, Rayong

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is mainly grown for its roots while leaves are mostly considered as a waste. However, cassava leaves are rich in protein, vitamins, and minerals unlike the roots that are essentially carbohydrates. Minerals found in cassava leaves include potassium, calcium, magnesium, phosphorus, sodium, manganese, iron, zinc, and copper. Cassava leaves also contain beta-carotene (vitamin A), thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), and ascorbic acid (vitamin C) (Achidi et al. 2008; Latif and Mulle 2015). Nevertheless, consumption of cassava leaves as a source of essential nutrients is not much explored in the Philippines because of its high level of cyanogens and its antinutritive properties.

This study was conducted to establish best agronomic practices for production and harvesting of cassava leaves that will not compromise the tuber yield. With proper processing techniques, the cyanide content of cassava leaves can be significantly reduced that can be used for food and feed industry. This would increase the income of cassava farmers and would potentially reduce the production cost of feed industry.

MATERIALS AND METHODS

Six local cassava varieties (ie. Binulak, Lakan 1, Sultan 6, Rayong, KU 50, and Golden Yellow) were selected for field experiment based on cyanide level, availability, and the variety planted by Filipino farmers as a result of the survey conducted in five regions in the Philippines. The frequency of harvest of cassava leaves and maturity of leaves harvested were studied for optimum leaf yield. The effect of leaf harvesting on cassava tuber yield was also investigated. Fifteen (15) healthy plants per plot from each variety were randomly selected for data gathering. Harvesting of leaves at 4, 6, 8, and 10 months after planting (MAP) was done and the tubers were harvested at 10 MAP. The leaves were numbered from the first fully opened one as Leaf 1 and counting sequentially down the stem. Leaf numbers 7-15 were harvested from Binulak, Lakan 1, and Sultan 6 (Year 1) while all the leaves except Leaf numbers 1-6 were harvested from Rayong 72, KU 50, and Golden Yellow (Year 2).

The experiment was composed of four treatments arranged in Split Plot in Randomized Complete Block Design with three replications. Each treatment consisted of 24 plants for each variety with a planting distance of 0.75 m x 0.75 m. The distance between varieties and replications was 1.5 m. Basal application of complete fertilizer (14-14-14) at a rate of 300 kg/ha was done and side dress using urea (46-0-0) at 100 kg/ha followed two months after planting.

RESULTS AND DISCUSSION

The harvest of leaves in Year 1 show that the mean fresh weight of harvested leaves of Binulak and Sultan 6 was significantly higher compared to Lakan 1. For Binulak and Lakan 1, the total weight of harvested leaves starting at 4 MAP was significantly higher than harvests at 6, 8, and 10 MAP. However, for Sultan 6, there was no significant difference in the total weight of harvested leaves among the four treatments. High total yield of leaves was obtained even at 8 and 10 MAP. The differences in the obtained yield may be attributed greatly to varietal differences. It was observed that the mature leaves of Binulak and Lakan 1 started to shed at 8 MAP unlike in Sultan 6 wherein more leaves were produced. Binulak and Lakan 1 have similar structure wherein they only have one major stalk. In contrast, Sultan 6 produces more stalks as it grows making the plant grow wider, laterally, thus producing more leaves.

As for Year 2, the total weight of harvested leaves from Rayong 72, KU 50, and Golden Yellow at 4, 6, and 8 MAP had no significant difference. It was also observed that leaf yield was inversely related to the tuber yield. Golden Yellow obtained the highest leaf yield but had the lowest tuber yield. On the other hand, Rayong 72 had the lowest leaf yield but obtained the highest tuber yield. The results show that for Rayong 72 and Golden Yellow, the harvest of leaves starting at 4 MAP did not significantly reduce the weight of harvested leaves at the succeeding months. But for KU 50, the deleafing frequency had a significant effect on the harvest of leaves. The highest weight of leaves was obtained when the harvest was done starting at 8 MAP.

The harvest of leaves at different plant age and frequency had no significant effect on the tuber yield. Sultan 6 had the highest tuber yield followed by Lakan 1 then Binulak. Similar results were obtained for Year 2 in terms of tuber yield. The harvest of leaves at different plant age and frequency had no significant effect on the tuber yield. Rayong 72 had the highest tuber yield followed by KU 50, then Golden Yellow.

CONCLUSION

Cassava growers may harvest the leaves of Binulak, Lakan 1, Sultan 6, Rayong 72, and Golden Yellow as early as 4 MAP without compromising much the leaf production and tuber yield in the succeeding months. For KU 50, the optimum plant age for deleafing is at 8 MAP. Thus, if cassava leaves would provide additional income for the farmers, it can be recommended that deleafing can be done between 4 to 8 MAP. This would give them the optimum yield of both leaves and tubers. Golden Yellow may have lower tuber yield but it produces significantly higher weight of leaves.

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SWEET ABULUG POMELO (SAP): THE LUCKY FRUIT OF CAGAYAN VALLEY, PHILIPPINES

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ABSTRACT

Sweet Abulug Pomelo brought good luck to farmers amidst pandemic. Mother trees are maintained in Foundation Grove of Department of Agriculture - Northern Cagayan Experiment Station, Maquire, Lucban, Abulug, Cagayan and was certified by Bureau of Plant Industry last 2008 as Siamese Abulug Pomelo as NSIC 2008 Pm 04 now popularly known as the Sweet Abulug Pomelo (SAP). After 3-5 years from planting, it can bear as many as100-300 fruits per tree. The sweetness reaches from 10.9 to 12.5 percent Brix. However, SAP is susceptible to Citrus Rind Borer (CRB) causing ugly appearance although the taste remains sweet. A verification trial on the different control of Citrus Rind Borer (CRB) was conducted and resulted to 90% CRB free damaged coupled with recommended cultural management. After 13 years, SAP Field Day was conducted last August 10, 2021 to showcase and promote SAP and attended by 114 participants and ten (10) partner Government agencies. Hence, the promotion of SAP through field day resulted in generating a net income of Php.1, 017,270.00 / ha / year.

INTRODUCTION

Obtaining sustainable food system in this time of new normal is a challenge to everyone. But in Cagayan Valley, amidst this threat of pandemic, we see an opportunity. The Sweet Siamese Pomelo now popularly known as Sweet Abulug Pomelo (SAP) was registered as NSIC 2008 Pm 04 by Bureau of Plant Industry (BPI) under the administration of Celerina T. Miranda last 2008. Although this high value commodity is already existing long before this pandemic and it seems low promotion with limited market penetration, but this time, it is making its own identity. The demand for its fruit is very high specifically this is a very good source of Vitamin C which everyone needs in boosting immune system to fight Covid19. Likewise, its juiciness and sweetness is comparable to Davao pomelo in spite of its unattractive appearance because it remains green even when ripe.

MATERIALS AND METHOD

Verification on Insect Pest Management was conducted last January 2021 in a 7 years old Fruit Bearing SAP at the NCES Foundation Grove following the Package of Technology for SAP except for the treatments such as:

1) Fruit Bagging – Plastic net with 15 inches' x 45 inches' bag size was used for fruit bagging
2) Chemical Spraying - Parker Neem insecticide was used against Citrus Rind Borer (CRB) and other pest attacking pomelo.
3) Frequency of Spraying: From Flushing to Ball size: 2times a month (every 14 days interval)
4) Insect Trapping –super net insect attractant sticker was sprayed to an empty plastic bottle and hanged near the tree using bamboo pole was installed for insect trapping
5) Biological Control Agent – The three Entomopathogenic Fungi (EPF), *Metarhizium spp.*, *Isaria*, *Beauveriabassania*, were applied. Each specie was applied to 10 sample trees following the recommended application.

Verification trial on 1-year-old SAP intercropped with various Cash crops with an area of 2,500 sq.m , 39 trees was conducted following the Package of Technology for SAP and cashcrops. Crop used were Corn (IES Glut 4), Upland Rice (Calatrava), Peanut (3 seeded), Sweet Potato (Swerte), Cassava (Golden Yellow), Bush Sitao (Los Banos white) and Pineapple.

Hosting the Field Day with 150 participants

-Farm tour was conducted in the Foundation Grove where the bearing and non-bearing pomelo were maintained. Likewise, the result of the trials on the different technologies was showcased. -The growth and performance of SAP model tree was showcased. -Testimonies from successful SAP growers inspired the participants to venture in SAP production. Packaging and Labelling of the SAP was developed to encourage potential buyers to purchase the product. Awarding and distribution of 6,000 seedlings to FCA was done during the field day

- Online advertising like social media platform (Facebook, Instagram), Direct Marketing, Product giveaways and samples, and Branded promotional gifts.

RESULTS AND DISCUSSION

Result shows that the net income of different Pest Management Practices in controlling the insect pest of SAP especially Citrus Rind Borer (CRB) the gross income derived from the 5 to 7 year-old SAP was Php. 1,248,000.00. Chemical Spraying practice gave the highest net income with 909,570.00 followed by insect trapping with Php. 899,170.00 . The treatments are not different among each other in terms of ROI. Therefore, any of the treatments can be recommended to SAP growers. Cash crops are a viable source of additional income to pomelo growers. Intercropping is a practice to maximize utilization of land use in SAP plantation. Result shows that the top three cash crops used with the highest net income were the Pineapple, Sweet Potato and bush sitao with Php.155,543.00, Php. 81,779.00, and Php. 80,250.00, respectively. The Field day conducted brought a significant impact to the pomelo growers. Farmer's option on Pest Management Practices for SAP was established aside from Chemical Pest Control. The different Pest Management Practices employed against Citrus Rind Borer improved the fruit quality of SAP up to 90% CRB damage free. Various crops were identified that can be grown in between non-bearing pomelo. There was an increase in demand of SAP fruit as source of Vitamin C during the pandemic, as well as planting materials. 6,000 SAP seedlings to Farmer Cooperative/Associations and 1,000 to Municipal Wide Backyard Tree planting in Abulug, Cagayan were distributed to recognize Abulug as the Home of SAP.
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CONCLUSION

Opportunity comes when least expected and even at the midst of hard times. It was in this pandemic, the health crisis brought by COVID-19 that each one struggle to boost one's immune system to combat the virus. The closure of many borders limits the entry of fruits and vegetables as part of essential hence, the supply decreased and the demand is high resulting to increase in price. Now, the hidden treasure of Abulug sparks and shines due to the call of the moment. The DA initiatives with other partner agencies made SAP a lucky fruit of Cagayan Valley, Philippines. The field day conducted at DA-NCES and NVES was carried out successfully in promoting SAP and now adopted as one of the priority crops to consider. This high value crop is now an integrated part as tourist attraction of Cagayan Valley. Government support system played significantly in the promotion of SAP that enable farmers to earn a million of pesos from the sale of fruits. SAP farming is a productive endeavour that keeps farmers alive in the new normal as indicated by the demand of seedlings as well as fruits. Growing fruit trees is environment friendly and minimizes soil erosion.

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CORN COB AND CORN HUSK BIOCHARS ENHANCE SOIL PROPERTIES AND GROWTH OF CORN (*ZEA MAYS* L.) IN FERTILIZED CLAY LOAM SOIL

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ABSTRACT

Biochar, produced from biomass pyrolysis under a limited oxygen environment, is a carbon (C)-rich material that improves soil quality and increases crop yield. An experiment laid out in split-plot in a completely randomized design (CRD) was conducted to investigate the growth responses of corn to corn cob biochar (CCB) (15 t/ha), and corn husk biochar (CHB) (15 t/ha) in Lipa clay loam (*Typic Eutrudepts*) applied with organic fertilizer (OF) (10 t/ha) and inorganic fertilizer [recommended rate (RR):120 N;60 P_2O_5-60; K_2O per ha]. Results show that CCB and CHB are rich in essential macro and micro-essential elements. Biochars have high average surface area, high pore radius, and pore volume. The field emission-transmission electron microscopy (FE-TEM) and X-ray energy dispersive spectroscopy (EDS) show a very high concentration of C (88-90%) and other elements (Cu, Na, K, Cl, S, Si, P, Ca, O). CCB and CHB applied with organic and inorganic fertilizers improved clay loam soil, such as soil pH, CEC, EC, and nutrient status in the soil, which is evident in the enhanced growth of the plants, such as increased leaf chlorophyll concentration, corn ear biomass, root biomass, and plant height. The CCB and CHB with organic and inorganic fertilizer applications are recommended for clay loam soil to improve soil health and plant growth.

Key words: corn cob biochar, corn husk biochar, biochar properties, fertilizer, clay loam

INTRODUCTION

One of the challenges nowadays is to feed the growing population while maintaining the integrity and sustainability of our natural resources, such as our soil systems. Biochar improves soil health and enhances plant growth (Lehmann et al. 2011). Biochar is a carbon (C)-rich material produced by biomass pyrolysis under limited oxygen (Lehmann et al. 2011; Villegas-Pangga 2021). It comprises recalcitrant C structures that prevent decomposition and help sequester C in soils, improving soil properties and agronomic properties (Lehmann et al. 2011). It has a large, charged surface area of great potential to adsorb heavy metals and organic contaminants. Its application decreases the bioavailability, toxicity, and mobility of organic and inorganic pollutants. It becomes beneficial to immobilize contaminants with high concentrations, allowing cultivated soils to have improved quality and enhanced crop yields. More studies have stated the benefits of biochar; however, there is limited data about the effects of biochar physico-chemical properties, specifically in clay loam soil and the crops planted in it. This study was conducted to determine the effect of corn cob biochar and corn husk biochar application on the growth of corn grown in clay loam soil with organic and inorganic fertilizers. Specifically, this study hypothesizes that the addition of CCB and CHB with organic and inorganic fertilizers will enhance corn growth.

MATERIALS AND METHODS

A Typic Eutrudepts clay loam soil of volcanic origin was air-dried, cleaned, and sieved (2mm), and potted. The pot with 30cm diameter x 30cm height x 20cm base was perforated to drain excess moisture and filled with 12kg soil. Corn cob and corn husk were air-dried (7 days) and chopped into small pieces (3-5 cm) before heat treatment. The air-dried corn cob and corn husk were pyrolyzed in the biochar-producing cookstove for 60 min and 30 min heating residence time, respectively, at 300-650°C (Villegas-Pangga, 2021).

The chemical analyses of soil, CCB, CHB, OF, and plant tissues were conducted at the Analytical Service Laboratory, UPLB, Laguna, Philippines. The Brunauer–Emmett–Teller (BET) analysis for CCB and CHB were conducted to determine the physical adsorption of gas molecules on their solid surfaces, which serves as the basis for a critical analysis technique for measuring the average surface area, pore size, and pore volume of corn cob and corn husk biochars using the Quanta Chrome Nova 22200BET automated N multilayer physisorption system at the Nanotechnology Laboratory, University of the Philippines Los Baños. The Transmission Electron Microscope (TEM)-TEM Imaging & Energy Dispersive X-ray Spectroscopy (EDS) were performed to determine the composition of C, and other abundant essential elements located in discrete spots of the CCB and CHB surface at

the Industrial Technology Development Institute-Department Science and Technology. The data gathered in this study that was laid out in split-plot in a completely randomized design were analyzed using a two-way analysis of variance and least significant difference through the Statistical Tool for Agricultural Research (STAR) 2.0.1 software developed by the International Rice Research Institute to determine the differences between treatment means at a 5% level of significance.

RESULTS AND DISCUSSION

The CCB and CHB generally have high pH, similar to the pH ranges reported for biochar made from rice straw, water hyacinth, mahogany flower, sugarcane bagasse, sugarbeet, cauliflower leaf, and orange peel wastes (Villegas-Pangga, 2021). It consists of high OC while containing high essential macro-elements (N, P, K Ca, Mg) and micro-elements (Fe, Zn, Cu, Mn). Ash content in biochars ranged from 0.35 to 59.05 %, rich in available nutrients, especially K, Ca, Mg, and Na. High organic C in fresh biochars and its well-developed pore structure may enhance water retention and provide a shelter for soil microorganisms. Both biochars had high porosity that could retain water, consequently increasing water holding capacity and helping infiltrate excess water through larger pores which provide essential sites for sorption and reaction (Xiao et al. 2018) thus increasing soil fertility and yield (Ding et al. 2016). The CCB and CHB samples contained essential elements (C, Cu, Na, K, Cl, S, S, Si, P, Ca, O). C is 88-90 (wt%). The ash content of biochars is rich in K+, Ca2+, Mg2+, and Na+ (Rajkovich et al. 2012).

Generally, soil parameters such as pH, OC, available P, exchange K, and EC, CEC are improved by biochars mixed with organic and inorganic fertilizer applications in Lipa clay soil. Biochar application could increase pH, which is an essential factor affecting nutrient availability (Wang et al. 2014). Biochar additions could improve CEC by 4-30%. The improvement of soil properties is highly related to high surface area, the magnitude of functional groups, and the liming effect (Ding et al. 2016). Both biochars with fertilizers added significantly improved chlorophyll concentration, upper and lower plant biomass, and root-shoot ratio. In the present study, a 54% increase was recorded at a 15 t/ha application rate. The properties of biochar in this study showed positive responses from the plants.

CONCLUSION

CCB and CHB applied with organic and inorganic fertilizers improved fertility of clay loam soil, such as soil pH, CEC, EC, and nutrient status in the soil, which enhanced growth of the plants, such as increased leaf chlorophyll concentration, corn ear biomass, root biomass, and plant height. The CCB and CHB with organic and inorganic fertilizer applications are better recommended for clay loam soil as these improved both soil health and plant growth.

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EVALUATION ON THE EFFECTIVENESS OF PHEROMONE TRAPPING AGAINST TWO LEPIDOPTEROUS PESTS OF ONION IN STO. DOMINGO, NUEVA ECIJA

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ABSTRACT

The effectiveness of pheromone trap as part of monitoring of pests in onion was examined by dispensing synthetic female sex pheromone (lure) in three locations at Barangay. Dolores, Sto. Domingo, Nueva Ecija by using one lure per used 6 liters plastic water container with 4 cm water and 1 teaspoon detergent. The set up was replicated four times. Two common pests of onion namely; onion armyworm, *Spodoptera exigua* (Hubner) and cutworm, *Spodoptera litura* (Fabricius) Boursin were detected from Day 1 to Day 45. Consistent with the result, the installed pheromone traps caught significant numbers of onion army worm and cutworm at 1 % level of significance ($p<0.01$) compared to the traps without pheromone. These results show that the pheromone treatment provides effective monitoring and possible reduction of population thru mass trapping of these two pests in onion. The trap data can also serve as a basis for Pest Alert SMS messages to stakeholders particularly during high catches for intensified field monitoring of eggs and larvae in onion fields and other crops in the area. including the timely application of integrated pest management measures.

Keywords: onion, pheromone trap, *Spodoptera litura*, *Spodoptera exigua*, pest monitoring

INTRODUCTION

Onion is extensively cultivated in the Philippines and grown in 22 provinces. There is an increasing trend in onion production as well as in farm gate price with an annual average rate of 2% to 7% from 2015 to 2019. Profitability is very attractive with return of investment of 147% in onion multiplier and 197% for bulb onion (PSA 2019). The top 3 major productions of onion in the country in 2019 came from Region III (138,795.26 MT), Region I with 38,827.62 MT) and Region IV-B with 35, 055.30 MT. Onion production is highly dependent on inorganic fertilizers and insecticides. Fertilizer usage is 26 to 30 bags per hectare while pesticide spraying is up to 16 times throughout the growing period. On the other hand, the damage caused by lepidopterous pests in Nueva Ecija was estimated at 20% reduction of the total yield (Cayabyab 1999). As of May 2017, 13 towns in Nueva Ecija have reported the presence of this pest in 1,076 hectares of onion fields (DA-RCPC III 2017) while in Pangasinan four municipalities were affected with a total of 409.4 ha infested (DA-RCPC I 2017). This pest has a short life cycle and may be resistant to insecticides due to non-stop and indiscriminate use. Until now these two pests are still infesting onions in Nueva Ecija and Pangasinan. Field monitoring is necessary for the rapid detection of the presence of onion army worm (OAW) including cutworm and for timely interventions to effectively protect onion while minimizing harm to the environment. Monitoring involves the active tracking of the presence, population assessment, and movement of a pest (FAO and CABI 2019). Pheromones are used in monitoring and belong to a class of semiochemicals that insects and other animals release to communicate with other individuals of the same species (Karlson and Luscher 1959). These signals can be effective in attracting faraway mates and can also be very persistent for days. This study sought to evaluate the effectiveness of selected pheromone traps against two lepidopterous pests in onion crop.

MATERIALS AND METHODS

Pheromone traps were set-up in three different sites of Brgy. Dolores, Sto. Domingo, Nueva Ecija with four replicates. The onion army worm pheromone lure contains Z9, E12-14:AC (90%) and Z9-14:OH (10%) active ingredients while the cutworm pheromone lure has (Z,E)9,11- Tetradecadien-1-yl acetate. The pheromones on screen wire mesh were attached to a wire and placed at the center of a 6-liter plastic water container with 4 cm water and 1 teaspoon detergent. It was stabilized by using two-bamboo sticks. Adult male moths trap catches were monitored every day for 45 days from June 28, 2019- August 11, 2019. Each trap was checked and captured moths were stored in 70% ethanol. The mean count was analysed for variance (ANOVA) among the three treatments using the Statistical Tool for Agricultural Research (STAR) software in randomized complete block design (RCBD). The repeated measures ANOVA was determined using the Minitab 17 Software.

RESULTS AND DISCUSSION

Higher density of the cutworm was observed than the onion army worm while no male moths were collected in the treatment without pheromone for all trial in three sites. Over-all, the population of *Spodoptera litura* was recorded with the highest density among the three treatments in the three different sites. At different observation intervals there were significant effects on pheromone trapping to each of lepidopterous onion insect pest but still, variation on mean counts from the different treatments per site (e.g *Spodoptera litura vs*. *Spodoptera exigua* vs. control) were observed. The mean count of *Spodoptera litura* caught in pheromone trap is significantly different from the control and mean count of *Spodoptera exigua* during trapping in all sites. Trapping resulted in detection of *Spodoptera litura* and *Spodoptera exigua.* This monitoring function is the keystone of integrated pest management. In this study, the mean count of *Spodoptera litura* was higher compared to mean count of *Spodoptera exigua* due to off- season of onion crop during the monitoring. However, alternate crops of both pests such as corn and vegetables were planted in the surrounding areas of the sites. Aside from monitoring as the most important application of pheromones, other uses include: mass trapping of insects to remove large numbers of insects from the breeding and feeding population and the disruption of mating in populations of insects. Massive reductions in the population density of pest insects ultimately help to protect the crop since there will be higher number of female cutworms and onion army worms that will not be mated. The normal egg production of adult female *Spodoptera exigua* ranges from 300-500 eggs (Capinera 1999). On the other hand, adult female *Spodoptera litura*'s fecundity varies from 2000 to 2600 eggs, and oviposition days vary from 6 to 8 days (Rao et al., 1989). The total caught adult male *Spodoptera litura* from three sites was 9,752 while the total caught adult male *Spodoptera exigua* was 541. This means that using the pheromone trap, 9752 adult female and potential $1.9x10^7$- $2.4x10^7$ F1 population of *Spodoptera litura* were reduced from the three sites while 541 adult female plus has a potential of 162,300-270,500 F1 population of *Spodoptera exigua* were reduced from three sites. The result of the study is similar with the report of Takai and Wakamura (1995) for the control of beet armyworm, *Spodoptera exigua* (Hübner) in both open field and green house. The rate of mating inhibition in the field and greenhouse were 97% and 20-50% respectively.

CONCLUSION

The effectiveness of pheromone trapping was evaluated as crucial role for integrated pest management in onion crop. Higher density of cutworm was observed from three sites compared to onion army worm. Pheromone traps can be used to monitor and at same time mass trap the cutworm and onion army worm male moths even before planting onions. Both lepidopterous pest also infest other crops such as some vegetables and corn. The trap data can also serve as a basis for Pest Alert SMS messages to stakeholders particularly during high catches in the traps for intensified field monitoring of eggs and larvae in onion fields and other crops in the area.

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GROWTH, YIELD AND ANTIOXIDANT PROPERTY OF LETTUCE (*Lactuca sativa* L.) APPLIED WITH ORGANIC AND CHEMICAL FERTILIZER

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ABSTRACT

A pot experiment was conducted to determine the effect of organic and chemical fertilizers on lettuce in growth, yield, antioxidant property as well as the occurrence of insect pests and diseases. Randomized Complete Block Design was used in the study with three replications. Seeds were directly sown on the pots with fertilizer and control treatments. Plants grown under organic fertilizer had significantly higher germination percentage compared to plants grown under control and chemical fertilizers. Organic fertilizer produced significantly longer and wider leaves during the first 4 weeks. However, chemically fertilized plants surpassed organically grown plants on the fifth week until harvest but the difference was only significant on the sixth week. Further, plants treated with chemicals had significantly heavier weight resulting to significant higher yield compared to those grown under organic fertilizer and control treatments. In terms of antioxidant property of lettuce particularly vitamin C, plants treated with inorganic fertilizer had significantly higher antioxidant content compared to plants grown organically. Pest and disease damage were not significantly affected by the kinds of fertilizer. Incidence of diseases and pest infestation were recorded low in all treatments.

Key words: lettuce, antioxidant, organic, chemical fertilizer

INTRODUCTION

Lettuce production in the Philippines was increasing at an average annual rate of 2.2 percent and 0.3 percent from 2011 to 2015 (Philippine Statistics Authority, 2017). This attests the need to produce more lettuce because of its increasing demand. The knowledge on the higher advantages of lettuce drives the need to study the different management of this crop. This study aimed to determine the effects of two kinds of fertilizer on the growth, yield and antioxidant property of lettuce grown in container. Specifically, it aimed to 1) Determine and compare the growth performance of lettuce applied with organic and chemical fertilizer; 2) Determine and compare the yield performance of lettuce applied with organic and chemical fertilizer; 3) Evaluate and compare the total antioxidant content of lettuce as affected by different fertilizers; 4) Evaluate pest and disease damage; and 5) Determine the economic benefit of growing lettuce in container. The study was limited on identifying the growth parameters of lettuce in terms of germination percentage, length, and width of leaves; and the yield components which are the total weight, and yield per hectare. The antioxidant property of lettuce was also covered by the study. However, the determination of antioxidant was limited to the total activity of vitamin C or ascorbic acid through quantitative analysis using phosphomolybdenum assay.

MATERIALS AND METHODS

Red Rapid variety of lettuce was used in the study with three (3) treatments: vermicompost ($T_{1)}$, complete fertilizer (T_2) and control (T_3) replicated thrice. Recommended rate of fertilizer was applied. Fifty (50) grams of vermicompost was applied as basal fertilizer and 6.4 grams of complete fertilizer (14-14-14) was applied per pot. The seeds were directly sown in pots filled with ordinary garden soil. All data were statistically analyzed using the Analysis of Variance (ANOVA) for Randomized Complete Block Design (RCBD). The data with significant and highly significant results were subjected to Least Significance Difference (LSD) Test in Statistical Tool in Agricultural Research (STAR) Version: 2.0.1 to determine the significant differences among treatment means.

RESULTS AND DISCUSSION

Plants grown under organic fertilizer had significantly higher germination percentage compared to control and chemical fertilizer treatments. The lowest germination percentage was obtained from chemically treated plants. This may be attributed to soil temperature and moisture. It was observed that the granular chemical fertilizers were not fully decomposed two weeks after application thereby causing high temperature. It was also observed that pots treated chemically were prone to soil clogging. On the other hand, pots treated with vermicompost were moist and well-drained. Organic fertilizer had produced significantly longer and wider leaves during the first 4 weeks. This result is consistent with earlier studies which revealed that lettuce grown in soil amended with inorganic fertilizers

had shorter leaves while the treatments with organic fertilizers increased the length and width of the largest leaves although the differences were not significant (Liu et al. 2014). However, chemically fertilized plants surpassed organically grown plants on the fifth week until harvest but the difference was only significant on the sixth week. This result may be attributed to the availability and accessibility of nutrients necessary for plant growth and development. After 5 weeks of application, the granular chemical fertilizer was fully transformed into soluble form which made them available for plant absorption. On the other hand, the nutrients in vermicompost were utilized at the early stage of lettuce growth. Thus, plants under chemical treatment showed higher increase in leaf length and width. This result conformed with the findings of Hernandez et al (2007) that after 5 weeks, plant growth and development in inorganic exceeded organic which showed a small increase in growth and development during the following 3 weeks. Similarly at harvest, vermicompost had no effect on leaf area (Leon 2012). Plants treated with chemicals had significantly heavier weight resulting to significant higher yield compared to those grown under organic fertilizer and control treatments, which si consistent with the findings of Hernandez et al (2007).

Chemically treated plants significantly contained more antioxidants compared to plants grown organically. This observation agrees with the findings of de Oliveira Pereira et al (2016) that showed conventional lettuce samples had a higher ascorbic acid value than organic and certified organic samples. Organic lettuce showed higher effectiveness in antioxidant capacity and higher levels of phenolic compounds than lettuce produced in the conventional system (Silva et al. 2018). The difference between these results may be attributed to the use of different cultivars and environmental conditions where the experiments were conducted. The exposure of plants to both biotic and abiotic stress may contribute to the greater effects of antioxidant activity. Incidence of diseases and pest infestation were recorded low in all treatments. Pest and disease damage were not significantly affected by the kinds of fertilizer and may be due to the environmental conditions the lettuce crops were exposed to. The site of the field experiment and the pot culture lessened the entry of insect pests. The net shade also protected the crops from heavy rains and from direct heat of the sun which prevented diseases caused by too much sunlight and too much water. The application of either organic or chemical fertilizer is feasible. Organically grown lettuces elicited better return next to chemically treated plants. Negative returns were obtained from unfertilized plants. The profitability of lettuce production is supported by the study of Bureau of Plant Industry - National Crop Research and Development Center which reported that the net profit of lettuce production in a hectare is equivalent to PhP 244,820.00 with an ROI of 182.82%.

CONCLUSION

The two kinds of fertilizer have positive influence on the growth, yield and antioxidant property of lettuce grown in container. Organic fertilizer performed better during the seed germination and seedling development whereas chemical fertilizer worked better at the later stage of lettuce growth. Thus, higher yield values were obtained from plants under chemical treatments. Further, highest absorbance of vitamin C was reflected in chemically treated plants. Moreover, insect pest management and disease management are easier in container gardening. Lettuce production with the application of fertilizer, whether organic or chemical is profitable.

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MORPHOMETRIC VARIATION IN ISO-FEMALE REARED MANGO TEPHRITID FLIES, *BACTROCERA DORSALIS* HENDEL

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ABSTRACT

The *Bactrocera* tephritid fruit flies, aside from being known pests of mango, are well-known to exhibit crypsis. Identifying the pest species have been difficult due to the morphological variations exhibited by the species. The study aimed to identify the species of tephritid flies pre-emerged from incubated-infested mango fruits, and find out which morphological character varies among the offspring from iso-female rearing. All fruit flies that emerged from incubated mangoes collected from 2014 to early 2017 in selected areas were identified as *Bactrocera dorsalis*. Female fruit flies from mango-growing localities were reared and representative isofemale offsprings were randomly selected. Comparison of the morphological characters of isofemale-reared individuals were measured to determine degree of variability among offspring using lengths of the body, thorax, midtibia, and wing. Measurements and resulting indices of the wing following terminologies of McAlpine (1981) were determined. Differences were evaluated using CRD, and analyzed using ANOVA (SAS v.9.1). All measured characters and most wing indices were not significantly different except for "d" vein and "4v" index; the latter being an index, based on the "d" vein. The study proves that variation among the offspring of *Bactrocera dorsalis* vary on this character, M1 between dm-cu and r-m.

Key words: mango fly pest, *Bactrocera* spp, wing measurements, wing indices, offspring morphometry

INTRODUCTION

The mango fruit fly is one of the most important insect pests in mango. Fruit fly damage is one of the major causes of our mangoes being banned for export to other countries. Since the 1980's, technologies such as sterile insect technique and male annihilation technique have been used to ensure that the pest is controlled. Determining the fruit fly (Tephritidae) species infesting mangoes is needed in mitigating area-wide pest management strategies effective only as species-specific. New technologies such as the Release of insects carrying dominant lethal or RIDL necessitates that other possible pest species' occurrence is known. Mango fruit fly is one of Philippines' worst horticultural pests due to invasiveness and crypsis (morphologically indistinguishable species). Two species of fruit flies have been reported to infest Philippine mangoes; *Bactrocera dorsalis* and *Bactrocera occipitalis*. These two are mostly observed by the authors to be commonly caught in methyl eugenol traps. *B. occipitalis* has also been reported by Drew in numerous publications as found in mangoes. It is not sure however, whether this was a natural infestation, or mango fruit has been used as a host to rear the species. It has also been proven before that these two species are hard to delineate morphologically (Mahmood 2004a) and Delomen et al. (2013). Comparison of morphological characters of individuals coming from one gravid female or "mother" has never been done before, hence this research. Using the offspring from one female and comparisons amongst offspring of one female may resolve issues on mango fruit fly identification and provide accurate database wherein further investigations on pest profiling could take off. The use of stable characters was duly emphasized by Ernst Mayr, and it was hypothesized that the variable character amongst offspring, between sexes, and among areas would likely be variable and therefore unstable. Unstable characters cannot be used on coming up with keys for identification.

The objectives of the research were to identify the species of Tephritid fruit fly pre-emerged from incubated-infested mango fruits, and find out which morphological character varies among the offspring from iso-female rearing.

MATERIALS AND METHODS

Sites selected for collection had the highest production of mango in Nueva Ecija and had not been subjected to pesticide application. The sites were located in the municipalities of Cuyapo (15.7861111 ^{o}N and 120.65000 ^{o}E), San Jose (15.8037018 ^{o}N and 120.9991032 ^{o}E), and Talavera (15.6136111 ^{o}N and 120.9250001 ^{o}E). To initiate the culture, purportedly maggot-infested mangoes were collected and placed in emergence canisters. This was done from 2014 to early 2017. Once enough females were emerged, iso-female rearing commenced using one pretreated or disinfested mango fruit (treated by dipping in hot water for twenty minutes) per one gravid female fruit fly. Resulting offspring from the iso-female rearing were allowed to mature, and these were prepared for morphometric examination. A total of 2,128 fruit flies were successfully reared coming from 34 females. Of these, 290 offspring composed of five males and five females from 34 "mother" females were measured using the accompanying software for Optika microscope.

Lengths of the body, thorax, mid-tibia, and wing were taken as well as length of *a* (2nd costal section between subcostal break and R_{2+3}), *b* (3rd costal section between R_{2+3} and R_{4+5}), *c* (M_1 between dm-cu and wing margin), *d* (M_1 between rm-cu and dm-cu), *e* (CuA_1 between M_1 and wing margin), *f* (dm-cu between M_1 and CuA_1), and *i* (distance between distal end of R_{2+3} and M_1). Wing indices were computed based on the previous measurements: C = *a/b*, 4c = b/d, 4v = *c/d*, 5x = *e/f*, ac = *b/i* and M = *e/d*. Analysis of variance (ANOVA, SAS v.9.1) considered collection sites, mothers, and sex of offspring as factors achieving a multi-level modelling or nested design.

RESULTS AND DISCUSSION

There was only one species of fruit flies collected from the different collection areas after more than two years of continuously incubating infested mangoes and rearing emerging larvae and pupae to adults from 2014 to early 2017. Succeeding collections were done for verification. The species from the three collection sites in Nueva Ecija was *Bactrocera dorsalis.* In the collection data of researches by Drew (1972; 1989; and 2011) and others, *Bactrocera occipitalis* has been documented from mango. The results of this research proved that *B. occipitalis* was not present in the infested mangoes and had not been reared-out. Further studies on the possible co-existence of *B. dorsalis* and *B. occipitalis* can be done in other mango-growing áreas for purpose of área-wide management using newer technologies in pest management

Analysis of variance showed that lengths of body and thorax were significant in three áreas, while lengths for midtibia and wings did not vary significantly. Among the letter measurements only *d* varied significantly. In the índices, although 4c and 4v were based on *d*, only 4v was significantly different based on the sites. In comparing the males and females, body and thorax lengths were significantly different. Taxonomic keys do not rely on lengths of body or thorax in determining identity which was supported by the findings in this research. Wing vein *d* was found significant based on sites and sex. This vein *d* had been crucial in the morphometric studies conducted by Adsavakulchai et al. (1999), and Delomen et al. (2013). Of the characters used, the most likely to be variable and therefore prove unstable in coming-up with keys even amongst offspring of one female is *d*.

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PROFITABILITY EVALUATION OF RICE-DUCK-KUHOL (RIDUKU) FARMING SYSTEM IN KANANGA, LEYTE

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ABSTRACT

Rice-Duck-Kuhol (RIDUKU) is an organic farming system that cultivates rice, ducks, and kuhol together. In 2005, the practice was introduced in Kananga, Leyte to substitute the use of agrochemicals in rice farming. However, since its introduction, only one farmer was adopting the farming system. A profitability case study of RIDUKU was conducted to determine its costs and returns, and the results were compared to 30 local farmers who adopted conventional farming. The study also identified problems and constraints, and provide solutions for the adoption of RIDUKU. Results of the study showed that gross revenue in RIDUKU was 40% higher than the conventional farming system, equal to Php149,131.00/ha, and Php60,808.80/ha, respectively; 11% lower in the cost of production equal to Php101,820.57/ha, and Php113,839.22 respectively; and a positive net income of Php47,310.43 against a negative net income of Php (-53,030.32) in the conventional farming. While the adoption of RIDUKU was high (73.3%) among the conventional farmers, it showed a Benefit-Cost ratio of 1.32, and a return on investment equal to 41.18%. The expected net present value was equal to Php127,045.00 at a 12% discount rate. RIDUKU can be a good investment, and an alternative to a more environmentally sustainable rice produce.

Key words*:* RIDUKU, organic farming system, rice, duck, kuhol

INTRODUCTION

Organic agriculture is one of the most dynamic and rapidly-growing sectors of the global food industry of which organic farming is one of its several approaches to sustainable agriculture (FAO 1999) because of its commercial viability, and may provide solutions to the current problems in conventional agriculture (Scialabba 2000). In recent years, several novel species-diversified farming systems such as rice-fish, rice-duck have been documented to be highly effective in controlling crop diseases, insect pests, and weeds in paddy fields with less pesticide and herbicide application. Rice-Duck-Kuhol farming system, also known as RIDUKU is an innovative farming system in rice that integrates duck raising to address problems on kuhol, but uniquely utilizes kuhol (Golden Apple Snail) however, little is known about this new farming system. This study was undertaken to provide insights to farmers on the profitability of the RIDUKU farming system using Net Present Value (NPV) approach, Benefit-Cost Ratio, and Return of Investment (ROI), and identify the problems and constraints associated with the adoption of RIDUKU by rice farmers engaged in conventional farming.

MATERIALS AND METHODS

This study was carried out as a case study in March 2019 at Brgy. Montebello, Kananga, Leyte, to determine the profitability of RIDUKU. Since its introduction in 2005, only one farmer was adopting the farming system. He introduced RIDUKU in the area in order to substitute the use of chemical fertilizers and agrochemicals in rice farming. Thirty (30) local farmers engaged in the conventional farming system in rice were also randomly identified from the list provided by the Barangay Captain of Brgy. Montebello. These farmers were utilizing agro-chemicals such as fertilizers and pesticides nevertheless, they were possible adopters of the innovative farming system. A one-on-one interview with the RIDUKU farmer was conducted to know its farm management practices, costs and returns, and profitability, and the results were compared to the 30 local farmers who adopted the conventional farming. A structured questionnaire was use to interview the 30 local farmers. Statistical Package for the Social Sciences (SPSS) version 23 was used in analyzing the collected data, and were presented in tables, analyzed and interpreted using descriptive statistics.

RESULTS AND DISCUSSION

RIDUKU farming system included activities like herding of ducks in the paddy rice field for 90 days during land preparation until the growth stage of the rice crop, and breeding of kuhol. Conventional farming system utilizes agro-chemicals like fertilizers and pesticides, often in heavy doses. It relies on its income from harvested and threshed palay. A comparative computation of income of the two farming systems was based on the total yield harvested per production system, on a one-hectare paddy rice field, and multiplying the total harvest on a per sack basis. The prevailing price in the area was P720.00 per sack, average of 48 kilograms of threshed palay. Gross

revenue under the RIDUKU farming system was equal to Php 149,131.00 per hectare. The higher income in the RIDUKO farming system was due to the integration of ducks into its farming system and contributed an additional income from the sales of eggs amounting to Php 48,600.00. It also incurred a non-cash revenue equivalent to Php 15,283.00 since it utilized ducks in the land preparation and in the control of weeds and kuhol rather than hiring laborers. RIDUKU cash requirement was Php 71.880.42, for duck feeds, for a total of 130 days for two cropping seasons while confined for 65 days. It did not incur a non-cash costs since the practitioner payed for hired laborers.

Conventional farming system on the contrary, only relied on its income from harvested paddy rice. Conventional farming system which had a gross revenue of Php 60,808.80 per hectare. It had only Php 42,508.47 cash requirements for the purchase of seeds, fertilizers, chemicals, payment of hired labor, and irrigation fee. It incurred four (4) types of non-cash costs; thresher share, prendis, landowner share, and harvester or tenant share.

The total production cost for the RIDUKU and conventional farming systems were Php 101,820.57 and Php 113,839.22, respectively. Net income accounted to RIDUKU was equivalent to Php 47,310.43 and a net loss of (-Php 53,030.42) was accounted to the conventional farming system. The expected net present value of RIDUKU was Php 127.045.00. Benefit-Cost ratio was 1.32 which meant that for every one peso invested in RIDUKU, we could expect a benefit of one peso and thirty-two centavos. ROI was equivalent to 41.18%. Conventional farming system did not calculate for NPV since it did not incur any fixed cost that were expecting returns from its used.

Twenty-two or equivalent to 73.3% were not willing to adopt the RIDUKU farming system in their paddy rice fields. According to them, it is expensive and time consuming, more volume of fertilizer was required and inconvenient, and effect is slow.

CONCLUSIONS

Rice-Duck-Kuhol farming system is an alternative method in rice production. It practices an organic way of producing rice by integrating ducks in the production system that can contribute to an additional income, other than rice. Ducks are beneficial not only to the rice crop, the paddy rice soil, but their eggs and meat are good source of additional income. RIDUKU can be a considered as a good investment and an alternative to agro-chemical production of rice that is more environmentally sustainable. On the contrary, the conventional farming system utilizes agro-chemicals like fertilizers and pesticides, often in heavy doses. This type of farming system in rice production relied on its income from harvested and threshed palay.

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VETIVER (*Vetiveria zizanioides)* AS PHYTOREMEDIATOR ON CHROMIUM AND NICKEL GROWN IN LOWLAND RICE SOILS AFFECTED BY MINING ACTIVITIES

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ABSTRACT

The use of vetiver (*Vetiveria zizanioides)* is one of a few plant species meeting all the criteria required for phytoremediation. However, very limited studies have been done to use this plant as phytoremediator in lowland rice soils. The study was conducted to evaluate the Cr and Ni accumulation and translocation factor and to determine the effect of phytoremediation on the soil properties using different time duration of vetiver grown for 3 and 8 months in the field. The field experimental set-up was conducted for two cropping seasons. Results revealed that vetiver had a Bio-accumulation factor of 0.97 for Cr and 1.07 for Ni after 3 months of growth while 1.48 for Cr and 1.38 for Ni after 8 months planted in a contaminated field indicating higher concentration of Cr and Ni in the plant than in the soil. The translocation factors at both 3 and 8 months were below 0.2 mg kg $^{-1}$ suggesting that vetiver accumulates Cr and Ni in the roots and very small amount are translocated to the shoots, making the plant safe for forage purposes. Furthermore, the longer the vetiver grown in the field the lesser the amount of Total and Available Cr and Ni were detected in the field thus soil pH and organic matter content were improved.

Key words: bio-accumulation, translocation, phytoremediation, soil pollutants

INTRODUCTION

Soils polluted with heavy metals have become common across the globe due to geologic and increase in anthropogenic activities like mining. In Philippines, particularly in Santa Cruz, Zambales mining industry has recently been booming due to its abundant deposits of chromite and nickel laterite minerals. Overburden and acid drainage from chromium (Cr) and nickel (Ni mining) industry are transforming productive agricultural lands to unproductive wasteland in some villages of the area. The Environmental Justice Atlas, 2015) reported that agricultural production in the municipality has been affected since mining operations started in 2006. Farmers tried to remedy the contaminated fields by applying more fertilizer, which jacked up their production costs but hardly made a difference.

Based on the identified problem of the area, there is an urgent need to determine cost effective techniques in mitigating Cr and Ni in a lowland rice field. Soil rehabilitation using vetiver as phytoremediator is a practical and low-cost measure that can be used to inhibit the availability of Cr and Ni in the soil and mitigate the metal uptake by the crops due to its high biomass and deep rooted which are more efficient in absorbing certain heavy metals and chemicals. The accumulation of Cr and Ni, particularly in an agricultural soil brings disorder of soil function which, in turn, affects crop growth. These metals can be transferred to crops therefore posing a risk to human health. The study was conducted based on the hypothesis that the removal of these metals will improve soil health. The results of this study can serve as a basis for mitigating heavy metal pollution in the affected area. Thus, it was conducted to evaluate the Cr and Ni accumulation and translocation factor and to determine the effect of phytoremediation on the soil properties using different time duration of vetiver grown for 3 and 8 months in the field.

MATERIALS AND METHODS

Ocular survey was conducted in the vicinity of a mining area in Santa Cruz, Zambales. The basic criterion for the selection of the study site was the presence of an irrigated rice field. The field experiment was conducted from June 2017 to February 2018 in barangay Lomboy, Santa. Cruz, Zambales. For the first cropping season (2017 WS), the area was planted with vetiver. During the second cropping season (2018 DS) the area previously planted with vetiver plant was divided into two (2) sub-blocks. One sub-block was terminated while the other sub-block was remained in the field. Soil sampling was conducted before planting with vetiver and after terminated the plant per cropping season. Samples were analyzed for various soil properties like Soil pH, Organic matter, Total and Available Cr and Total and Available Ni. Plant tissue of vetiver samples were analyzed for Cr and Ni accumulation and translocation.

RESULTS AND DISCUSSION

After 3 months, soil pH increased to 6.21 and soil organic matter content to 2.6%. Total Cr and Available Cr were reduced by 186 mg kg^{-1} (4.3%) and 160 mg kg^{-1} (8.53%), respectively. Total and available Ni on the other hand were reduced by 261 mg kg^{-1} (9.86%) and 246 (14.26%) mg kg^{-1}, respectively. Moreover, effect of vetiver grown after 8 months show a continuous increase in soil pH by 7.12 and organic matter to 2.8. Total Cr and available Cr were continually reduced by 307 mg kg^{-1} (7.12%) and 287 mg kg^{-1} (15.30%). Total and available nickel were reduced by 537 mg kg^{-1} (20.28%) and 511mg kg^{-1} (29.62%). Results indicate that vetiver is very efficient in increasing soil organic matter content due to the unique characteristics of its root system. It has been considered an ideal plant to build up organic matter in poor or degraded soils (Truong 2007). Moreover, vetiver also significantly reduced the amount of total and available Cr and Ni indicating its potential in the phytoremediation of heavy metals.

The BAF of vetiver after 3 months is 0.97 for Cr and 1.07 for Ni which means that vetiver has the capacity to absorb Cr and Ni from the soil and store them in their system, particularly in the roots. Allowing vetiver to grow for 8 months increased BAF to 1.48 for Cr and 1.38 for Ni indicating higher concentration in the plant than in the soil. This further shows the potential of vetiver in mitigating heavy metal pollution in soils.

The translocation factor (TF) at 3 and 8 months are very low, indicating that vetiver accumulates Cr and Ni in the roots and very small amount of Cr and Ni were translocated to the shoots, making them safe for forage purposes. Higher TF was obtained for Ni as compared to Cr both at 3 and 8 months of growth. These values are below 0.2 mg kg^{-1} which is the prescribed limit given by the Codex Standard (2001). Results implied that vetiver is a good phytoremediator due to their extensive rooting system that can take up considerable amounts of heavy metals using ion channels and metal transport proteins in their roots. This type of plant is suited in polluted soils due to its metal accumulating ability coupled with metal tolerance and high shoot biomass (Truong and Baker, 1998; Chen 2000).

CONCLUSION

Based on the results of the study, the rice field was highly contaminated with Cr and Ni due to its naturally occurring mineral from the parent material and the presence of mining activities. Remediation technique using vetiver was ideal and sustainable alternative on the permanent removal of pollutants or recovery to rice paddies. Results showed that the longer the vetiver grown in the field the greater increased in soil pH and percent organic matter with a greater decreased of the amount of total and available Cr and Ni in the field. The bioaccumulation factor indicate that vetiver has the capacity to absorb and store metals on their system particularly the roots. With the continuous cultivation of vetiver in the field higher bio-accumulated metals were observed due to its increasing biomass. However, the translocation factor in both cropping seasons show that it is below 0.02 mg kg^{-1} indicating vetiver plant had a lower translocated Cr and Ni to their shoots thus it is safe for forage purposes. Furthermore, it was observed that the longer the time of vetiver in the field the lower is the translocation factor due to that fact that vetiver has high capacity to store and uptake metals according to its bio-availability.

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EFFECT OF HARVESTING CASSAVA LEAVES AT DIFFERENT PLANT AGE AND FREQUENCY ON CASSAVA LEAF PRODUCTION AND TUBER YIELD

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ABSTRACT

Cassava leaves are rich in protein and other essential nutrients, thus, it has the potential to be an alternative source of protein for human and animal consumption. This study investigated the effect of harvesting cassava leaves at 4, 6, 8, and 10 months after planting (MAP) on the leaf production and tuber yield. Leaf numbers 7-15 were harvested from Binulak, Lakan 1, and Sultan 6 (Year 1) while all the leaves except leaf numbers 1-6 were harvested from Rayong 72, KU 50, and Golden Yellow (Year 2). The results showed that harvesting of cassava leaves starting at 4 MAP had no significant effect on the fresh weight of the harvested leaves as well as on the tuber yield for all the six cassava varieties. Therefore, deleafing every two months did not reduce the production of cassava leaves and tubers. Sultan 6 and Rayong 72 had the highest tuber yield but Binulak and Golden Yellow obtained the highest total fresh weight of harvested leaves. Thus, it can be recommended that the harvest of cassava leaves can be done starting at 4 MAP and every two months thereafter without compromising the tuber yield.

Key words: *Manihot esculenta* Crantz, Binulak, Lakan, Sultan, Rayong

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is mainly grown for its roots while leaves are mostly considered as a waste. However, cassava leaves are rich in protein, vitamins, and minerals unlike the roots that are essentially carbohydrates. Minerals found in cassava leaves include potassium, calcium, magnesium, phosphorus, sodium, manganese, iron, zinc, and copper. Cassava leaves also contain beta-carotene (vitamin A), thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), and ascorbic acid (vitamin C) (Achidi et al. 2008; Latif and Mulle 2015). Nevertheless, consumption of cassava leaves as a source of essential nutrients is not much explored in the Philippines because of its high level of cyanogens and its antinutritive properties.

This study was conducted to establish best agronomic practices for production and harvesting of cassava leaves that will not compromise the tuber yield. With proper processing techniques, the cyanide content of cassava leaves can be significantly reduced that can be used for food and feed industry. This would increase the income of cassava farmers and would potentially reduce the production cost of feed industry.

MATERIALS AND METHODS

Six local cassava varieties (ie. Binulak, Lakan 1, Sultan 6, Rayong, KU 50, and Golden Yellow) were selected for field experiment based on cyanide level, availability, and the variety planted by Filipino farmers as a result of the survey conducted in five regions in the Philippines. The frequency of harvest of cassava leaves and maturity of leaves harvested were studied for optimum leaf yield. The effect of leaf harvesting on cassava tuber yield was also investigated. Fifteen (15) healthy plants per plot from each variety were randomly selected for data gathering. Harvesting of leaves at 4, 6, 8, and 10 months after planting (MAP) was done and the tubers were harvested at 10 MAP. The leaves were numbered from the first fully opened one as Leaf 1 and counting sequentially down the stem. Leaf numbers 7-15 were harvested from Binulak, Lakan 1, and Sultan 6 (Year 1) while all the leaves except Leaf numbers 1-6 were harvested from Rayong 72, KU 50, and Golden Yellow (Year 2).

The experiment was composed of four treatments arranged in Split Plot in Randomized Complete Block Design with three replications. Each treatment consisted of 24 plants for each variety with a planting distance of

0.75 m x 0.75 m. The distance between varieties and replications was 1.5 m. Basal application of complete fertilizer (14-14-14) at a rate of 300 kg/ha was done and side dress using urea (46-0-0) at 100 kg/ha followed two months after planting.

RESULTS AND DISCUSSION

The harvest of leaves in Year 1 show that the mean fresh weight of harvested leaves of Binulak and Sultan 6 was significantly higher compared to Lakan 1. For Binulak and Lakan 1, the total weight of harvested leaves starting at 4 MAP was significantly higher than harvests at 6, 8, and 10 MAP. However, for Sultan 6, there was no significant difference in the total weight of harvested leaves among the four treatments. High total yield of leaves was obtained even at 8 and 10 MAP. The differences in the obtained yield may be attributed greatly to varietal differences. It was observed that the mature leaves of Binulak and Lakan 1 started to shed at 8 MAP unlike in Sultan 6 wherein more leaves were produced. Binulak and Lakan 1 have similar structure wherein they only have one major stalk. In contrast, Sultan 6 produces more stalks as it grows making the plant grow wider, laterally, thus producing more leaves.

As for Year 2, the total weight of harvested leaves from Rayong 72, KU 50, and Golden Yellow at 4, 6, and 8 MAP had no significant difference. It was also observed that leaf yield was inversely related to the tuber yield. Golden Yellow obtained the highest leaf yield but had the lowest tuber yield. On the other hand, Rayong 72 had the lowest leaf yield but obtained the highest tuber yield. The results show that for Rayong 72 and Golden Yellow, the harvest of leaves starting at 4 MAP did not significantly reduce the weight of harvested leaves at the succeeding months. But for KU 50, the deleafing frequency had a significant effect on the harvest of leaves. The highest weight of leaves was obtained when the harvest was done starting at 8 MAP.

The harvest of leaves at different plant age and frequency had no significant effect on the tuber yield. Sultan 6 had the highest tuber yield followed by Lakan 1 then Binulak. Similar results were obtained for Year 2 in terms of tuber yield. The harvest of leaves at different plant age and frequency had no significant effect on the tuber yield. Rayong 72 had the highest tuber yield followed by KU 50, then Golden Yellow.

CONCLUSION

Cassava growers may harvest the leaves of Binulak, Lakan 1, Sultan 6, Rayong 72, and Golden Yellow as early as 4 MAP without compromising much the leaf production and tuber yield in the succeeding months. For KU 50, the optimum plant age for deleafing is at 8 MAP. Thus, if cassava leaves would provide additional income for the farmers, it can be recommended that deleafing can be done between 4 to 8 MAP. This would give them the optimum yield of both leaves and tubers. Golden Yellow may have lower tuber yield but it produces significantly higher weight of leaves.

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INDIGENOUS VEGETABLES IN *BULANGLANG* MIXED VEGETABLE DISHES: SUPPORTING BIODIVERSITY AND PROMOTING NUTRITION

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ABSTRACT

"*Bulanglang* dishes" are variants of traditional oil-less dishes of boiled Philippine indigenous vegetables (IVs) of which 46 recipes were documented from IV inventories across 20 provinces in 2019-2020: *bulanglang* (Tagalog); *dinengdeng*, *inabraw* (Iluko); *law-uy* (Bisaya); *linapwahan*, *laswa* (Ilonggo); *s'nobow*, *semla lemnaw* (T'boli, B'laan). They indicate potential contributions to nutrition and biodiversity with up to 14 vegetable ingredients per dish. Frequency counts revealed 47 IV species from 19 taxonomic families as ingredients – part of local biodiversity conserved through use. Analysis of 13 representative recipes using *Menu Eval Plus* (DOST-FNRI) online application revealed contributions to macro and micronutrients. Contributions of 10% or more to daily recommended energy intake (REI) and recommended nutrient intake (RNI) of adults 19-29 years old based on the Philippine Dietary Reference Intake per 100g serving were noted from *inabraw* (calcium, iron, vitamin A, niacin, vitamin C); *bulanglang* (vitamin C); *dinengdeng* (calcium, phosphorus, iron, niacin, vitamin C); *law-uy butong nga gulay* (vitamin A, vitamin C); and *laswa* (calcium, phosphorus, vitamin C). Lighter leafy vegetables appeared to contribute less. Nutrition and biodiversity conservation-through-use can be achieved by increasing: 1) the amounts of leafy IVs, 2) level and frequency of consumption, and 3) the diversity of IV ingredients.

Key words: dinengdeng, inabraw, laswa, Recommended Energy Intake (REI), Recommended Nutrient Intake (RNI)

INTRODUCTION

Bulanglang, a mixed vegetable dish with variants around the Philippines based on the local preferences and availability of locally adapted, readily-available IV ingredients with a simple basic preparation of boiling the vegetables. In Quezon province, it is usually prepared by boiling vegetables in rice washing, a practice perceived to enhance flavor. *Dinengdeng*, the Ilocano variant, is flavored with *bagoong* or dried shrimps. Leaves are the most utilized plant part, followed by fruits and flowers (Favor 2019) resulting in vitamin A (Gascon and Orr 1973). There are few literatures that mention *bulanglang* and some of its variants, but data is scarce on the IVs ingredients in these dishes. Moreover, data is scarce on the dietary contributions of *bulanglang*-type dishes.

The study aimed to discuss the contributions of *bulanglang*-type dishes to biodiversity and nutrition. Specifically, 1.) to describe the diversity of IVs associated with the mixed vegetables dish *bulanglang* and its variants and assess its contributions to biodiversity; 2.) to assess the contribution of *bulanglang*-type dishes to the daily recommended nutrient/energy intakes of adults 19-29 years old; and, 3.) to make recommendations for policy directions in promoting *bulanglang*-type dishes for health and biodiversity conservation.

MATERIALS AND METHODS

Six (6) variants or recipes of *bulanglang* from different regions identified during a 2018-2019 Documentation Project were included in the study – *bulanglang* (Southern Luzon), *inabraw* (Northern Luzon), *dinengdeng* (Ilocos Region), *laswa/linapwahan* (Western Visayas), *law-uy* (Central Visayas), and *s'nowbow* and *semla lemnaw* (Mindanao). The source data of the recipes was the Inventory of Indigenous vegetables done in 2018-2019.

To assess the diversity of indigenous vegetables (IV) in *bulanglang* variants, frequency counts of both the species per dish variant and taxonomic families of IV species in *bulanglang* variants were conducted.

To assess the contribution of 100 g serving size of *bulanglang* variants to the daily Recommended Nutrient Intake (RNI)/Recommended Energy Intake (REI) of adults 19-20 years old, 13 representative recipes from the six identified variants were used. Contributions to energy, protein, calcium, phosphorus, iron, vitamin A, thiamin, riboflavin, niacin and vitamin A requirements were computed using the Menu Eval Plus online application of the Food and Nutrition Research Institute (FNRI) of the Philippines Department of Science and Technology (DOST).

RESULTS AND DISCUSSION

Contributions to biodiversity. Ingredients of documented *bulanglang* dishes include 47 IV species from 18 taxonomic families. The IV ingredients per recipe ranged from 1 to 14 species. Sustained utilization of IVs in *bulanglang* recipes reflect conservation these species through use. Across all provinces, at least five IV species are ingredients. Fabaceae was the most reported species (9). At least one legume is an ingredient in all recipes documented except in *semla lemnaw*. Legumes serve as a protein source, while growing them allow soil nitrogen fixation to help regenerate the soil, supporting growth of diverse flora and allowing other life forms to thrive. Tree vegetables like *himabao* [*Brousonettia luzonica* (Blanco) Bureau] also contribute ecosystem services that trees provide.

Nutritional contributions. The range of contributions of the 13 recipes of different *bulanglang* variants to the REI/RNI of adults with ranges reaching at least 10% upper level were noted: 12% calcium, 7-16% iron, 11-13% vitamin A, 14-16% niacin, 17-20% vitamin C from *inabaraw*; 11-50% vitamin C from *bulanglang*; 2-17% calcium, 2-13% phosphorus, 2-11% iron, 1-10% niacin, 3-37% vitamin C from *dinengdeng*; 12-14% vitamin A, 9-10% vitamin C from *butong nga gulay* recipe for *law-uy*; 11-15% calcium, 7-10% phosphorus, 3-13 vitamin C for *laswa*. The rest contributed <10% to the REI/RNI of an individual, but in general, the recipes registered contributions to the different macro and micronutrients required by the body. Lower percent contribution from *bulanglang* variants with mostly leafy vegetables is expected due to its approximate weight of 40g per cup basis for computation compared to heavier non-leafy vegetables with an weight basis of 240g per cup. Increasing the amounts of vegetables in each dish, increasing the number of servings consumed will enhance nutrition contributions. A lower percent contribution of a specific *bulanglang* variant highlights the importance of diet diversification, including diversification in *bulanglang* ingredients. While a higher percent contribution is favorable, it is also important to note the source of the macro or micronutrient. In the case of *dinengdeng* in Ilocos Norte, the calcium phosphorus contributions of 17% and 13%, respectively, were attributed to fish paste which is high in sodium. High sodium intake for people with heart conditions as well as edema and ascites might exacerbate their conditions; therefore, caution must be taken. Key IVs with relatively high contributions to essential macro and micronutrients effect on the percent contribution of *bulanglang* variants include horseradish tree pods (*Moringa oleifera* Lam.), unripe papaya (*Carica papaya* L.), bottle gourd [*Lagenaria siceraria* (Molina) Standl.], lima bean (*Phaseolus lunatus* L.), okra [*Abelmoschus esculentus* (L.) Moench.], sweetpotato [*Ipomoea batatas* (L.) Lam.], hummingbird tree flowers [*Sesbania grandiflora* (L.) Pers.], amaranths (*Amaranthus viridis* L., *Amaranthus spinosus* L.), morning glory leaves (*Ipomoea triloba* L.), and ricebean [*Vigna umbellata* (Thunb.) Ohwi & H. Ohashi].

CONCLUSION

Bulanglang variants contribute macro and micronutrients based on the amount and type of IV ingredients used. Some of the key vegetables noted to have relatively high effect on the percent contributions of bulanglang variants include horseradish tree pod (*Moringa oleifera* Lam.), unripe papaya (*Carica papaya* L.), bottle gourd [*Lagenaria siceraria* (Molina) Standl.], lima bean (*Phaseolus lunatus* L.), okra [*Abelmoschus esculentus* (L.) Moench], sweetpotato [*Ipomoea batatas* (L.) Lam.], hummingbird tree flowers [*Sesbania grandiflora* (L.) Pers.], amaranths (*Amaranthus viridis* L., *Amaranthus spinosus* L.), morning glory leaves (*Ipomoea triloba* L.), and ricebean [*Vigna umbellata* (Thunb.) Ohwi & H. Ohashi]. Lower contribution does not signify inferiority of a particular variant, but rather highlights the importance of diet diversification.

Policy recommendations to promote *bulanglang*-type dishes builds on the observation that IV ingredients are highly adapted to localities, requiring minimal management and the recipes require simple preparation. Thus, their potential for nutrition and biodiversity conservation-though-use can be exploited through recommending: 1) increase in the amounts of leafy IVs, 2) increase in the amounts and frequency of consumption, and 3) increasing the diversity of IV ingredients used as well as the diversity of variants served.

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ASSESSING THE ACCURACY OF DAILY SOLAR RADIATION FROM NASA-POWER REANALYSIS DATASET OVER REGION IV-A, PHILIPPINES

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ABSTRACT

Complete and reliable time series of global solar radiation data (Rs) is vital for its applications in meteorology, hydrology, agriculture, and renewable energy. However, in the case of CALABARZON region in the Philippines, solar climatic variables are scarce. Out of seven DOST-PAGASA stations in the region, only UPLB station has the available ground-based solar radiation data with temporal record from 1977 to 2011 (35 years), and for this reason, exploration of other sources of solar radiation data was necessary. One of the most important data types available today that could help overcome scarce ground-based climatic observations is reanalysis and gridded meteorological data derived from global atmospheric models and satellite images. In this study, daily solar radiation data from Goddard Earth Observing System (GEOS) reanalysis datasets that can be accessed through National Aeronautics and Space Administration-Prediction of Worldwide Energy Resource (NASA POWER) were assessed considering different intervals of atmospheric transparency index. The accuracy of NASA-POWER Rs was compared against the registered ground-based data in the region including the Rs registered in UPLB station and the deployed automatic weather stations (AWS) of DOST-ASTI and DA-BSWM from period 2012 to 2019. On the average, Rs of NASA POWER is off by 17.05%, 17.79% and 51.51% to the ground-based data during days with clear, partially cloudy, and cloudy type of sky, respectively. Overall, NASA POWER Rs reanalysis dataset has an acceptable remark based on the obtained RRMSE and NSE which demonstrated that this data could be a valid substitute in case of missing or availability limitations of Rs observations in the region.

INTRODUCTION

The availability of accurate data of global radiation (Rs) at a specific region are highly crucial to regional crop growth modeling, evapotranspiration estimation, and irrigation system development (Zhang et al., 2018). However unlike other meteorological data such as rainfall and temperature, Rs data is not always available owing to the high cost of measuring equipment, maintenance, and calibration requirement (Cenk et al., 2017; Despotovic et al., 2015). In the case of Region-IVA of the Philippines, solar climatic variables are scarce. Out of seven DOST-PAGASA stations in the region, only UPLB station has the available ground-based solar radiation data with temporal record from 1977 to 2011 (35 years), and for this reason, exploration of other sources of solar radiation data was necessary. One of the most important data types available today that could help overcome scarce ground-based climatic observations is reanalysis and gridded meteorological data derived from global atmospheric models and satellite images (Aboelkhair et al., 2019). In this study, daily solar radiation data from Goddard Earth Observing System (GEOS) reanalysis datasets that can be accessed through National Aeronautics and Space Administration-Prediction of Worldwide Energy Resource (NASA POWER) (https://power.larc.nasa.gov/data-access-viewer/) were assessed considering different intervals of atmospheric transparency index.

MATERIALS AND METHODS

Aside from the Rs data of DOST-PAGASA in UPLB station, data acquired from installed Automated Weather Stations (AWS) of DOST-ASTI and DA-BSWM were also used . These AWS were deployed in the region from 2012 to 2019 and were being used in weather forecasting activities and disaster decision-support for agencies and local governments. The Rs data from these AWS were collected in sub-daily basis and expressed in W/m2, thus necessary conversion and validation test were done to ensure that proper information was used as the baseline Rs data. The quality assurance procedures on AWS-collected Rs data, consolidated by Estévez et al. (2011) were followed in this study. For general analysis and evaluation of Rs of NASA POWER against ground-based data, the values of clearness index were regrouped in three ranges of clearness index (Kt): clear sky ($0.70 \leq Kt \leq 0.90$), partially cloudy ($0.30 \leq Kt < 0.70$), and cloudy ($0.03 \leq Kt < 0.30$) (Sayago et al., 2019). The data are classified according to data source and type of the day. Overall, the total number of samples considered for evaluation is 20,141 by which 10.72% (n=2,159) is under clear sky, 78.20% (n=15,750) is partially cloudy, and 11.08% (n=2,232) is cloudy.

RESULTS AND DISCUSSION

The Rs data of NASA POWER generally tend to underestimate the ground data collected from DOST-PAGASA but overestimate the data from DA-BSWM and DOST-ASTI as indicated with the signs of their average mean error. The values of average R2, RMSE, and ME ranged between 0.63 – 0.73, 3.63 – 4.45 MJ/m2-day, and -2.29 – 2.27 MJ/m2-day, respectively. There is relative consistency on the daily Rs of NASA-POWER and ground observations over Region IV-A especially on days with clear and partially cloudy skies. These ranges of statistical indicators are consistent with the results of conducted evaluations in other locations. Bai et al. (2010), who evaluated NASA POWER Rs against 39 weather stations in China, got an average R2 of 0.8, RMSE of 3.1 MJ/m2-day, and ME of 0.7 MJ/m2-day, while White et al. (2011) who assessed the data using 295 stations in USA obtained a higher correlation with R2 of typically 0.85 to 0.95 and RMSE of 2.0 to 3.0 MJ/m2-day. More recent publications prove the promising accuracy of NASA-POWER like Negm et al. (2017) who had assessed the Rs data using 40 stations in Sicily, Italy and obtained a range of R2of 0.65 to 0.94, RMSE of from 2.0 to 4.8 MJ/m2-day, and ME of -0.67 to 2.6 MJ/m2-day. Moreover, Sayago et al. (2019) with 31 meteorological stations across Spain, verified a good fit of the estimates from NASA -POWER with R2 of 0.85 to 0.96 and RMSE ranging from 1.78 to 4.62 MJ/m2-day.

CONCLUSION

On the average, Rs of NASA POWER is off by 17.05%, 17.79% and 51.51% to the ground-based data during days with clear, partially cloudy, and cloudy type of sky, respectively. Overall, NASA POWER Rs reanalysis dataset has an acceptable remark based on the obtained RRMSE and NSE which demonstrated that this data could be a valid substitute in case of missing or availability limitations of Rs observations in the region. NASA POWER could give historical and real time estimates of Rs data that could be used for optimization of design of solar energy conversion systems, architecture projects, irrigation projects, among others.

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CONIDIAL PRODUCTION OF BEAUVERIA BASSIANA AND METARHIZIUM ANISOPLIAE IN CORN AT DIFFERENT INCUBATION PERIODS

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ABSTRACT

Entomopathogenic fungi are promising biological control agents against major insect pests of crops. Production of high yields of infective propagules is essential for field application and formulation. The potential yield and quality of propagules maybe affected by the incubation period of inoculated grain substrates. This paper presents the conidial yield of two entomopathogenic fungi grown in corn substrate incubated at 25-27°C until 28 days. In each 250g substrate, a 10-mm mycelial plug of Beauveria bassiana was inoculated in pre-cooked corn while 1 x 106 conidia/ml of Metarhizium anisopliae was inoculated in corn. Total conidial yield was determined by harvesting dry conidia and washing the substrates with 0.1% Tween 80 solution. Fungal growth in substrate bags was visible at 3-4 days after inoculation. Initial results indicate that conidial yield and germination of these entomopathogenic fungi declined as incubation of inoculated corn substrates was prolonged. Results suggest that optimum conidial production was 7 to 14 days after inoculation. During this period, conidial yield of B. bassiana was 8.38 x 1010 to 9.82 x 1010 conidia/kg of corn substrate whereas M. anisopliae had 6.39 x 1011 to 1.07 x 1012 conidia/kg of corn substrate with 84-89% conidial germination. These findings highlight the influence of incubation period on the conidial production of the two entomopathogenic fungi.

Key words: biological control, entomopathogenic fungi, fermentation, mass production, solid substrate

INTRODUCTION

Mycobiocontrol utilizes fungi to lower the insect population and reduce crop damage. Entomopathogenic fungi (EPF) are effective biological control agents of economically important insect pests of crops with over 750 fungal species that can provoke fungal infections in insect populations (Sandhu et al. 2012). Production of high yields of infective propagules is essential for biological control program (Agale et al. 2018). Infective inocula are necessary for field application and formulation. EPF can be mass produced using liquid or solid state fermentation with a common practice of culturing in solid substrates due to their availability and ease of handling (Jaronski 2014; Sandhu et al. 2012). Some of the most commonly used grains are corn and rice. Substrate production is usually affected by temperature, incubation period and substrate used, among others. The conidial yield and viability are influenced by these factors. Hence, this study was carried out to investigate the conidial yield of Beauveria bassiana and Metarhizium anisopliae in corn substrate, which is commonly used to mass produce EPF.

MATERIALS AND METHODS

In this experiment, conidial yield of B. bassiana and M. anisopliae were determined in corn substrate. In each 250g substrate, a 10-mm mycelial plug of B. bassiana was inoculated in pre-cooked corn while 1 x 106 conidia/ml of M. anisopliae was inoculated in corn. Corn substrates were incubated at room temperature for 28 days after inoculation. Conidial yield was determined at 7 days interval using wet and dry method. Corn substrates were dried. Dry conidia were harvested by sieving. To obtain the total yield, the substrate was washed with 0.1% Tween 80 solution after sieving. The following data were gathered: conidial yield in terms of mass of dry conidia and total yield, and conidial germination. Conidial concentration was determined with the use of a hemocytometer. Conidial germination was assessed by spread plating fungal suspension of B. bassiana and M. anisopliae in potato dextrose agar (PDA) and water agar (WA), respectively. Each treatment was replicated five times. Data was analyzed using one-way ANOVA and treatment means were compared using Tukey's HSD.

RESULTS AND DISCUSSION

Fungal growth in corn substrate was visible at 3-4 days after inoculation. White fungal growth was observed in B. bassiana. Total yield of B. bassiana declined from 9.82 x 1010 to 6.50 x 1010 conidia/kg of substrate at 7 and 28 days after inoculation, respectively. On the other hand, substrate bags inoculated with M. anisopliae were initially with white fungal growth and later turned green during sporulation. The total conidial yield increased at 14 days after inoculation at 1.07 x 1012 conidia/kg of substrate and subsequently declined to 5.52 x 1010 conidia/kg of substrate at 28 days after inoculation. Highest mass of dry conidia of B. bassiana was harvested 21

days after inoculation, however, conidial concentration was in declining trend from 7 to 28 days after inoculation. In M. anisopliae, dry conidia and concentration peaked at 14 days after inoculation and subsequently declined thereafter. The moisture content in the substrate bags may have affected the resulting weight of dry conidia. Based on these findings, the optimum conidial production is at 7 to 14 days after inoculation with 84-89% conidial germination. The decreasing trend in conidial production in both EPF may be due to the declining nutrition in the substrate as these are fully consumed during prolonged incubation period (Posada-Florez 2008). Likewise, conidia may have germinated due to moist condition in the substrate bags.

Jaronski and Mascarin (2017) noted that the typical production cycle in solid substrate fermentation is 7-14 days followed by drying of the culture and removal of conidia. Posada-Florez (2008) observed that conidial concentration of B. bassiana is higher if harvested earlier in rice substrate. Dry conidia can be used in formulation and field application. Since the conidial yield declines after further incubation, dry conidia can be harvested and stored. Proper drying of conidia is essential to maintain good shelflife (Jaronski and Mascarin 2017; Moslim et al. 2005). Recorded conidial yield in this study differ with other findings. Bich et al. (2018) recorded highest sporulation of B. bassiana (4.62 x 107 conidia/g) and M. anisopliae (2.22 x 106 conidia/g) in rice substrate after four weeks of incubation. On the other hand, Moslim et al. (2005) recorded highest yield of M. anisopliae in maize. About less than 1 x 1010 spores/g of B. bassiana was recovered in cooked rice (Posada-Florez 2008). Furthermore, Moslim et al. (2005) recommended to harvest conidia between 30-40 days after inoculation. Variation in conidial yield maybe due to differences in inoculum, photoperiod, nutrients, and moisture content during mass production.

CONCLUSION

The maximum conidial yield can be obtained in B. bassiana and M. anisopliae up to 14 days after inoculation. Many factors are known to affect conidial yield. Hence, experiments will be conducted to optimize mass production in solid substrate.

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DEVELOPMENT OF SWEET AND SPICY PALAPA ORGANIC SAKURAB (*Allium chinense*)

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ABSTRACT

Sakurab Scallion Palapa (*Allium chinense*) comes close to the traditional Maranao apetizer, it is made of stewed scallion bulbs. Palapa is hawked in humble eateries and restaurants in almost every corner of the provinces of Lanao. This study was conducted in Cotabato State University,Sinsuat Avenue,Cotabatto City, April 2019, Philippines to determine the acceptability of sweet and spicy *Palapa organic Sakurab* in the University. Specifically, it sought to answer the acceptability of *Sweet and Spicy Palapa* ,organic sakurab in terms of taste, physical appearance, color and aroma. Experimental research design was used. Descriptive survey method was used and followed with 50 respondents. Data were analysed using percentage and frequency counts. Findings showed that the overall mean rating on the acceptability of sweet and spicy *palapa* in terms of color was 1.34 (very acceptable), in terms of aroma was 1.32 (very acceptable), in terms of taste was 1.28 (very acceptable) in terms of physical appearance was 1.22 (very acceptable). The study concluded that *spicy palapa* is highly acceptable. The research study shall be continued to focus on health advantages, life span of sweet spicy *palapa,* commercialization of the product, and use as a meal appetizer, as well as technical support, and motivation, to the researcher. Some of the people who responded said it was their first time eating organic *sakurab palapa*. It was a good concept or a good product to launch a new business in order to capitalize on a market opportunity and make it successful. A separate study shall be conducted focusing on sakurab farming.

Key words: Spicy Palapa, Palapa, Organic sakurab, Marano, *Allium chinense*

INTRODUCTION

Salad onion is the common name for scallion green onion (*Allium chinense* L.). It is known as "Sakurab" among the Meranaos and has been transformed into a popular seasoning called "Palapa," which non-Meranaos have adopted. A mature green onion bulb has 47.0 calories, 1.4 grams of protein, 7 mg of sodium, 180 mg of potassium, 32 mg of calcium, 44 mg of phosphorus, 16 mg of magnesium, 70 mg of sulfur, 25 mg of chlorine, and 28 mg of vitamin C. In China, diluted squeezed juice is used to combat pests. Alliums, such as scallion green onions, have a unique thiosulfate that has anti-thrombotic properties, including antioxidant activity, lower serum cholesterol, and improved in vitro antiplatelet action. This latter action is beneficial to cardiovascular health since it reduces the likelihood of platelets clumping together in the blood. It belongs to the same botanical family as garlic and leeks, and its oil is stimulant, diuretic, and expectorant both internally and externally, which increases the peristaltic action of the bulb in the intestines.

A variety of condiments spice up most of the dishes in the southern Philippines, but nothing matches the exceptional taste shigasakurab gives. The taste is unique to your buds, making you crave more rice. Sakurab, or native shallots, is a vegetable that looks like scallions and is used as an atypical Filipino cuisine seasoning. Sakurab is a popular ingredient in the Maranao condiment palapa, which is made with species, salt, and ginger. Sakurab is also utilized in traditional cuisine on the islands of Mindanao and Visayas, where it is more popularly known as sibujing. Palapa sakurab from the Maranao people of Lanao del Sur. Palapa sakurab from the Maranao people of Lanao del Sur is an important cultural symbol of the Maranao people and is an ubiquitous accompaniment at every meal. Sakurab is sold in almost every street and alley in the province, proof that the palapa's existence is intertwined with the history and culture of the people of the lake. A traditional Maranao appetizer that is essential to any meal. Exquisite Marano cuisine and hospitality are palpable. A condiment made of traditionally cultivated spices, locally known as "palapa," is one of their distinctive culinary symbols. It is made of steamed scallion bulbs, or sakurab in marano. The study sought to tdetermine the acceptability of organic sweet and spicy palapa sakurab as a preserve in terms of taste, physical appearance, color, and aroma, and it was determined by comparing it to established products.

MATERIALS AND METHODS

The respondents were six (6) faculty and forty-four (44) students from Cotabato State University, who were interviewed after tasting the products. The data collection instrument used in the study was a questionnaire, and it was used to compare the two products. Respondents were required to indicate whether the samples were very acceptable, acceptable, fairly acceptable, or not acceptable in terms of preferences. To address the study's objectives, the data was analysed using descriptive statistical methods such as percentage, frequency, and means.

RESULTS AND DISCUSSION

The respondents' profile revealed that 84% were between the ages of 19 and 24, 10% are between the ages of 25 and 30, 4% are between th ages of 31 and 36, and 2% are between the ages of 43 and 48. The majority of the respondents were female, with 44 percent being male. The respondents with the highest educational attainment were college graduates (12%) and students (88%). This also reveals that 82 percent of those polled were single, with only 18 percent married. The majority of the respondents (86%) are Muslims, whereas 14% are Roman Catholics. The study also indicated that 88 percent of the respondents are students at Cotabato State University, while 12 percent are faculty.

The sweet and spicy palapa organic sakurab is very acceptable to 40 to 80 percent of respondents, it is new and appealing to their taste. In terms of physical appearance, 40 to 80 percent of respondents were eager to purchase the goods and that it is quite acceptable. The sweet and spicy palapa organic sakurab has a really appealing color. The aroma is also well received with 40% to 80% agreeing that the sweet and spicy palapa organic sakurab are quite satisfactory. Organic sweet and spicy palapa could be marketed. Some of the respondents stated that it was their first time trying sakurab palapa. It was a good concept or product to launch a new business to capitalize on a market opportunity and turn a profit. The sweet and spicy palapa organic sakurab, as well as the normal spicy palapa, were found to be very acceptable because it is new and extraordinarily well accepted in terms of taste, color, aroma, and physical appearance,

CONCLUSION

The sweet and spicy organic palapa sakurab processing has evolved as a result of the addition of new components. The sweet and spicy palapa organic sakurab is very much acceptable. There is no difference in taste or appearance between the organic sweet and spicy palapa and the established salty and spicy palapa. The established salty and spicy palapa and sweet spicy palapa organic sakurab have no color or aroma differences. Organic sweet and spicy palapa was discovered to have the highest percentage of approval and is favoured by respondents.

EFFECT OF VARIOUS COMBINATIONS OF LEAF CLIPPING, CYTOKININ APPLICATION, AND TIMING OF FERTILIZATION ON YIELD OF PINEAPPLE

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ABSTRACT

The market demand for pineapple is continuously increasing; hence, research on different production practices is still being done to improve production to take advantage of increasing large-scale markets. Leaf clipping and application of cytokinin increases yield in some crops by increasing the photosynthetic capacity. Proper timing of fertilizer application is important to maximize its benefits. This study explored the potential effects of leaf clipping, cytokinin introduction, and fertilizer scheduling in pineapple production. Leaf clipping or cutting 4 to 6 inches from the leaf tip of pineapple was done 16 weeks after planting. Cytokinin application was 48 hours after leaf clipping. A similar amount of fertilizer was applied to all plants for the whole cropping production but at two different schedules, weekly and bi-monthly. Leaf clipping, cytokinin application, and fertilizer application schedule did not significantly affect the growth parameters, such as plant height, the number of leaves, root length, stumps biomass, and the leaf nutrient content of pineapple plants. Leaf clipping, regardless of cytokinin application and schedule of fertilizer application, increased the Normalized Difference Vegetation Index (NDVI) and improved the yield of pineapple by about 6.04 to 9.17 metric tons/ha, or a 5.11 to 7.94% yield increase. Leaf clipping with bi-monthly fertilizer application resulted in the highest yield of 124.69 metric tons/ha.

Key words: assimilates, MD2 cultivar, fertilizer scheduling, Normalized Difference Vegetation Index

INTRODUCTION

The pineapple industry grew and flourished with steady progress. At present, market demand for pineapple continues to increase; however, the current pineapple cultural practices in the country cannot cope with market demand. Hence, work on different production practices is being done to improve production to take advantage of the increasing large-scale markets. The leaf clipping technology may increase the yield of pineapple in conjunction with cytokinin and the timing of fertilizer application. Source tissues are generally responsible for acquiring resources from the outside environment. Greater source capacity leads to poor crop performance as fertilization produces more foliage and reduced productivity. Leaf clipping technology is proven effective in increasing yield in some crops such as rice, sorghum, pearl millet, corn, and soybean (Liu et al. 2017; Srinivasan et al. 2016). Leaf clipping changes the canopy structure resulting in an adequate green leaf area index and effective blade spatial arrangement. This increases the canopy photosynthesis as light transmission to lower canopy strata increases, resulting in improved light conditions within the canopy. Greater light penetration in the canopy results in more assimilates (Dong et al. 2000) and finally brings about higher yield (Hachmann et al. 2014).

Leaf clipping brings wound stress to pineapple; hence cytokinin application may be necessary as it is known to activate dormant buds to allow recovery after injury or damage (Müller et al. 2011). Cytokinin has also increased yield in some crops like maize, cherry tomatoes, winter wheat, and bread wheat (Gao et al. 2017; Shahzad et al. 2016; Yang et al. 2016). Proper timing of fertilizer application increases yield, reduces nutrient losses, increases nutrient use efficiency, and prevents environmental damage (Sela 2018). Dividing the amount of fertilizer required and applying in smaller quantities more frequently during the active growing season is a good practice for better nutrient uptake, rather than applying larger amounts less often (UCANR 2018). This study was undertaken to determine the production (growth and yield) performance of pineapple as affected by leaf clipping, cytokinin application, and timing of fertilization.

MATERIALS AND METHODS

The study was conducted in Polomolok, South Cotabato, from September 2017 to April 2019. The pineapple crown (medium size ranging from 300-350 g) of the MD2 cultivar was used as planting materials. The study was a six-treatment experiment in a Randomized Complete Block Design replicated four times with 164 sample plants. Fisher's Least Significant Difference (LSD) was used to determine differences between and among treatment means.

Leaf clipping at 4 to 6 inches from the leaf tip was done 16 weeks after planting. Application of cytokinin (Fast Gro with 0.04% a.i.) using an automatic knapsack sprayer at a rate of 5.26 L/ha was done within 48 hr after clipping. For fertilizer application, the total monthly rate (following the farm practice) was divided into 2 for the bi-monthly (14 days) application, while the monthly rate was divided into 4 for the weekly (7 days) application. Fertilization was applied foliar using a knapsack sprayer starting 14 days after planting. Forced flowering was done 12 months after planting through spraying of ethephon and harvested 18 months after planting or six months after forcing.

RESULTS AND DISCUSSION

Pineapple plants applied with fertilizer every two weeks without or with leaf clipping and cytokinin application were the tallest plants at eight months after planting (8 MAP). The bi-monthly fertilizer application may have provided the right amount of nutrients for uptake, making the plants grow taller. On the other hand, results indicate that clipping and cytokinin application did not influence the pineapple plant height. Also, leaf production of pineapple was not influenced by clipping of the leaf, cytokinin spraying, and at a closer gap of fertilizer application at seven days. Root length had a significant difference among treatments at four months after planting. Clipped plants fertilized weekly showed the longest root but comparable to clipped plants fertilized weekly and applied with cytokinin. At eight months after planting, root length ranges from 30.8 to 34.7 cm in all treatments, which was insignificant. Root length incremental increase in a span of 4 months is marginal, entailing that root length was not influenced by leaf clipping. Leaf clipping, cytokinin, and timing of fertilizer application did not significantly affect most of the nutrients in the leave of pineapple plants. It is not apparent if cytokinin application and leaf clipping influenced the leaves' N, K, and B contents. Leaves from pineapple plants applied with fertilizer weekly have more N, K, and B content than applied bi-monthly. The weekly foliar application resulted in more nutrients absorbed by the leaves than when applied bi-monthly. Leaf clipping significantly increased the pineapple yield by about 6.04 to 9.17 tons/ha, or a 5.11 to 7.94% yield increase per hectare. Leaf clipping increases yield as it removes competing sinks for assimilates. Moreover, leaf clipping increased the NDVI of pineapple plants which indicates that leaf clipping increased the photosynthetic activity of the plants. The clipping of vegetative organs like leaves allows plants to capture light effectively and use it efficiently by increasing the photosynthetic capacity (Zhu et al. 2004), leading to an increase in net photosynthetic rates (Anderson et al. 2013).

CONCLUSION

Leaf clipping combined with the bi-monthly application of foliar fertilizer effectively increased the yield of pineapple and resulted in higher net income. With this result, leaf clipping technology is proven effective in increasing the yield of pineapple.

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BANANA BUNCHY TOP VIRUS CAUSED ABACA BUNCHY TOP IN LEYTE, PHILIPPINES

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ABSTRACT

Bunchy top is a serious constraint to the abaca production, not only in Leyte, but also in all major abaca growing regions in the country. Managing such disease has been so challenging due to the complex nature of the viral pathogen, and its early disease detection and correct virus identification is integral in developing an effective disease management strategy. Hence, this study sought o determine the occurrence and identity of the causative agent of abaca bunchy top in Leyte. Virus isolates from bunchy top infected abaca plants were collected from four different locations in Leyte, analyzed by polymerase chain reaction, and identified via next generation sequencing (NGS). The virus was highly detectable using an optimized PCR conditions using a Banana bunchy top virus (BBTV) specific primer pair BBT1/BBT2 at 10 ng template concentration of Dellaporta-derived DNA extracts. Analyses of full-length genome sequences derived by NGS-Illumina MiSeq platform revealed that the bunchy top virus infecting abaca in Leyte, Philippines is *Banana bunchy top virus* (BBTV). The knowledge generated from this study has important implication in designing PCR primers for specific and sensitive detection of BBTV causing bunchy top of abaca and in virus resistance breeding programs against the disease.

Key words: Dellaporta-derived DNA extracts, polymerase chain reaction (PCR), next generation sequencing (NGS), BBT1/BBT2 primer pair

INTRODUCTION

The Philippines enjoys the monopoly of supplying about 87.4% of world's demand of abaca fiber (PhilFIDA 2020). However, meeting the increasing demand for abaca fiber has presently been difficult because of the declining abaca productivity due largely to virus diseases, specifically the abaca bunchy top. The disease is caused by *Banana bunchy top virus* (BBTV) (Bajet and Magnaye 2002; Furuya et al. 2006; Magee 1953; Ocfemia 1931) and *Abaca bunchy top virus* (ABTV) (Sharman et al. 2008). It is transmitted by an aphid *Pentalonia nigronervosa* Coquerel (Ocfemia 1927). The data generated from this study will ultimately be useful in the formulation of an effective and durable abaca bunchy top (ABT) management through virus resistance breeding. To attain a durable ABT resistance, the abaca varieties must be bred with resistance to the range of virus species infecting the crop and that must be strategically deployed to areas where they can resist infection which all require a correct and early detection of the virus. Hence, this study aimed to determine the occurrence and identity of the causative agent of bunchy top in Leyte, Philippines, specifically to detect the BBTV or ABTV using optimized PCR conditions, and sequence the genome of the Leyte bunchy top virus (BTV) isolates through next generation sequencing (NGS).

MATERIALS AND METHODS

The total nucleic acid from bunchy top-infected abaca leaf samples collected from four municipalities of Leyte (Abuyog, Baybay City, Kananga, and Tanauan) was extracted using three different DNA extraction protocols, namely: CTAB DNA extraction (Doyle and Doyle 1990) modified for abaca, PCR Dellaporta Miniprep (Dellaporta et al. 1983), and Sarkosyl method (Su 2000). The total NA extracts at eight concentrations (undiluted, 1000, 100, 10, 1.0, 0.01, 0.001, and 0.0001 ng/uL) were subjected to PCR using four BBTV-specific primers (BBT1/BBT2, CBT3F/CBT3R, CR-SL(F)/CR-SL(R), F3/FPCR4, and JO2/GO1) and seven ABTV specific primers (AbBTVC5A/AbBTVC5B, CRMA/1108C6B, SLCR2/CRMB, 767C1A/767C1B2, 767C2A/CRMB, 1108C3A/1108C3B2, and 1108C4A/1108C4B). The same set of samples were also subjected to PCR using an internal control DNA, a *Musa* sequence tagged microsatellite site with primers AGMI025/AGMI026 (Lagoda et al. 1998; Mansoor et al. 2005) to test the reliability of the virus detection via PCR. The identity of the causative virus of abaca bunchy top in Leyte was confirmed by next generation sequencing (NGS).

RESULTS AND DISCUSSION

Optimized PCR Condition for the bunchy top virus (BTV) *in Leyte.* The Leyte BTV isolates were highly detectable by PCR using the BBTV-specific primer pair BBT1/BBT2. Among the seven ABTV-specific primers used, only

the SLCR2/CRMB primer pair yielded a single band DNA amplification, but the detection level was way lower than that of BBT1/BBT2 primer pair. The level of virus detection was affected by the DNA extraction method. Dellaporta DNA extracts resulted in the highest virus detection level, indicating that Dellaporta method can yield good quality DNA with no or less PCR inhibitors. This agrees with the report of Bevilacqua (2008) that the method produced quality DNA suitable for subsequent PCR-based applications and other downstream applications (Piamonte and Sta Cruz 2018; Sta Cruz et al. 2016). The sensitivity of virus detection by PCR increases with the dilution of the DNA extracts. The BTV detection was highest at 10 and 1.0 ng/µL DNA template.

Reliability Test. The use of internal control DNA in PCR assays will ensure the correct and reliable virus detection. This will lessen, if not avoid, the chance of getting false negatives in PCR results. A sample can be confidently declared negative of the virus when the internal control DNA is positively detected in the sample. The internal control DNA is derived from a DNA sequence of the plant host of the virus and should always be detectable in the DNA extracts of both healthy and virus-infected plants, unless affected by PCR inhibitors. The presence of PCR inhibitors in the DNA extract will also inhibit or reduce the level detection of the internal control DNA. A similar trend of results was obtained in detecting the plant host DNA (internal control DNA) to that of detecting the BTV in the DNA extracts via Dellaporta, Sarkosyl and CTAB methods at eight DNA concentrations. There was no amplification of the host DNA in the undiluted Sarkosyl and CTAB DNA extracts, only in the Dellaporta DNA extracts. The level of detection also increases with the dilution of the DNA extracts. The amplification was obtained when the DNA extracts were diluted. The host DNA was highly detectable by PCR at 100 ng/uL DNA concentration using AGMI025 and AGMI026 primer pair.

Genome Sequence of BTV in Leyte, Philippines. The full-length genome sequences of BTV Leyte isolates were derived via NGS - Illumina MiSeq platform at the Philippine Genome Center, University of the Philippines Diliman, Quezon City. The obtained genome sequences were aligned to BBTV (BBTV MS18_PH_2008, KM607655.1) (Stainton et al. 2015) and ABTV (Q1108, EF546807.1) (Sharman et al. 2008) reference genomes. To declare whether the BTV Leyte isolates are BBTV or ABTV, the species demarcation set for nanoviruses by Vetten et al. (2004) was used. This is the same species demarcation that Sharman et al. (2008) used in proposing another species of bunchy top virus infecting abaca, naming as *Abaca bunchy top virus* (ABTV). The BBTV and ABTV shared only 54-76% in overall nucleotide sequence identity and 79-81% amino acid sequence identity for putative coat protein, enough to be declared as distinct species (Sharman et al. 2008). In this study, the pairwise nucleotide identity analyses revealed that the BTV Leyte isolates has 98.8% to 99.6% overall nucleotide identities to BBTV and only 70 to 71.3% overall nucleotide identities to ABTV. This confirmed that the bunchy top virus infecting abaca in Leyte is *Banana bunchy top virus* (BBTV). The knowledge generated can be utilized in designing PCR primers for specific and sensitive detection of BBTV causing bunchy top of abaca and in virus resistance breeding programs.

CONCLUSION

The virus causing bunchy top in Leyte, Philippines was highly detectable using an optimized PCR conditions using a BBTV-specific primer pair BBT1/BBT2 at 10 ng template concentration of Dellaporta-derived DNA extracts. Analyses of sequences derived by NGS-Illumina MiSeq platform revealed that the BTV species infecting abaca in Abuyog, Baybay City, Kananga, and Tanauan municipalities of Leyte, Philippines is BBTV.

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PLANT GROWTH REGULATORS AGAINST PRE AND POSTHARVEST DISEASES OF MANGO

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ABSTRACT

With attention to the risk of improper use of chemicals in the field and after harvest and the consumers' demand for safe and nutritious fruits and fruit products, studies on alternative disease management that reduces the excessive use of fungicide in mango fruit production are essential. Plant hormones play a central role in the regulation of plant immune responses. This study explored the potential of applying plant growth regulators in mango fruit production in controlling the field and postharvest diseases in mango. The farmers' practice of mango fruit production in Davao del Sur was followed but modified by substituting the fungicide application with different combinations of plant growth regulators (PGRs) from panicle elongation (at 14th day from flower induction) until full bloom (at 28th day from flower induction). The PGRs used in the study are auxin, cytokinin, gibberellic acid, and salicylic acid. The application of PGR reduced the severity of blossom blight by 48.33-71.67% compared to farmers' practice, resulting in higher fruit setting and retention. The yield of PGR treated mango trees was 48.14-81.91 kg per tree, which is generally slightly higher than trees following the farmers' practice (54.52 kg). The fruit disease incidence and other postharvest qualities of the fruits from PGR treated trees were comparable to those from other trees following the farmers' practice and integrated crop management system. This study showed that PGRs help increase plant resistance to diseases thus might help reduce the use of fungicide during fruit production.

Keywords: anthracnose, auxin, cytokinin, gibberellic acid, salicylic acid, fungicide

There is intensive use of agrochemicals in mango production, especially during flowering, fruit set, and fruit growth. Diseases such as anthracnose, Alternaria rot, bacterial black spot, blossom blight, and powdery mildew, are the primary production constraints in virtually all areas where mango is grown. Practical strategies for managing these diseases are often limited. The disease management in commercial production relies heavily on synthetic pesticides, primarily where disease-conducive environments exist and when export quality fruit are desired (Ploetz 2004). With attention to the risk of improper use of chemicals in the field and after harvest and the consumers' demand for safe and nutritious fruits and fruit products, studies on alternative disease management that reduces the excessive use of fungicide in mango fruit production are essential. The use of plant growth regulators in mango production may help decrease the heavy dependence on chemical disease control measures. Plant growth regulators (PGR) are chemical substances that govern all the development and growth factors within plants. The application of plant growth regulators to crops modifies hormonal balance and growth, leading to increased yield, enhanced crop tolerance against abiotic stress, and improved physiological traits of crops. The plant hormones ethylene, jasmonic acid, and Salicylic Acid (SA) play a central role in regulating plant immune responses. In addition, other plant hormones such as auxins, abscisic acid (ABA), cytokinins, gibberellins, and brassinosteroids recently emerged as key regulators of plant immunity (Denance et al. 2013). Plants have developed molecular mechanisms to detect pathogens and pests and to activate defense responses, and that plants utilize hormone cross-talk to optimize defenses (Pieterse 2009). This study explored the potential effects of applying plant growth regulators in mango fruit production in controlling the field and postharvest diseases in mango.

The study was conducted in Mabuhay, Bansalan, Davao del Sur, Mindanao, Philippines. The 23 years old 'Carabao' mango trees were used in the experiment. **The Philippine 'Carabao' mango is known worldwide as the best tasting variety of mangoes.** The study was a seven-treatment experiment in a Completely Randomized Design (CRD) replicated six times. Three commercially available PGR products (P) were used in the study. Treatments were the following: T1 – Control (no fungicide and pesticide), T2 – Integrated Crop Management (ICM), T3 – Auxin+ Cytokinin + Gibberellic Acid (GA) (P1), T4 – Auxin + Cytokinin + GA + Calcium Boron (CaB) (P2), T5 - SA + GA + CaB (P3), T6 - P1 and P2 (sequential application), and T7 – Farmers Practice. PGR were used as substitute to fungicide and were applied at panicle elongation, pre-bloom, and full bloom for T3-T5. For T6, the P1 PGR was applied at panicle elongation then P2 applied at pre and full bloom. Paclobutrazol as growth regulator was applied 3 months before flower induction at the rate of 4 mL per linear meter of the mango tree canopy to manipulate uniform and early maturity of the mango leaves in preparation for flower induction. Calcium nitrate was used to induce flowering at a rate of 8 kg per 200 L of water and was sprayed 3 months after

the application of paclobutrazol. Application of pesticides was based on spray schedule, pest population and weather condition. Fruits were bagged and harvested at 50 days and 107 days, respectively, after flower induction.

The exogenous application of PGR, except the sequential application, as a substitute to fungicide following the farmers' practice in mango production effectively reduced the severity of blossom blight. Fruit set at 40 days after flower induction (DAFI), fruit retention at 50 DAFI, and yield of mango trees following farmers' practice and ICM were not statistically different from those applied with PGR. The result implies that the reduced application of fungicide through substituting with PGR is possible with positive effects on the yield of mango trees. The plant hormones SA, auxin, cytokinin, and GA are among the key regulators of plant immunity (Denance et al. 2013). Plants have resistance mechanisms or responses to biotic stress regulated by plant hormones, where their pathways are linked to each other in a complex and ambiguous network. Infection of plants with disease pathogens results in changes in the level of various plant hormones, resulting in changes in the expression of genes and activation of defense responses. The exogenous application of plants with some hormones results in reprogramming the host metabolism, gene expression, and modulation of plant defense responses against microbial challenge (Bari and Jones 2008).

The visual quality and the incidence and severity of anthracnose, stem-end rot, and scab during storage at the ambient condition of mangoes from PGR treated trees were comparable to those mangoes produced using ICM and farmer's practice. Plant growth regulators and even synthetic chemicals did not control the incidence and severity of disease at postharvest. The application of PGR effectively lowers the severity of blossom blight, resulting in comparable fruit set, fruit retention, and yield with those following farmer's practice and ICM. However, the PGR did not control the postharvest disease with similar results to other treatments except control or with no pesticide application. This study showed that PGRs help increase plant resistance to diseases thus might help reduce the use of fungicide during fruit production.

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COMBINED MYKOPLUS BIOFERTILIZER AND INORGANIC FERTILIZER INCREASED STRAW AND RICE GRAIN YIELD IN TWO FIELD SITES IN NEGROS OCCIDENTAL, AS WELL AS SIGNIFICANTLY INCREASED THE RESIDUAL P AND N CONTENT IN THE SOIL AFTER CROPPING

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ABSTRACT

Agricultural practices rely heavily on chemical inputs that causes deleterious effect. Use of biofertilizers, as good agricultural practice, aims to supplement fertilizers. Efficacy testing of Mykoplus biofertilizer on lowland rice was done in two sites in Negros Occidental, arranged in Randomized Complete Block Design with eight treatments and four replications. Treatments include chemical fertilizer at full recommended rate (100%RR) based on soil analysis, 70%RR and 50%RR, then, same fertilizer rates plus Mykoplus, untreated (Control) and only Mykoplus. Highest straw weight and grain yield was obtained in Mykoplus+100%RR at 17.85 t/ha, and 7.07 t/ha, respectively (Kabangkalan site), and 14.94 t/ha, 6.37 t/ha, respectively (Murcia site). This gave 16% increase in straw yield and 18% increase in grain yield compared to 100%RR alone. Mykoplus+70%RR also gave comparably high straw yield at 15.41 t/ha (Kabangkalan) and 14.6 t/ha (Murcia) but slightly lower grain yield at 5.42 t/ha (Kabangkalan) and 4.83 t/ha (Murcia). Mykoplus alone, significantly out yielded the control in both sites with 33.4% average increase. At end of cropping, microbial count was similar between treatments on Nutrient Agar medium, but Mykoplus treated soil had significantly higher N_2 fixers and P solubilizers. Furthermore, soil in the Mykoplus+100%RR had higher %P (70% in Kabangkalan and 4% in Murcia) and 3% higher %N compared to 100%RR (Murcia).

Key words: Rice, Biofertilizer, MykoPlus, Microbial Inoculant, Residual nutrients

INTRODUCTION

Rice is the staple food for majority of 105 M Filipinos, and many of the country's work force depends on this commodity. However, for nearly a decade (2011-2020), palay production was not enough to feed the exponentially increasing Philippine population (Statistica, 2021). Bridging this rice yield gap through many biotechnological interventions such as: new high yielding varieties, pest control managements, etc. need further assistance. The use of biological fertilizers or microbial inoculants as supplements to chemical fertilizer has been widely studied to boost yield and preserve the soil, but needs further popularization for greater adoption. Biofertilizers are preparations that contain live or latent cells of efficient strains of microorganisms, which could be nitrogen fixers, phosphorus solubilizers, etc. that accelerate certain microbial processes to release more nutrients for the crop. The objective of this study was to determine the efficacy of biofertilizer Mykoplus in the growth and yield of lowland rice.

MATERIALS AND METHODS

Field efficacy testing of biofertilizer Mykoplus was done in 1) Barangay Biniquil, Kabangkalan city and 2) Central Philippine Adventist College, Barangay Alegria, Murcia, both from Negros Occidental, using lowland rice (*Oryza sativa*) NSIC RC 222. Experiments were arranged in a Randomized Complete Block Design (RCBD) with eight treatments and four replications. Treatments were 1) Control (unfertilized and untreated) 2) 100% Recommended Rate (100%RR) based on soil analysis, 3)70%RR, 4) 50%RR, 5) 100%RR+Mykoplus, 6) 70%RR+Mykoplus, 7) 50%RR+Mykoplus and 8) Mykoplus alone. The 100%RR chemical fertilizer rate were: 30-60-40 kg NPK/ha (Kabangkalan) and 70-40-40 kg NPK/ha (Murcia). Mykoplus was applied at different stages of plant growth (sowing, transplanting and a month after transplanting) by seed coating or spraying, at a recommended rate of nine packs/ha where each pack weighs 300 g. Rice was planted at 20 cm by 20 cm, with plot dimensions of 20 sq m/plot/treatment (4m x 5m), with outer rows (16 plants) for panicle counts, and inner 4 sq m as harvest area (100 plants). At harvest, bacterial count was determined through dilution plate count. Mycorrhizal spores were counted under a stereo microscope after wet-sieving and centrifugation in sucrose solution (Guillen,2021). Soil chemical analysis was done prior to and at end of cropping. Data collected were statistically analyzed using International Rice Research Institute-Statistical Tool for Agricultural Research (IRRI-STAR, version 2.01, 2013)

following RCBD for Analysis of Variance (ANOVA) and Least Significance Difference (LSD) and or Tukey's Honest Significant Difference (HSD) test for mean comparison.

RESULTS AND DISCUSSION

Agronomic traits and yield: Rice plants applied with biofertilizer Mykoplus alone significantly increased straw weight by 17% and grain yield by 33% *vs* the control. Treatment 100%RR+Mykoplus (T5) had the highest number of panicles (339 panicles/sq m), heaviest straw weight (17.8 t/ha) and highest grain yield (7.07 t/ha) in Kabangkalan site, with an average increase of 16% in straw weight and 19% in grain yield compared to 100%RR (T2) in the two sites. Treatment 70%RR+Mykoplus (T6) also had significantly higher number of panicle and straw weight and grain yield, with an average increase of 19% in straw weight and 6% in grain yield *vs* 70%RR (T3). Treatment 50%RR+Mykoplus (T7) also increased straw weight with an average of 23% and grain yield by 8% vs 50%RR (T4).

Economic analysis: Considering cost of production, gross and net yield, the highest net income was in 100%RR+Mykoplus (T5) at Php 47,520, which also had the highest Return on Investment (ROI) of 98.94% and highest benefit due to Mykoplus inoculation at Php 11,700/ha (Murcia). Similarly, in Kabangkalan site, highest net income was in 100%RR+Mykoplus at Php 53,026, with ROI of 107.15% and benefit due to Mykoplus inoculation of Php 14,296/ha. Treatment 70%RR+Mykoplus (T6) gave the next highest ROI at 81.95% and 75.22%, in both sites.

Microbial count: Total bacterial count detected through spread plating in Nutrient agar was not significantly different between treatments in both sites, with average count of 1.1×10^7 cfu/g soil. However, in differential medium for nitrogen fixers (Burk's and Mannitol Medium), the control consistently and significantly had the lowest value at 3.1×10^5 and 3.6×10^6 cfu/g soil, respectively (Kabangkalan site). Meanwhile the highest count was in treatment 70%RR+Mykoplus (T6) at 9.5×10^5 cfu/g (Burk's medium), 9.1×10^6 cfu/g (Mannitol medium). Best microbial growth in National Botanical Research Institute Phosphate (NBRIP) medium was observed in all treatments with MykoPlus (100%RR, 70%RR, 50%RR) and Mykoplus alone with bacterial count ranging from at 3.75×10^6 to 4.8×10^6 cfu/g soil. Whereas, bacterial count in the uninoculated control, 100%RR and 70%RR only ranged from 3.1×10^5-7.4×10^5 cfu/g soil (Kabangkalan site). This indicates that microorganisms in Mykoplus may have persisted in the soil as shown in the differential medium and assisted in crop growth.

Mycorrhizal spore count: Mycorrhizal spore count based on 20 g soil samples per treatment, replicated four times, was not significantly different between treatments. Variety of endomycorrhizal spores were observed in most of the treatments in addition to the endomycorrhizal species in Mykoplus. This could possibly be due to the unsterilized soil used in the field trial. This indicates abundance of natural mycorrhiza in the rice rhizosphere.

Residual soil nutrients and pH: Soil pH became more acidic after cropping irregardless of treatments. Lowest pH was in treatments receiving 100%RR (T2 and T5), possibly due to high amounts of chemical fertilizer applied. Soil % N after cropping was higher in Mykoplus applied treatments (T5 to T8) and comparable with treatment 100%RR (T2) and 50%RR (T4) in Kabangkalan site. Residual % P was higher in Mykoplus applied treatments *vs* same rate of chemical fertilizer alone. For example, treatment T5 (100%RR+Mykoplus) had 70% higher %P than T2 (100%RR), T6 (70%RR+Mykoplus) had 4% higher %P *vs* T3 (70%RR), and T8 (Mykoplus alone) had 11% higher %P *vs* 1 (control). This indicates greater availability of nitrogen and phosphorus in the soil for next cropping's use.

CONCLUSION

Beneficial microorganisms in Mykoplus helped in increasing the availability of nutrients for the crop and increasing residual amount in the soil after cropping. Mykoplus applied alone out yielded the control, giving 33% increase in rice grain yield. However, greater and more significant growth promotion and economic benefits were obtained when incorporated with 100%RR and 70%RR. Biofertilizer Mykoplus application can be considered as a good agricultural practice leading towards sustainable farming.

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