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INCIDENCE AND DISTRIBUTION OF PEPPER-INFECTING VIRUSES IN THAILAND

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ABSTRACT

Incidence and distribution of plant viruses infecting peppers in Thailand were surveyed from 2014-2016 to obtain updated information to support development of effective diagnosis and disease management. Field surveys for virus infection were conducted in 15 provinces encompassing 40 pepper-growing locations. Total numbers of 1,319 samples from 33 varieties were tested for virus infection using enzyme-linked immunosorbent assay with locally produced and commercial antibodies specific to chilli veinal mottle virus (ChiVMV), cucumber mosaic virus (CMV), potato virus Y (PVY), tobacco mosaic virus (TMV), tomato leaf curl New Delhi virus (ToLCNDV), tomato necrotic ring virus (TNRV), watermelon silver mottle virus (WSMoV) and pepper mild mottle virus (PMMoV). Of this sample number, 567 were positive to at least one pepper virus. Mean disease incidence (DI) indicated the highest value in the northern (93.41±8.62) followed by northeastern (92.60±9.99) and central (73.80±25.04) regions. The highest DI was in Uttaradit, Phitsanulok and Surin provinces (100%). Overall identified virus incidence was 42.99% whereas the highest value was in Chiang Rai province (72.31%). CMV was the major species among 3 surveyed regions (29.11%) followed by ChiVMV (11.83%), tospovirus serogroup IV (6.67%), PMMoV (5.53%), ToLCNDV (1.21%), PVY (1.14%), TMV (0.53%) and TNRV (0.45%). Mean disease severity showed the highest value in the northeastern (3.00±0.58) followed by northern (2.55±0.54) and central (2.05±0.86) regions. The Yok Siam pepper variety was found to be very susceptible to all viruses examined. Among mixed infections, incidence rate of ChiVMV + CMV was the highest at 41.8%. The information from this research provides useful information to support development of effective disease diagnosis and management for peppers in Thailand.

Key words: disease survey, plant virus, *Capsicum* spp.

INTRODUCTION

Pepper (*Capsicum* spp.) originated in regions of Southern Peru and Bolivia in South America. It is now one of the most widely cultivated vegetable crop worldwide and is grown extensively under various environmental and climatic conditions in more than 60 countries, covering a total annual production of approximately 34.5 million tons worldwide. The majority of chilies and peppers are produced in Asia and Europe with 64.9% and 11.5%, respectively (Lin et al. 2013; FAOSTAT 2016). The growing areas of peppers in Thailand covered more than 18,000 hectares and doubled by 2019, with over 60% being planted to small erect-fruited chili in 2016. The main production area for small pepper is in the northeast followed by the north and the east of Thailand (DAE 2019). Peppers are used in some local dishes all over the country although the types and quantities used vary in different geographical areas. Two main pepper types are grown in Thailand; the Cayenne or big group (*C. annuum*) and the small hot group (*C. annuum* and *C. frutescens*) (Kraikruan et al. 2008). Generally, these are dried, ground and then processed into viscous liquid or powder, which is then used as spice. Viral diseases are considered a major limiting factor in pepper production contributing to yield losses and low quality. Many viruses known to infect peppers cause a wide range of symptoms such as mosaic, mottle, deformation, leaf spots, stunt, vein banding, necrosis, blistering and ring spots.

Pepper viruses in 13 provinces in Thailand were surveyed from 1989 to 1991 (Kittipakorn et al. 1993). Examination was conducted using enzyme-linked immunosorbent assay (ELISA) and the results indicated the prevalence of chilli veinal mottle virus (ChiVMV), cucumber mosaic virus (CMV), potato virus Y (PVY), tobacco etch viruses (TEV), pepper mottle virus (PepMoV), tobacco mosaic virus (TMV), and pepper mild mottle virus (PMMoV). Updated information regarding infecting viruses is necessary for development of proper diagnostic tools and resistant cultivars for different pepper crops and locations. In addition, another part of our project sought to produce on-site test kits for single and mixed infections of major pepper viruses. Therefore, this study was conducted to investigate incidence and distribution of plant viruses infecting peppers in major production areas in the north, northeast and central Thailand.

MATERIALS AND METHODS

Survey of virus incidence. Field surveys of pepper virus infection were conducted from April 2014 to September 2016 in major pepper-growing areas among 15 provinces in the northern (Chiang Rai, Chiang Mai, Phrae, Uttaradit and Phitsanulok provinces), central (Nakhon Pathom, Kanchanaburi, Ratchaburi and Phetchaburi provinces) and northeastern regions of Thailand (Chaiyaphum, Nakhon Ratchasima, Buri Ram, Surin, Si Sa Ket and Ubon Ratchathani provinces) (Meteorological Department of Thailand 2020). Twenty samples were randomly collected per the area of one rai (2.53 rai = 1 acre) based on the guideline for surveillance for plant pests in Asia and the Pacific (McMaugh 2008). When plants were grown in rows within 1 rai, Format 1 was applied however, if plants were grown more than 1 rai, Format 2 was applied (Fig. 1). Pepper leaves were randomly collected at the fruiting stage (4-8 months) from the middle part of plant. Symptoms were recorded and percentage of infection was visually estimated based on the extent of leaf damage and disease scoring scales of 0-5.

Fig. 1. Field sampling design per the area of one rai based on the guideline for surveillance for plant pests in Asia and the Pacific (McMaugh 2008).

Virus identification using ELISA method. All collected pepper leaf tissue samples were diagnosed for virus infection using locally produced antibodies specific to ChiVMV, CMV, PVY, TMV, tomato leaf curl New Delhi virus (ToLCNDV), tomato necrotic ring virus (TNRV), watermelon silver mottle virus (WSMoV) and a purchased antibody to PMMoV (Agdia USA).

Indirect plate-trapped antigen ELISA (PTA-ELISA) was performed according to Crowther (2001) with minor modification. Wells in microtiter plates were coated with 50 µL/well of clarified, ground plant sap in sodium carbonate buffer, pH 9.6 (1:10 w/v) and incubated overnight at 4ºC. Leaf extract from healthy pepper leaf tissue was used as a negative control while positive controls were purchased from Agdia (USA). The coated wells were washed with phosphate buffered saline (PBS) and then 4% dried skimmed milk in PBS was added with 37ºC incubation for 1 h to block non-specific reaction. An optimum dilution of each virus-specific antibody was added at 50 µL/well and incubated for 2 h at 37ºC. Goat anti-rabbit IgG conjugated with alkaline phosphatase at a 1:30,000 dilution was added and incubated for 1 h at 37ºC. Each step was followed by three washes with PBST (PBS with 0.05%.Tween 20) at 5 min incubation. The enzyme reactivity was measured after 60 min incubation at 37°C with *p*-nitrophenyl phosphate (1 mg/mL) in diethanolamine buffer, pH 9.8. For anti-PMMoV antibody, double-antibody sandwich ELISA was performed according to the manufacturer (Agdia USA). Reactions from both PTA-ELISA and DAS-ELISA were measured spectrophotometrically at 405 nm using a MultiscanEX ELISA reader (Labsystems, Finland). A sample was considered positive if the absorbance was greater than twice of the negative control.

Disease incidence and severity. In each field, incidence and severity were estimated visually at several selected sampling sites. Virus symptom severity was scored on a scale of 0-5, based on the extent of leaf damage and percentage number of leaves showing symptoms such as mosaic, mottle, chlorotic blotches, leaf distortion, systemic chlorosis and yellow. The rating scales were as follows: L0 = no disease on leaf (0%), L1 = very mild (1-20%), L2 = mild (21-40%), L3 = severe (41-60%), L4 = very severe (61-80%) and L5 = almost dead (81-100%) (Fig. 2). Disease incidence, severity and infection index were calculated according to Allen et al. (1983). The data for DI and DS were analyzed by analysis of variance (ANOVA) at the significance level of 0.05 followed by Tukey's honest significant difference test.

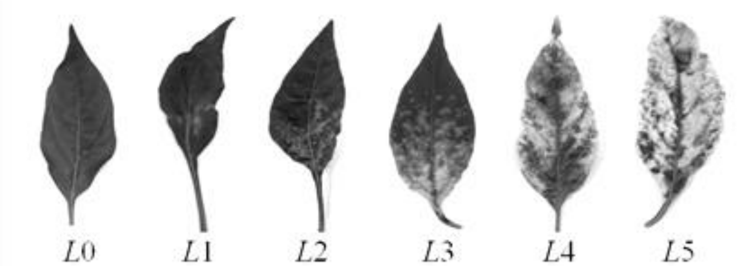

Fig. 2. Disease assessment score to determine the severity of symptoms: *L*0 = 0% (no disease on leaf), *L*1=1-20% leaf area (very mild), *L*2=21-40% leaf area (mild), *L*3=41-60% leaf area (severe), *L*4=61-80% leaf area (very severe) and *L*5=81-100% leaf area (almost dead).

RESULTS AND DISCUSSION

Detection and identification of virus diseases in the pepper samples. Pepper leaf samples collected from field surveys in fifteen provinces including forty locations were tested with specific antibodies to eight viruses as mentioned above. The results showed that 42.99% of the overall collected samples (567/1319) were positive to at least one virus. The incidence of viruses was highest for CMV (29.11%) followed by ChiVMV (11.83%), tospovirus serogroup IV (6.67%), PMMoV (5.54%), ToLCNDV (1.21%), PVY (1.14%), TMV (0.53%) and TNRV (0.46%) (Table 1). Based on the identified virus incidence and distribution, CMV was the major species among 3 surveyed regions. Since anti-WSMoV PAb, used for the detection of tospoviruses, recognizes more than one of the Tospovirus serogroup IV including capsicum chlorosis virus (CaCV) and WSMoV/GBNV (groundnut bud necrosis virus) positive samples from Agdia (USA), specific identification of the infecting species requires a more specific method.

Table 1. Detection of virus incidence in pepper samples collected from 15 provinces in Thailand, 2014-2016 survey

Region	**Province**	**No. of samples collected**	**No. of virus incidence**								**Positive samples**	**Virus incidence (%)**
			ChiVMV	**CMV**	**PMMoV**	**PVY**	**TMV**	**TNRV**	**ToLCNDV**	**WSMoV***		
North	Chiang Rai	130	66	33	34	8	0	4	0	7	94	72.31
	Phrae	165	34	98	1	1	2	1	1	3	107	64.85
	Uttaradit	20	3	6	0	0	0	0	0	4	9	45.00
	Chiang Mai	75	3	0	10	0	4	1	5	10	25	33.33
	Phitsanulok	86	0	25	0	0	0	0	0	0	25	29.07
Total		476	106	162	45	9	6	6	6	24	260	54.6
Virus incidence (%)			22.27	34.03	9.45	1.89	1.26	1.26	1.26	5.04		
Central	Kanchanaburi	331	17	82	23	6	0	0	2	46	133	40.18
	Ratchaburi	18	3	5	0	0	0	0	0	0	7	38.89
	Nakhon Pathom	200	5	8	0	0	0	0	5	0	15	7.50
	Phetchaburi	14	0	0	0	0	0	0	0	0	0	0.00
Total		563	25	95	23	6	0	0	7	46	155	27.5
Virus incidence (%)			4.44	16.87	4.09	1.07	0	0	1.24	8.17		
North-east	Si Sa Ket	80	0	51	0	0	0	0	0	0	51	63.75
	Buri Ram	40	10	14	1	0	0	0	1	3	26	65.00
	Surin	40	15	15	2	0	0	0	2	4	23	57.50
	Nakhon Ratchasima	50	0	26	0	0	0	0	0	8	28	56.00
	Chaiyaphum	30	0	13	2	0	1	0	0	3	16	53.33
	Ubon Ratchathani	40	0	8	0	0	0	0	0	0	8	20.00
Total		280	25	127	5	0	1	0	3	18	152	54.3
Virus incidence (%)			8.93	45.36	1.79	0	0.36	0	1.07	6.43		
Total		**1,319**	**156**	**384**	**73**	**15**	**7**	**6**	**16**	**88**	**567**	
Virus incidence (%)			**11.83**	**29.11**	**5.53**	**1.14**	**0.53**	**0.45**	**1.21**	**6.67**		

* Anti-WSMoV antibody utilized in this experiment recognizes CaCV and WSMoV/GBNV positive samples from Agdia.

The highest percentage of virus incidence (72.31%) was observed in Chiang Rai province with six viruses detected including ChiVMV (66/130), PMMoV (34/130), CMV (33/130), PVY (8/130), Tospovirus serogroup IV (7/130) and TNRV (4/130); followed by Buri Ram (65%), Phrae (64.85%), Si Sa Ket (63.75%), Surin (57.50%), Nakhon Ratchasima (56%), Chaiyaphum (53.33%), Uttaradit (45%), Kanchanaburi (40.18%), Ratchaburi (38.89%), Chiang Mai (33.33%), Phitsanulok (29.07%), Ubon Ratchathani (20%) and Nakhon Pathom (8%). There was only one province, Phetchaburi, where no virus incidence was found in any sample (Table 1). Conversely, all viruses examined were detected in the samples from Phrae with the highest incidence of CMV (98/165) followed by ChiVMV (34/165) while the rest of virus incidences were observed in low numbers (1-3 positive samples).

Mixed infections. Twenty-seven combinations of mixed virus infections in the pepper samples are presented in Table 2. Interestingly, the highest number of mixed-infected samples was found in Chiang Rai at 41.8% (56/134) in which almost all of them contained ChiVMV. Among mixed infections, the combination of ChiVMV and CMV was found to be highest as 41.8%. The highest number of virus species in mixed infection was eight species whereas most of them contained 2-3 species of viruses (126/134).

Virus incidence and severity were highly variable among geographical locations. Symptoms observed in the surveyed areas were diverse which consisted of mosaic, mild mottle, mottle, chlorotic blotches, leaf distortion, systemic chlorosis and yellowing. However, the prominent symptoms of individual virus infection were: mosaic, mild mottling and systemic chlorosis associated with ChiVMV, CMV, PMMoV and PVY; mosaic and leaf distortion associated with TMV; leaf mottle and distortion associated with TNRV; mosaic, systemic chlorosis and yellowing associated with ToLCNDV; chlorotic blotches, systemic chlorosis and yellowing associated with Tospovirus serogroup IV. Some of the negative samples in ELISA displayed virus-like symptoms such as mosaic, blistering, deformation and size reduction. This might indicate the other causal agents for these symptoms, not only other viruses but also physiological disorders, therefore symptoms are not recommended to be used for virus identification.

The earlier survey indicated the presence in descending order of prevalence of ChiVMV (56.96%), CMV (26.67%), PVY (24.35%), TEV (11.88%), PepMoV (9.97%), TMV (4.86%), and PMMoV (1.92%) (Kittipakorn et al. 1993). From 2016 to 2019, pepper viruses including begomoviruses, ChiVMV, CMV, PVY, TMV and TNRV were detected in 13 provinces of Thailand (Laprom et al. 2019). These surveys revealed the incidence of the viruses infecting pepper in Thailand was high, being identified in nearly 70% (1,482/2,149) of the collected samples. The highest virus incidence was found in the central (96%), followed by northern (74.4%) and northeastern (52.8%) regions, respectively. Begomoviruses (32.7%), ChiVMV (21.5%), CMV (25.7%), TNRV (19.5%) were detected at varying rates whereas PVY, TMV and TSWV were not detected (31.0% negative samples). Compared to our observations, the results are consistent for ChiVMV and CMV which were present in all regions surveyed and these viruses were the most common in the mixed infection in both the present survey and Laprom et al. (2019). The percentage of PMMoV-infected samples increased from 1.92% from the previous survey (Kittipakorn et al. 1993) to 5.53%, especially in the north. Although the value was not very high, this virus can remain infective for many months in alternative weed hosts, infects some other solanaceous plants and be easily spread by contact (Agrios 2005). Therefore, it can lead to contamination in seed production and export. Since there was no antibody for this virus available in Thailand then, polyclonal and monoclonal antibodies were produced from PMMoV antigen and rapid test kits were developed for this virus and 4 pepper-infecting tobamoviruses (Phatsaman et al. 2020). Begomoviruses were not reported in the 1989-1991 survey which might be due to no evidence of symptoms associated with this pathogen observed in the production fields. However, increasing incidence and prevalence of begomoviruses were reported, not only in peppers but also in tomatoes and cucurbits, in 2014-2019 (Charoenvilaisiri et al. 2020; Laprom et al. 2019; Malichan et al. 2019).

Table 2. Mixed infection detected by ELISA in peppers collected from Chiang Rai (CRI), Chiang Mai (CMI), Phrae (PRE), Uttaradit (UTT), Phitsanulok (PLK), Nakhon Pathom (NPT), Kanchanaburi (KBI), Phetchaburi (PBI), Ratchaburi (RBR), Chaiyaphum (CPM), Nakhon Ratchasima (NMA), Buri Ram (BRM), Surin (SRN), Si Sa Ket (SSK) and Ubon Ratchathani (UBN) provinces.

Mixed infection of viruses	**Northern region**					**Central region**				**Northeastern region**						**Virus-infected samples**
	CMI	**CRI**	**PLK**	**PRE**	**UTT**	**KBI**	**NPT**	**RBR**	**BRM**	**CPM**	**NMA**	**PBI**	**SRN**	**SSK**	**UBN**	**(%)***
ChiVMV, CMV	0	18	0	25	1	2	3	1	1	0	0	0	5	0	0	41.8
CMV, Tospovirus gr.IV	0	0	0	0	1	11	0	0	0	0	6	0	1	0	0	14.2
ChiVMV, Tospovirus gr.IV	0	3	0	0	0	4	0	0	0	0	0	0	2	0	0	6.7
ChiVMV, PMMoV	1	7	0	0	0	0	0	0	1	0	0	0	0	0	0	6.7
ChiVMV, CMV, PVY	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	4.5
CMV, PMMoV	0	1	0	0	0	1	0	0	0	1	0	0	1	0	0	2.9
ChiVMV, PVY	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	2.2
ChiVMV, TNRV	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2.2
ToLCNDV, Tospovirus gr.IV	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	2.2
ChiVMV, PVY, Tospovirus gr.IV	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1.5
ChiVMV, CMV, PVY, Tospovirus gr.IV,	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	1.5
ChiVMV, CMV, Tospovirus gr.IV	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1.5
ChiVMV, CMV, PMMoV	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1.5
ChiVMV, CMV, ToLCNDV, Tospovirus gr.IV	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0.7
ChiVMV, TNRV, Tospovirus gr.IV	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
ChiVMV, CMV, PMMoV, TNRV	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
ChiVMV, PMMoV, Tospovirus gr.IV	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7

Mixed infection of viruses	Northern region					Central region					Northeastern region					Virus-infected samples
	CMI	CRI	PLK	PRE	UTT	KBI	NPT	RBR	BRM	CPM	NMA	PBI	SRN	SSK	UBN	(%)*
ChiVMV, CMV, PMMoV, TNRV, Tospovirus gr.IV	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0.7
PMMoV, Tospovirus gr.IV	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
CMV, PMMoV, Tospovirus gr.IV	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0.7
CMV, PVY, Tospovirus gr.IV	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0.7
ChiVMV, TMV, Tospovirus gr.IV	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
PMMoV, TMV	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
ChiVMV, CMV, PMMoV, PVY, TMV, Tospovirus gr.IV, TNRV, ToLCNDV	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0.7
TMV, ToLCNDV	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
TMV, ToLCNDV	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0.7
TMV, ToLCNDV, Tospovirus gr.IV	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.7
Total	**8**	**46**	**0**	**27**	**3**	**24**	**3**	**1**	**3**	**3**	**6**	**0**	**10**	**0**	**0**	

* Percentage of the particular mixed infection among the total number of mixed infected samples (134 samples).

Disease incidence and severity. The highest disease incidence (100±0.00) recorded was in Uttaradit, Phitsanulok and Surin provinces followed by Nakhon Ratchasima (98.22±2.52), Chiang Rai (96.97±4.29), Si Sa Ket (95.00±10.00), Buri Ram (92.50±10.61), Chaiyaphum (90.00±14.14), Phrae (89.01±8.31), Kanchanaburi (84.38±20.06), Chiang Mai (80.00±0.00), Ubon Ratchathani (77.50±3.54), Nakhon Pathom (50.50±0.00) and Ratchaburi (38.89±0.00). A significant effect of region location was observed with the highest incidence recorded in the northern region (93.41±8.62a) followed by northeastern (92.60±9.99a) and central regions (73.80±25.04b) which was significantly different from the former 2 regions.

Disease severity investigation, based on mean severity values, showed the highest severity in the northeastern (3.00±0.58a) followed by northern (2.55±0.54ab) and central regions (2.05±0.86b). Variation of severity was observed among the pepper samples with the highest DS in Surin (3.58±0.53) and the lowest in Ratchaburi province (0.94±0.06). Region-based analysis of mean infection index showed that virus diseases of peppers displayed moderate to very severe infection indices and corresponded to the disease severity reported above. The highest infection index was in the Northeast (60%) followed by the North (50.94%) while the Central had the lowest value (41.05%).

Incidence of viruses detected in different pepper varieties revealed the most susceptible variety was Yok Siam in which all viruses analyzed could be detected (Table 3). Most varieties were infected with at least one virus species except three varieties including the Saban-Nga, Rocket Chili and Chai Buri. However, some of these varieties might have been present in too small sample number to substantiate this conclusion. The severity was also affected by the growth stage of the pepper plant. In the northeastern region, most pepper plants had an average age of 3 months and were susceptible to the viruses, as shown by the highest DS value, followed by the northern and central regions where the average pepper plants during the collection periods were aged 5 and 6 months, respectively. The younger the age of the peppers, the higher the risk of virulence. Analysis of the relationship between disease incidence and severity from forty locations in fifteen provinces revealed that the severity increased linearly as incidence increased (data not shown).

Expansion and intensification of pepper cultivation also affected DI and DS. For example, peppers in the northeast were grown for industrial seasonings and most of them were in the Small hot group. The planting areas were large with frequency of planting spacing aiming to increase the number of pepper plants as observed in Chaiyaphum, Nakhon Ratchasima, Surin and Sisaket provinces. These planting conditions would likely enhance the field spread of viruses from the infested plant to neighboring plants. In the central region (Kanchanaburi and Nakhon Pathom provinces), peppers were planted wide apart with a furrow system for easy watering, whereas in the north, the Big pepper group was planted in separate rows to avoid fruit damage when handling such as in Phrae but in Chiang Mai, these were grown in greenhouses. This condition reduces potential transmission by insect vectors for several viruses and might be the reason for less severity of diseases in the Big pepper group.

In addition, the growing season also influenced disease occurrence and severity. Climate change is expanding the geographic range suitable for the viruses and vectors. Analysis of the relationship between DI and DS showed that severity increased linearly with increased incidence. Although the pepper-growing season in Thailand varies by geographical areas and it is not possible to definitively identify the proper pepper-planting period. Growing in late summer before the rainy season in order to break the cycle of disease is recommended to avoid virus infection (Pakuthai et al. 2015).

Table 3. Incidence of viruses detected in different pepper varieties using ELISA method.

Species	Type	Variety	Region*	No. of samples collected	Number of virus incidence								Total no. of virus species
					TMV	PMMoV	ChiV MV	PVY	Tospovirus gr. IV**	TNRV	CMV	ToLCNDV	
Capsicum annuum	Bell gr. / Big gr.	Spider	N	17	1	0	0	0	4	0	0	3	3
C. annuum	Bell gr./ Big gr.	Sunnyez	N	58	3	10	3	0	6	1	0	2	6
C. annuum	Cayenne/ Big gr.	Jomthong 2	N	1	0	1	0	0	0	0	0	0	1
C. annuum	Cayenne/ Big gr.	Manee-Karn	N	1	0	1	0	0	0	0	0	0	1
C. annuum	Cayenne/ Big gr.	Pichai	N/C	30	0	0	3	0	7	0	10	0	3
C. annuum	Cayenne/ Big gr.	Prik Chi Fa	C	39	0	0	3	0	0	0	10	0	2
C. annuum	Cayenne/ Big gr.	Prik Num	C	19	0	0	0	0	9	0	0	0	1
C. annuum	Cayenne/ Big gr.	Saban-Nga	N	2	0	0	0	0	0	0	0	0	0
C. annuum	Cayenne/ Big gr.	Salika	N	15	0	3	12	0	1	2	4	0	5
C. annuum	Cayenne/ Big gr.	Wat Bot	C	102	0	0	0	0	1	0	34	0	2
C. annuum	Cayenne/ Big gr.	Yellow Pepper	C	18	0	0	1	0	0	0	7	0	2
C. annuum	Cayenne/ Big gr.	Yok 31	N	41	0	2	3	0	0	0	8	0	3
C. annuum	Cayenne/ Big gr.	Yok Sawan	N	7	0	3	5	0	2	1	0	0	4
C. annuum	Cayenne/ Big gr.	Yok Siam	N	174	2	2	37	1	5	1	98	1	8
C. annuum	Small hot gr.	Bird Eye Pepper	N/C	56	0	6	25	8	1	0	29	1	6
C. annuum	Small hot gr.	Chinda	C	86	0	4	1	0	10	0	30	2	5

Species	Type	Variety	Region*	No. of samples collected	Number of virus incidence								Total no. of virus species
					TMV	PMMoV	ChiVMV	PVY	Tospovirus gr. IV**	TNRV	CMV	ToLCNDV	
C. annuum	Small hot gr.	Double Hot	N	5	0	2	4	0	0	1	2	0	4
C. annuum	Small hot gr.	Haurue	C	11	0	0	0	0	3	0	0	0	1
C. annuum	Small hot gr.	Hot Chili	N/NE	161	0	4	11	0	1	0	14	3	5
C. annuum	Small hot gr.	Maxi	N	2	0	1	0	0	0	0	0	0	1
C. annuum	Small hot gr.	Taiwan	C	30	0	0	9	5	20	0	14	0	4
C. annuum	Small hot gr.	Pha-Nom-Pai	N	3	0	3	0	0	1	0	0	0	2
C. annuum	Small hot gr.	Red Hot	N	8	0	2	4	0	0	0	0	0	2
C. annuum	Small hot gr.	Rocket Chili	N	1	0	0	0	0	0	0	0	0	0
C. annuum	Small hot gr.	Smile Hot	N	5	0	3	2	0	0	0	0	0	2
C. annuum	Small hot gr.	Star Hot	NE	40	0	2	15	0	4	0	13	2	5
C. annuum	Small hot gr.	Super Hot	N/NE	78	1	5	10	0	3	0	26	1	6
C. annuum	Small hot gr.	Three Cherry Pepper	C	37	0	0	0	0	3	0	11	0	2
C. frutescens	Small hot gr.	Amphawa	N/NE	86	0	1	5	0	1	0	58	0	4

Species	Type	Variety	Region*	No. of samples collected	Number of virus incidence								Total no. of virus species
					TMV	PMMoV	ChiV MV	PVY	Tospovirus gr. IV**	TNRV	CMV	ToLCNDV	
C. frutescens	Small hot gr.	Chai Buri	C	16	0	0	0	0	0	0	0	0	0
C. frutescens	Small hot gr.	Hmong	C	112	0	15	3	1	2	0	5	1	6
C. frutescens	Small hot gr.	Karen	C	31	0	2	0	0	0	0	1	0	2
C. frutescens	Small hot gr.	Pop	NE/C	27	0	1	0	0	4	0	10	0	3
Total				**1319**	**7**	**73**	**156**	**15**	**88**	**6**	**384**	**16**	
Virus incidence (%)					**0.53**	**5.53**	**11.83**	**1.14**	**6.67**	**0.45**	**29.11**	**1.21**	

* Geographical regions of Thailand; North (N), Central (C), and Northeast (NE)

** Anti-WSMoV antibody utilized in this experiment recognizes CaCV and WSMoV/GBNV positive samples from Agdia.

Note: Some samples were infected with more than one virus.

CONCLUSIONS

This study focused on the incidence of plant viruses infecting peppers in Thailand from 2014-2016 in 3 regions to obtain useful information for further effective disease management and virus-resistance breeding programs. Peppers were infected with several viruses with different levels of incidence and severity. A significant effect of province and region was observed on disease incidence. The following categories could be the main reasons for disease incidence and severity: growing season, growth stage, pepper variety and also geographical area of sampling. Severity increased linearly with increased incidence. Organized epidemiological knowledge can help determine virus occurrence and forecast possible diseases in order to prevent viruses from infecting peppers as well as provide useful information for breeding programs. Efforts made for phytosanitary system should be focused on early detection and diagnostics which will facilitate successful pepper production in Thailand.

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EFFECTS OF SALINITY ON GROWTH, PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF TOMATO

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Salinity stress creates serious problems for tomato production in dry climate regions like Southeast Asia. Two sets of experiments were conducted, where the initial experiment was on effects of salinity on seed germination and seedling characteristics in the laboratory and the second experiment was to evaluate the effects of salinity on growth, physiological and biochemical responses of tomato. These experiments were carried out in the greenhouse of the Laboratory of Tropical Horticulture Science in Tokyo University of Agriculture during 2020 and 2021. Tomato (*Solanum lycopersicum* L. cv. Micro-Tom) was used as plant material and sodium chloride was used as salt. The experiment was designed in complete randomized design with 4 salt treatments (50, 100, 150, and 200 mM), and control (no sodium chloride). In the initial experiment, it was found that the germination rate, shoot and root length were significantly reduced under saline conditions. In the second experiment, the seeds were first grown in vermiculite, and 3-4 leaf seedlings were transplanted into a hydroponics system where the saline treatments were applied. It was observed that salinity, at the rates tested, decreased plant height, root length, the number of flowers, photosynthetic rate, transpiration rate, and stomatal conductance, but it increased leaf temperature. Moreover, sugars decreased under salinity, while organic acids, MDA and proline content increased. Proline and MDA are produced in response to salt stress. Accordingly, fruit yield was reduced under salinity as compared to control.

Key words: hydroponics, photosynthetic rate, sugars, organic acid, malondialdehyde (MDA), and proline.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is a vegetable with immense economic importance and is grown worldwide (Saito et al. 2011). The demand for tomatoes is increasing with the rapid increment in population, and in the near future, the demand for tomato will increase while there will be low production (Hernández-Pérez et al. 2020) due to land unavailability, and stagnation in yield due to biotic, and abiotic stress factors which are the main constraints for tomato producers around the globe. It is estimated that by the year 2050, salt stress alone will cause a 50% loss in yield of vegetables including tomato (Wang et al. 2003), and it will be a huge concern for southeast Asian countries.

The negative effect of salt stress on tomatoes is a result of retarded plant growth due to decline in photosynthetic rate (Giannakoula and Ilias 2013), which leads to a reduction in fruit size and total yield per plant, which are the most important factors for tomato producers. Moreover, in the case of growth, it has been reported that tomato plants under salinity stress have shorter plant height and lower leaf area. Also, leaf relative chlorophyll content (SPAD value) which shows the amount of nitrogen, the key element for enhancement of photosynthesis, could also be affected under saline conditions (Zhang et al. 2017). Tomato seeds are very sensitive to salinity as germination and vigor can be compromised due to small amounts of salt. Additionally, salinity is supposed to decelerate the growth of tomato seedlings at the initial growth stage (Singh et al. 2012). Plant roots become shorter and the ability to absorb water and nutrients decreases under salinity and it leads the plant to be stunted. Therefore, a disruption of photosynthesis in leaves will occur and there will be shortage of water in leaves for transpiration and cooling (Ismail et al. 1994). Furthermore, if leaf temperature increases, plants start to produce chemical compounds such as proline and malondialdehyde (MDA) to combat the shock, and electrons start to leak from the cells (Gharsallah et al. 2016). Interestingly, salinity in a small amount somehow can improve the fruit quality, and the reason is due to the concentration of sugars in small fruits. However, fruit quality in plants grown under high salinity is still questionable (Zhu et al. 2018). Plants grown under salinity, produce fruits with higher organic acids even in pre and post-harvest stages (Zushi and Matsuzoe 2006). Currently, in many countries people use the green tomatoes with high organic acids to make pickle and serve it as food supplement (Locato et al. 2013), and people in developed countries like Japan appraise fruit quality and like to buy fruits with higher Brix, sugar content, and superior taste (Johkan et al. 2014; Saito and Matsukura 2015).

Therefore, the current study was carried out to explore the effects of salinity on (a) germination and seedling growth of tomato, (b) growth, physiological characteristics and yield of tomato, and (c) biochemical attributes of tomato fruits.

MATERIALS AND METHODS

Two experiments were conducted from 2020 to 2021 to investigate tomato response under saline conditions in both seedling and fruit bearing stages. The seedling experiment was done in the laboratory of Tropical horticultural science from June to July 2020, while the second one was carried out from October 2020 to March 2021 under greenhouse conditions in the green house belonging to Tropical Horticultural Science laboratory, Tokyo University of Agriculture, Japan. Tomato (*Solanum lycopersicum* L. cv. Micro-Tom) seeds were used as plant material and sodium chloride as salt.

In the first experiment, tomato seeds were subjected to germination in Petri dishes under normal and saline conditions and placed in a growth chamber (CHF-405 Cultivation Chamber, Japan) to determine percent of germination and seedling growth. In each treatment, 100 seeds were used, and the experiment was designed in a complete randomized design (CRD) with three replications. The daily light was adjusted for 12 hours, and the temperature was set up at 25 ºC. Observation was done daily, and watering done when needed. In the second experiment, Micro-Tom seeds were cultivated first in rockwool under normal conditions, then at 3-4 leaf stage, transplanted to a hydroponics system in the greenhouse, and the salinity treatments were applied. The hydroponics system was set using solution reservoir separately for each treatment, a cork board to keep the plants on the solution and a pump for aeration once in 7:00 – 8:00 AM and once 04:00 – 05:00 PM. For hydroponics solution, tap water was used. OAT House 1 and 2 fertilizers (OAT Agrio Co., Ltd Japan) were used for making the hydroponics nutrient solution, while according to OAT Agrio company recommendation, OAT 1 (OAT green) was used 1 kg / 1000 liters of water and OAT 2 (OAT blue) as 1.5 kg / 1000 liters of water. The OAT House 1 contains 11 % NO3-N and 16.4 % calcium. The OAT House contains 10 % nitrogen (N), 8 % phosphorus (P), 27 % potassium (K), 4 % magnesium (Mg), 0.10 % manganese (Mn), 0.18 % iron (Fe), 0.002 % copper (Cu), 0.006 % zinc (Zn), and 0.002 % molybdenum (Mo).

Salt stress. Salinity treatments were set as: Control (no NaCl), T1 (50 mM NaCl), T2 (100 mM NaCl), T3 (150 mM NaCl), and T4 (200 mM NaCl). In experiment 1, saline solution was applied (according to mM) every two days. In experiment 2, tap water was used for irrigation, and its pH was adjusted between 5.8 – 6 using sodium hydroxide and hydrochloric acid. Then NaCl was added to the hydroponics solution for the appropriate molarity, mixed well and used for irrigation.

Parameters. In experiment 1, germination capacity was measured using the method by Bam et al. (2006). The relative injury rate in seeds was measured using the formula described by Fetouh and Hassan (2014).

Relative Injury Rate (RIR) = (GC % – GS%)/GC% 1

In formula 1, GC % represents the germination percentage in control plants, and GS% represents the germination percentage in salt treatment.

Root and shoot length was measured using ImageJ software after scanning the seedlings using a digital scanner (EPSON DS-G2000, Japan). Seedling vigor index (Masuthi et al. 2015) and seedling height reduction (Kandil et al. 2012) were calculated using the following formula:

Seedling vigor index (SVI) = L /G %.. 2

Seedling height reduction (SHR) = (LC-LS)/LC ×100.. 3

In formula 2, L represents the seedling length (cm), and G% represents the germination percentage. In formula 3, LC represents seedling length in control, and LS represents seedling length in salt treatment.

Growth, yield and physiological traits. Among the growth parameters, plant height and root length were measured once a week for 6 weeks starting at first flowering. The number of flowers per plant was counted and recorded weekly from the first flowering and continued for five weeks till the end of the flowering stage. Photosynthetic rate, transpiration rate, stomatal conductance, and leaf temperature were measured using an LCi-SD Portable Photosynthesis System (ADC Bioscientific, Hoddesdon, UK) at the fruit-bearing stage. Yield and its components like individual fruit weight, number of fruits per plant, and total yield per plant were recorded when the fruits ripened.

Leaf area measurement. Leaf area was measured using ImageJ software after scanning the leaves using a digital scanner (EPSON DS-G2000, Japan). Five leaves from each treatment were taken for leaf area measurement, and the data recorded for further analysis.

Electrolyte leakage. Electrolyte leakage (EL) was measured at flowering and fruit-bearing stages based on the method described by Hatsugai and Katagiri (2018). Fifteen leaves from each treatment were cut by a 1-cm diameter stainless steel cork borer and kept inside pure water in 2 ml tubes under 25±1°C for 20 minutes. An electrical conductivity meter (LAQUATWIN-S070, Horiba Scientific Ltd., Japan) was used to measure EC and the reading was recorded as EC1. Then the tubes were put in the water bath for 20 minutes under 70 °C. Then, cooled at room temperature and again EC was measured and recorded as EC2. Therefore, EL was calculated using the below formula:

Electrolyte leakage (%) = (EC1/EC2) ×100……………………………………….. 4

Sugars, and organic acid analysis. Sugar contents of tomato fruits (glucose, fructose, and sucrose) were measured with Shimadzu HPLC 2007 system. The amount of sugars were quantified using the method described by Agius et al. (2018). Mature tomato fruits were harvested, crushed in liquid nitrogen and 200 mg of the powder was placed in a 2 ml plastic vials and 1.8 ml of 5% aqueous ethanol was added. The samples were vortexed and centrifuged at 15000 rpm for 15 minutes at 4 °C. From the supernatant, 900 µl was collected and a 900 µl of acetonitrile was added, vortexed and poured into a 2.5

ml syringe equipped with polytetrafluoroethylene (PTFE) 0.20 µm syringe filter unit. The filtrate samples were placed into 2 ml HPLC vials and analyzed for sugars and organic acids by HPLC.

Citric acid and malic acid were measured by ion chromatography following the method described by Agius et al. (2018) with some modifications using a Shimadzu 10AVP (Japan) HPLC fitted with a column series SCR-102H×2 (length 8 mm × 300 mm × 2), at 40 °C and coupled with a Shimadzu CDD 10Avp conductivity detector. These measurements were made under circulation of a mobile phase composed of 100% 3mM $HClO_4$ at a flow rate of 0.8mL/min. Tomato fruit powder (200 mg) was mixed with 1.8 ml ultra-pure water, vortexed and centrifuged at 15000 g for 15 minutes at 4 °C. The supernatant was collected in a 2 ml plastic tube and transferred into a 2.5 ml syringe equipped with a 0.45 µm DISMIC cellulose acetate syringe filter, manufactured by membrane solutions Co. Ltd (Japan). The filtrate samples were poured into 2 ml glass vials for HPLC analysis of organic acids.

Fruit brix was measured by slicing tomato fruits and removing the jelly layer and seeds. The pulp was crushed, and the juice was filtered by two-layer mesh cloth. Brix was measured from the juice using an Asone refractometer and the amount was presented as percentage (%).

Color measurements. Fruit color was quantified using a Handy Colorimeter (NR-3000, Nippon Denshoku Ind., Ltd., Japan). The method used was CIE 1976 L*a*b* method (Anon. 1974, Kuehni 1976). The data was given as numerical a, b and L values separately. The maturity stage was decided by the color of control (no salinity) plants and as a-color shows redness, therefore we made our decision about fruit maturity by considering a-color (Kuehni 1976).

Ethylene production measurement. The fruit weight was measured, and the fruits were kept in 550 ml jars under a black cloth. After one hour, 1 ml gas from the jars was taken by plastic syringe and injected into the GC-FID (Gas Chromatograph-Flame Ionization Detector, GC-14B Japan) with the following parameter: column temperature = 80 °C, injector temperature = 180 °C and detector temperature = 200 °C. The GC column Sunpak-A (Shinwa chemical industries, Japan) was used for ethylene production measurement. The carrier gas was ultra-high-quality nitrogen, and column pressure was 6 kg cm^{-2}. The GC reading was then converted to nL g^{-1} h^{-1} using the following formula:

$$E = [(MR*V)/W]/h \quad \ldots\ldots\ldots \quad 5$$

where E is the amount of ethylene, MR is the GC reading, V is jar volume, W is fruit weight, and h is number of hours of keeping fruits inside jars under a black cloth.

Fruit firmness measurement. A Multilateral Tester model 2519-104 (INSTRON Company), was used to measure fruit firmness when the 1.0 cm diameter plunger pressed tomato fruit at 1 mm/sec speed. Five fruits were measure per treatment.

Malondialdehyde and proline. Malondialdehyde (MDA) was measured according to the method described by Wang et al. (2005) with some modification. Leaf tissue samples (2 g) were collected from each treatment in five replications and homogenized in 6 ml of 100 mM sodium phosphate buffer (pH 6.4) containing 0.5 g polyvinylpolypyrrolidone (PVPP). The homogenized solution was filtered through a cotton cloth, centrifuged at 15,000 rpm for 30 minutes at 4 °C and the supernatant was used directly for assay. The MDA content was determined by adding 2 mL of 0.5 % trichloro-butyric acid (TBA) in 15 % trichloro-acetic acid (TCA) to a 1 mL sample. The mixture was heated at 95 °C using a water bath for 20 minutes and cooled immediately. The absorbance of the supernatant was measured using a spectrophotometer (Hitachi U-1100, Japan) at 532 nm and 600 nm. The level of TBA equivalents (nmol.g^{-1}FW) was equal to {[(A532 – A600)/155,000] × 106}.

Proline content was analyzed by the modified procedure of Gharsallah et al. (2016). Ten leaves were taken from each treatment, and approximately 100 mg of the crushed leaves was homogenized with 250 µl each of methanol (analytical reagent grade) and chloroform (analytical reagent grade) and heated in a water bath for 5 minutes at 37 ºC. Ribitol solution (50 µl) and pure water (175 µl) were added, and vortexed. All samples were centrifuged for 10 minutes at 140 x 100 rpm, and 80 µl of the supernatant from each sample was vaporized using vaporizer, and was placed in a freeze dryer for 24 hrs. A solution of 20 mg of methoxamine hydrochloride and 1 ml of pyridine was prepared and 40 µl of the prepared solution was added to each sample and was heated at 37 ºC for 90 minutes. Finally, 50 µl of MSTFA solution was added to each sample and analyzed for proline using a gas chromatograph-mass spectrophotometer (GCMS-QP2010 Plus Shimadzu, Japan). The amount of free proline was quantified using a standard curve and expressed as µmole g^{-1} tissue fresh weight.

Statistical analysis. The experiment was laid out as a completely randomized design. The data were analyzed with analysis of variance (ANOVA), Pearson's correlation analysis, and the principal component analysis (PCA) method with language R 3.6.2 statistical software and data visualization by Python 3.7.4 (Jupyter Notebook, https://api.anaconda.org). The means were compared using Tukey's test at 0.05 level.

RESULTS AND DISCUSSION

Germination characteristics and seedling growth. Salinity stress caused severe injury to tomato seeds by decreasing germination and increased relative injury rate (Table 1). Likewise, seedling vigor index and seedling height were significantly reduced under saline conditions. About 91.4 % of relative injury rate was observed in high salinity (T4) while it was 0 % in control. Also, a significant seedling height reduction was observed in salinity treatments as compared to control.

Table 1. Germination characteristics and seedling growth parameters.

Treatments	Germination (%)	RIR (%)	SVI	SHR
Control	97.67 ± 1.52 a	0.00 ± 0.00 e	0.04 ± 0.006 c	0 ± 0.00 e
50 mM	84.33 ± 2.76 b	13.65 ± 2.93 d	0.02 ± 0.002 d	0.43 ± 0.03 d
100 mM	31.67 ± 4.04 c	67.57 ± 4.56 c	0.04 ± 0.005 c	0.55 ± 0.05 c
150 mM	15.83 ± 2.25 d	83.79 ± 6.83 b	0.06 ± 0.003 b	0.64 ± 0.04 b
200 mM	8.33 ± 3.35 e	91.47 ± 8.44 a	0.09 ± 0.007 a	0.73 ± 0.06 a
	***	***	**	**

RIR: Relative injury rate, SVI: Seedling vigor index, SHR: Seedling height reduction. Data is represented as 'mean ± SD' and different letters are shown according to the Tukey test at the 0.05 level. The asterisks show significant differences at ***p<0.001, **p<0.01 levels respectively.

Furthermore, control plants produced the longest seedling root and shoot (Fig. 1), which is consistent with the results of Singh et al. (2012). Furthermore, root and shoot growth was not affected severely in control, 50 mM NaCl, and 100 mM NaCl stress plants, while shoots were severely reduced under 150 mM and 200 mM NaCl stress (Fig. 1).

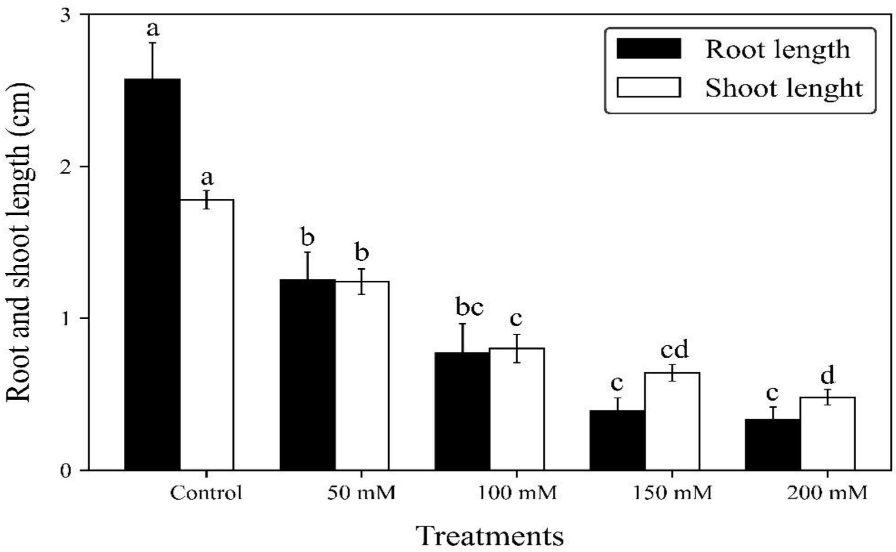

Fig. 1. Effect of salinity on root and shoot length of seedlings. The letters represent significant differences between treatments according to the Tukey test at 0.05 level.

Plant growth parameters. Plant height is the primary attribute that was affected by salinity. There was a significant difference between control and salinity-treated plants. Salinity decreased the root growth and plants produced shorter roots under saline conditions. A similar result was concluded by Ismail *et al*. (1994), that an increase in salinity is followed by a decrease in plant height, and root length. Furthermore, plants under salinity conditions had pale green leaves with lower SPAD values compared to control plants (Table 2). This result agrees with Parvin *et al*. (2015), but with a difference that we used higher concentration of salt.

Table 2. Effects of salt concentration on growth parameters of tomato.

Treatments	Plant height (cm)	Root length (cm)	Leaf area (cm^2)	SPAD value
Control	12.8 ± 1.48 a	17.40 ± 3.91 a	6.40 ± 0.45 a	75.56 ± 7.73 a
50 mM	10.86 ± 0.58 b	16.51 ± 2.31 a	4.26 ± 0.25 b	73.04 ± 6.21 ab
100 mM	8.22 ± 0.84 bc	14.72 ± 1.84 ab	3.00 ± 0.38 c	69.86 ± 3.27 b
150 mM	7.58 ± 1.79 bc	10.60 ± 2.30 bc	2.83 ± 0.27 c	65.98 ± 5.85 bc
200 mM	7.19 ± 0.88 c	8.50 ± 2.69 c	2.32 ± 0.30 c	61.40 ± 7.61 c
	***	*	**	**

The data in the above table are presented as means ± SD, and letters shown according to Tukey test at the 0.05 level. The asterisks show significant differences at ***p<0.001, **p<0.01, *p<0.05 levels respectively.

Moreover, salinity harmed flower production. Control plants had a higher rate of flower production compared to salinity-treated ones. Significant difference was observed only at two weeks while highly significant difference was observed at four weeks (Fig. 2). Similarly, Umar et al. (2018) indicated a decreased vegetative growth and number of flowers in tomato under salinity.

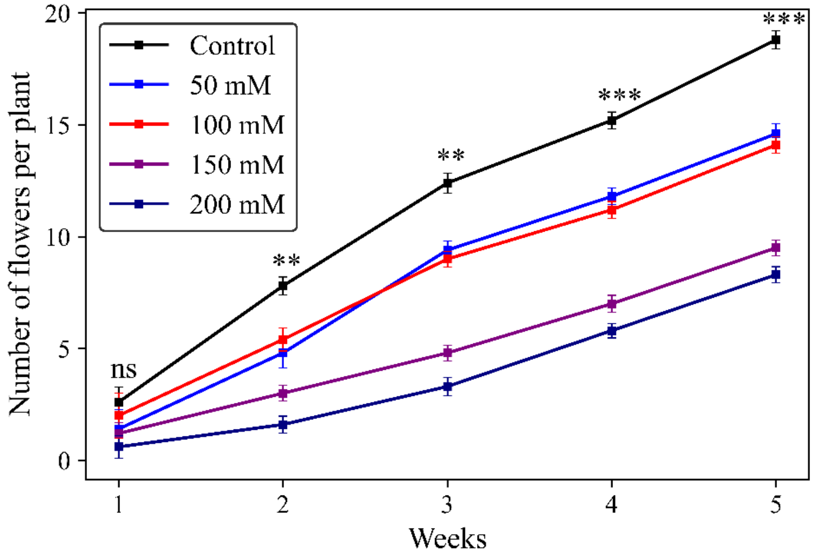

Fig. 2. Effect of salinity on the number of flowers in tomato plants. The asterisks show significant differences at ***p<0.001, **p<0.01.

Physiological attributes. Physiological parameters including photosynthetic rate, transpiration rate, stomatal conductance, and leaf surface temperature were highly affected by salinity. Principally, positive relation exists between photosynthetic rate, transpiration rate, and stomatal conductance, but they have a negative correlation with leaf surface temperature. Salinity increased significantly leaf surface temperature in tomato plants while this increment was followed by a decrease in photosynthetic rate, transpiration rate, and stomatal conductance. Obviously, when the transpiration is not normal, the plant cannot cool its canopy, so, the leaf surface temperature will increase. Moreover, stomatal conductance leads the photosynthetic materials to other parts of the plant, and salinity decreased it, therefore, photosynthetic rate also decreased (Table 3). A decrease in photosynthetic rate in tomato leaves under salinity was earlier demonstrated by Yang et al. (2020) and Ullah et al. (2020), but in our experiment we evaluated the photosynthetic rate under 200 mM NaCl which has not been reported previously.

Table 3. Effect of salinity on physiological parameters of tomato leaves.

Treatments	Photosynthetic rate (μmol CO_2 m^{-2} s^{-1})	Transpiration rate (mol CO_2 m^{-2} s^{-1})	Stomatal conductance (μmol CO_2 m^{-2} s^{-1})	Leaf surface temperature (°C)
Control	1.85 ± 0.08 a	0.37 ± 0.11 a	0.32 ± 0.05 a	24.70 ± 1.39 c
50 mM	1.11 ± 0.32 b	0.31 ± 0.06 ab	0.22 ± 0.07 b	26.50 ± 2.04 b
100 mM	0.85 ± 0.35 bc	0.21 ± 0.06 bc	0.13 ± 0.05 c	27.66 ± 2.51 b
150 mM	0.53 ± 0.14 cd	0.19 ± 0.09 bc	0.05 ± 0.02 cd	31.44 ± 0.44 a
200 mM	0.35 ± 0.10 d	0.09 ± 0.05 c	0.02 ± 0.01 d	32.36 ± 0.40 a
	***	**	***	**

The data in the above table are presented as 'mean ± SD' followed by letters that show significant differences between treatments according to the Tukey test at 0.05 level. The asterisks show significant differences at ***p<0.001, **p<0.01.

Principal component analysis revealed that 88.3% of data were in PCA1 and 8% in PCA2, showing is a good correlation between photosynthetic rate, stomatal conductance, and transpiration rate, but in contrary with leaf surface temperature (Fig. 3). Control plants had a high photosynthetic rate, transpiration rate, and stomatal conductance, but a lower leaf surface temperature. In contrast, saline-treated plants had a higher leaf surface temperature, while the photosynthetic rate, transpiration rate, and stomatal conductance were low.

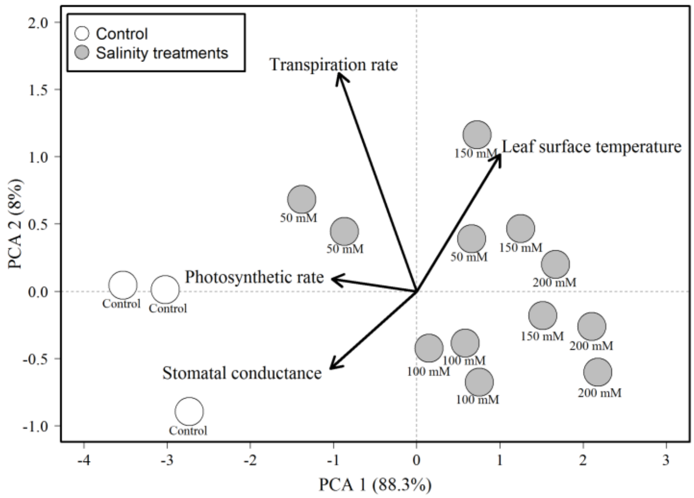

Fig. 3. Principal component analysis of physiological parameters.

Yield and its components. Fruit weight, number of fruits per plant, and yield per plant were highly affected by salinity. Individual fruit weight and the number of fruits per plant were significantly decreased under salinity conditions. There was a significant decrease in yield per plant under NaCl stress treatments as compared to control (Table 4). Salinity stress was observed by Zhang and co-workers (2017) to diminish tomato plant yield under NaCl stress (Control = 0.8 dS m^{-1} and sodium chloride solution with EC = 2.0 dS m^{-1}) when salt stress was applied separately during the vegetative, flowering and fruiting stages, while we used different salt concentrations starting from transplanting to harvest.

Table 4. Effect of salinity on yield and its components.

Treatments	Fruit weight (g)	Fruits/plant	Yield / plant (g)
Control	3.72 ± 0.74 a	16.0 ± 1.67 a	23.14 ± 3.54 a
50 mM	2.34 ± 0.58 b	9.4 ± 0.84 b	15.87 ± 1.74 b
100 mM	1.57 ± 0.38 bc	5.8 ± 2.26 c	11.52 ± 1.17 c
150 mM	1.28 ± 0.43 c	2.9 ± 0.84 d	10.09 ± 0.86 cd
200 mM	0.83 ± 0.20 c	1.8 ± 0.70 d	6.71 ± 0.53 d
	***	***	***

Yield components are represented as 'mean ± SD', and the letters show significant differences between treatments according to the Tukey test at 0.05 level, and the asterisks show significant differences at ***$p<0.001$, **$p<0.01$.

Electrolyte leakage and survival rate. Electrolyte leakage shows that electrons are leaked from plant parts as an after effect of injury. In the current experiment, electrolyte leakage increased with the increment of salinity. In the flowering and fruit-bearing stages, a significantly higher electrolyte leakage was observed in saline treatments compared to control. Therefore, the flowers wilted, and less number of fruits were formed in saline treatments. Furthermore, salinity decreased the survival rate (%) in tomato plants under saline treatments compared to control except for 50 mM NaCl stress (Table 5).

In leafy vegetables, salt stress increases electrolyte leakage in lettuce and spinach leaves (Hniličková et al. 2019), inasmuch as electrolyte leakage causes cell membrane damage and electrons leak from the cell. Therefore, electrolyte leakage is reported as one of the most important criteria to determine the cell injury. However, there is no specific previous study that shows the effect of NaCl on electrolyte leakage of tomato (*Solanum lycopersicum* L. cv. Micro-Tom), and our result is the first report.

Table 5. Effect of salinity on electrons leakage in leaves and survival rate of tomato plants.

Treatments	Electrolyte leakage (%)		Survival rate (%)
	Flowering stage	Fruit-bearing stage	
Control	14.88 ± 2.00 d	17.49 ± 2.79 d	100 a
50 mM	24.03 ± 3.08 c	34.16 ± 1.80 c	100 a
100 mM	27.74 ± 3.49 c	40.47 ± 2.07 b	80 b
150 mM	33.49 ± 1.81 b	44.65 ± 1.89 a	80 b
200 mM	42.76 ± 2.11 a	47.42 ± 1.59 a	60 c
	***	***	*

Data are shown as 'mean ± SD', and the letters show significant differences between treatments according to the Tukey test at 0.05 level, and the asterisks show significant differences at ***p<0.001, *p<0.05.

A strong negative correlation (r = - 0.84) was found between electrolyte leakage and number of flowers, as well as with the number of fruits (r = - 0.91) (Fig. 4). The reason is the cell membrane injury that caused flowers to die and decrease the number of flowers, therefore, less number of fruits were formed.

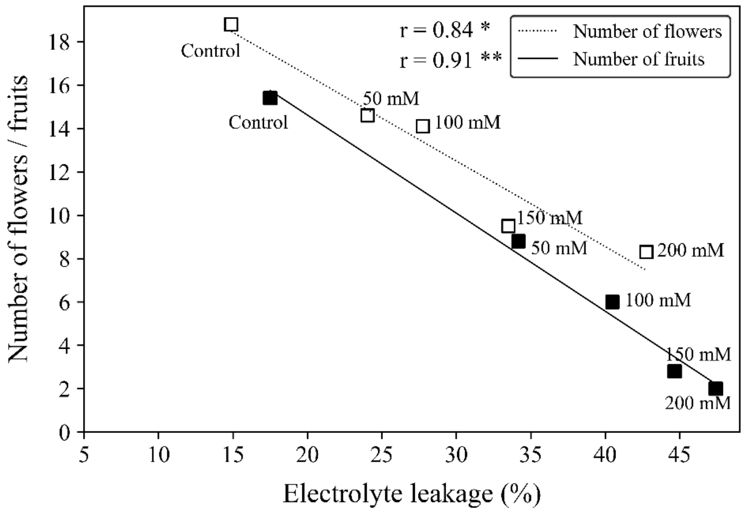

Fig. 4. The correlation coefficient of electrolyte leakage (%) with number of flowers per plant, and number of fruits per plant.

Sugar content. The most important quality indicator of tomato fruit is the presence of sugars (glucose, fructose, and sucrose). In the current study, low salinity (50 mM) improved the glucose content of tomato fruits, but high salinity level (>50 mM) gradually decreased the glucose content of tomato fruits. Furthermore, 50 mM and 100 mM NaCl improved fructose concentration, but in high levels of salinity

(150 mM and 200 mM) fructose decreased significantly as compared to control. Sucrose was highly affected by salinity, and there was a significant decrease in fruit sucrose content in salinity treatments compared to control. Therefore, salinity decreased sugar levels in tomato fruits (Table 6).

Moderate salinity (50 mM) can improve tomato fruit Brix level (Johkan et al. 2014) which is consistent with our results. A significant decrease in fruit Brix under high salinity (150 mM) has also been observed. It was also observed that 150 mM and 200 mM treatments decrease sucrose (Lu et al. 2010), and 60 mM NaCl salinity increased sugars in tomato fruits (Carvajal et al. 2000), which are consistent with results of this study.

Table 6. Effect of NaCl stress on the sugar content of tomato fruits.

Treatments	Sugars (mg/g)			
	Glucose	**Fructose**	**Sucrose**	**Total**
Control	42.73 ± 3.22 b	72.47 ± 3.09 c	16.65 ± 1.02 a	131.86 ± 3.97 b
50 mM	54.85 ± 3.76 a	85.08 ± 2.77 a	14.25 ± 0.76 b	154.18 ± 5.13 a
100 mM	42.04 ± 4.43 b	77.43 ± 5.60 b	12.64 ± 0.92 bc	132.11 ± 9.62 b
150 mM	34.14 ± 3.57 c	63.26 ± 1.71 d	12.12 ± 1.14 cd	109.51 ± 4.57 c
200 mM	25.01 ± 2.52 d	51.61 ±3.12 e	10.47 ± 0.61 d	87.09 ± 5.33 d
	***	***	**	***

Data are shown as 'mean ± SD', the letters show significant differences between treatments according to the Tukey test at 0.05 level, and the asterisks show significant differences at ***p<0.001, **p<0.01.

In addition, we observed an improvement in fruit Brix under low and medium salinity conditions was observed, but it was significantly decreased under 150 mM and 200 mM treatments compared to control. Furthermore, there was a negative relationship between fruit Brix and fruit fresh weight under low (T1) and medium (T2) salinity, but in treatments 150 mM and 200 mM, where tomato plants face high salt stress, both fruit fresh weight and fruit Brix decreased significantly (Fig. 5).

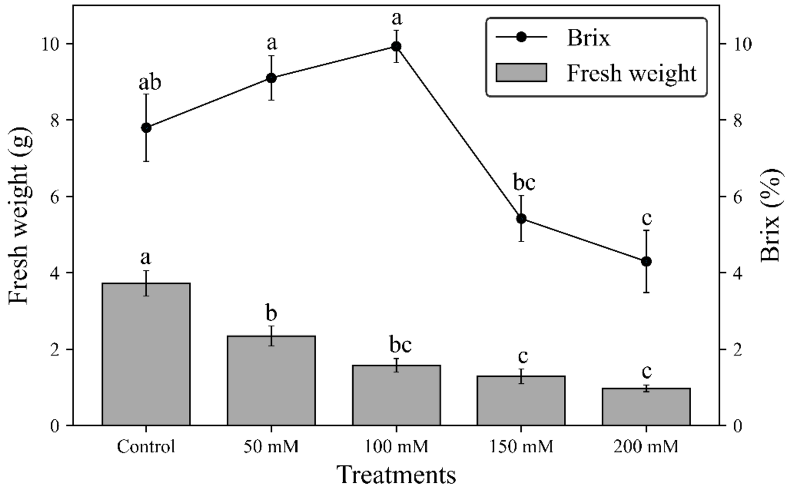

Fig. 5. Effect of salinity on fresh weight and Brix of tomato fruits. The letters represent significant differences between treatments according to the Tukey test at 0.05 level.

Moreover, salinity delayed the fruit ripening in tomato fruits (Table 8), and it is obvious that the red and ripened fruits have a higher amount of sugars. Therefore, the fruits in control plants were fully ripened and red, but in salinity treatments, the fruits were not fully ripened, and their color was ranged from pale red (50 mM, 100 mM, and 150 mM) to green (200 mM) (Fig. 6).

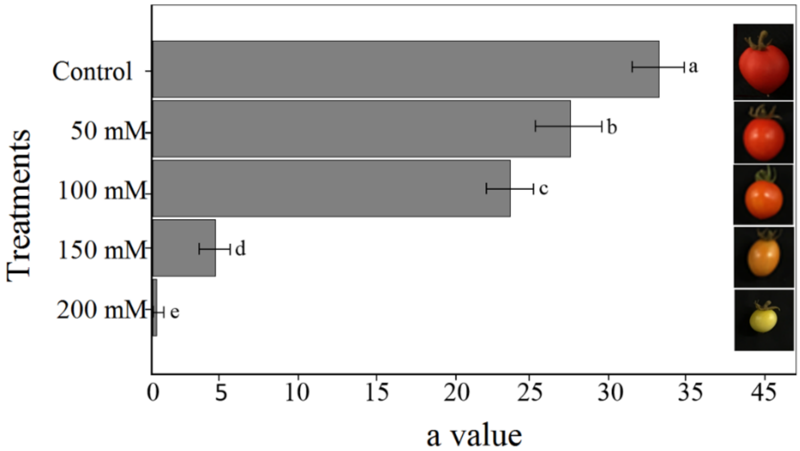

Fig. 6. The effect of salinity on tomato fruit color at ripening stage. The letters represent significant differences between treatments according to the Tukey test at 0.05 level.

Organic acid content. Organic acid was measured from the harvested fruits of the same age based on time of anthesis. There was a significant increase in organic acid accumulation in tomato fruits under NaCl stress conditions compared to control. There was a significant increase in citric acid and malic acid concentration compared with control (Fig. 7), this result agree with previous studies by Zushi and Matsuzoe (2006).

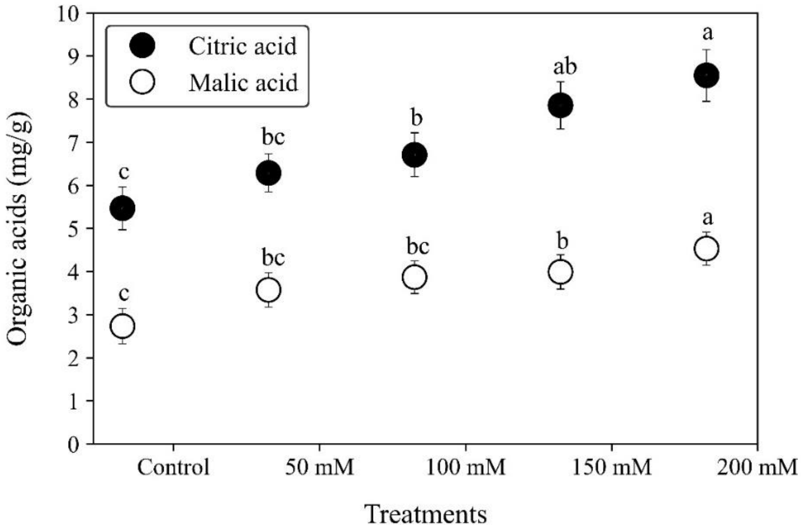

Fig. 7. Effect of salinity on organic acid content of tomato fruits. The letters represent significant differences between treatments according to the Tukey test at 0.05 level.

Malondialdehyde and proline content. Malondialdehyde (MDA) and proline are highly reactive chemical compounds produced to strengthen the plant to combat abiotic stress (Gharsallah et al. 2016). In the current study, MDA and proline concentration in tomato leaves which were grown under saline conditions produced significantly higher concentrations compared to control (Table 7). Plants grown under high salinity produced a higher concentration of proline, and the leaf size (leaf area) was smaller than control (Table 7).

Table 7. Malondialdehyde and proline content in tomato leaves.

Treatments (T)	MDA (10^{-5} nmol/g FW)	Proline (10^{-1} mg/g FW)
Control	4.92 ± 1.29 d	3.79 ± 0.31 d
50 mM	8.48 ± 0.61 c	4.16 ± 0.30 cd
100 mM	10.74 ± 2.46 c	4.99 ± 0.28 c
150 mM	14.75 ± 2.40 b	5.56 ± 0.12 b
200 mM	22.00 ± 2.23 a	6.27 ± 0.19 a
	***	**

Data are shown as 'mean ± SD', the letters show significant differences between treatments according to the Tukey test at 0.05 level, and the asterisks show significant differences at ***p<0.001, **p<0.01.

Ethylene production and fruit maturation. Fruit ripening has been delayed in the plants grown under saline conditions. There was a significant difference in the number of days to full fruit ripening between plants grown under control and salinity treatments. A higher number of days to fruit ripening was observed in the salinity treatments compared to control. The plants grown under control conditions took lesser days to mature their fruit compared to the ones in the salinity treatments. Furthermore, fruits produced by plants grown under the saline conditions with the same flowering date were green in color (Fig. 6) and had harder pericarp than in control (Table 8).

Ethylene production was also affected by salinity in tomato fruits. This is because of not fully ripened and unhealthy fruits in salinity treatments. There was a significant difference in ethylene production between control and saline treatments. The fruits which were grown under control had the highest ethylene production while the lowest ethylene production was measured from the plants grown under saline conditions (Table 8). Salinity induces ethylene production in plants (Zapata et al. 2017; Riyazuddin et al. 2020), but it was observed that when the control fruits were fully ripened, the salinity stressed ones were hard and not mature, therefore, their firmness was higher and ethylene production was lower compared to control ones. However, we measured ethylene production of salinity stressed plants' fruit when they became mature and red, we observed an increase in ethylene production compared to the previous time, because this time the salinity treatments fruits were ripened (Table 8).

Table 8. Effects of salt-stress conditions on fruit ripening characteristics of tomato.

Treatments (T)	Fruit firmness (N)	Ethylene production (nl/g/hr)		Days to ripening
		Control plants maturity	Stressed plants maturity	
Control	2.99 ± 0.48 d	0.81 ± 0.26 a	0.81 ± 0.26 c	92.4 ± 2.70 e
50 mM	3.86 ± 0.69 cd	0.51 ± 0.15 b	0.85 ± 0.09 bc	97.8 ± 1.30 d
100 mM	4.22 ± 0.52 c	0.38 ± 0.11 bc	0.89 ± 0.06 b	102.0 ± 3.43 c
150 mM	5.64 ± 0.86 b	0.21 ± 0.08 c	0.91 ± 0.12 b	109.6 ± 1.81 b
200 mM	7.80 ± 0.52 a	0.09 ± 0.06 d	0.94 ± 0.10 a	113.8 ± 2.49 a
	***	**	**	***

Data are shown as 'mean ± SD', the letters show significant differences between treatments according to the Tukey test at 0.05 level, and the asterisks show significant differences at ***p<0.001, **p<0.01.

CONCLUSION

Salinity stress retards tomato plant growth and has adverse consequences on physiological traits such as photosynthetic rate, SPAD value, transpiration rate, stomatal conductance, and leaf temperature. Furthermore, salinity decreases the water absorption in tomato roots which leads to low transpiration rate in leaves, and it increases the leaf temperature and electrolyte leakage. Therefore, a smaller number of plants can survive under high salinity. Moreover, sodium chloride salinity stress increases fruit firmness and decreases ethylene production, and due to the presence of sodium in fruit cells that helps to produce thicker cell membrane, therefore, this characteristic delays fruit maturity.

Tomato plants are very sensitive to salinity in the reproductive stage causing fewer fruits with higher levels of acids and low sugar content. In the future we would like to find some practical and easy approaches to enhance the tomato plant tolerability against salinity.

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HOST RESPONSE TO *Meloidogyne incognita* INFECTION OF SEMI-TEMPERATE CARROT, CELERY AND SWEET PEPPER CULTIVARS COMMONLY GROWN IN THE HIGHLANDS OF BENGUET PROVINCE, PHILIPPINES

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ABSTRACT

Although the Philippines is a tropical country, 80% of semi-temperate vegetables are produced in the highlands (Cordillera) in northern Luzon. Due to the increasing yield losses caused by root-knot nematodes (RKN; *Meloidogyne* spp.) to these crops, a greenhouse experiment was conducted to evaluate the host response (resistance and tolerance) to *Meloidogyne incognita* infection of semi-temperate carrot, celery, and sweet pepper cultivars commonly grown in the Cordillera. The cultivars were inoculated with 1,500 eggs of *M. incognita* and the plants harvested 8 and 16 weeks after inoculation (WAI). Host response was determined based on the resistance index (RI; combining severity of root galling and number of eggs masses), the percentage change in plant growth and yield between inoculated and uninoculated plants. During the early stage of infection, the carrot cultivars were classified as either highly resistant or immune at 8 WAI. However, post-infectional response at 16 WAI showed the cultivars Caroline, Chunhong and Lucky Kuroda were classified as intermediate while Argo Super Kuroda and Royal Chantenay as moderately susceptible, and New Kuroda, New Kurodagosun and Victoria as susceptible. The celery cultivars Tall Utah Supreme and Utah 52-70 R Imp showed some resistance at 8 WAI, however, both cultivars were classified as susceptible at 16 WAI. The sweet pepper cultivar California Wonder was classified as moderately susceptible while Smooth Cayene and Yolo Wonder were classified as susceptible and highly susceptible, respectively at 8 WAI. The production of semi-temperate vegetables in the Cordillera is highly valuable. Knowledge of their host response to nematodes will assist farmers to minimize yield losses.

Key words: nematode reproduction, resistance, root galling, tolerance, yield loss

INTRODUCTION

The cultivation of semi-temperate vegetables in the Philippines is restricted mainly to two provinces (Benguet Province and Mountain Province) in the highlands (Cordillera) in the northern part of Luzon at 500 to 1,000 m above sea level. Benguet Province produces about 200,000 metric tons (mt) of semi-temperate vegetables each year (PSA 2018), which is 10 times more than Mountain Province. In the Cordillera, farmer s commonly plant semi-temperate vegetables along mountain slopes and in valleys forming terraces of vegetables. Given this type of terrain, the farmers apply conventional agronomic practices on small farms of about 2,500 to 5,000 m^2. In Benguet Province, the most commonly cultivated semi-temperate vegetables are cabbage (*Brassica oleracea* var. *capitata*; 80,634 mt), carrot (*Daucus carota*; 54,387 mt) and Chinese mustard (*Brassica chinensis*; 40,418 mt) (Philippine Statistics Authority 2018). Other important crops cultivated in the highlands of Benguet Province include celery (*Apium graveolens*), cucumber (*Cucumis sativus*), onion (*Allium cepa*), snap beans (*Phaseolus vulgaris*) and sweet (or bell) pepper (*Capsicum annuum*).

Root-knot nematodes (*Meloidogyne* spp.) are obligate endoparasitic root pathogens that can cause serious damage to agricultural crops around the world (Jones et al. 2013). The root-knot nematode species *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* are of particular economic importance to vegetable production worldwide (Hallmann and Meressa 2018). In the Philippines, *Meloidogyne* spp. have been reported associated with a number of high value vegetable crops in both the lowlands (Pascual et al. 2017) and the highlands (Pedroche et al. 2012). The economically damaging potential of this group of plant-parasitic nematodes was established on numerous vegetables including cabbage, carrot, cauliflower (*Brassica oleracea*), celery, eggplant (*Solanum melongena*), okra (*Hibiscus esculentus*), sweet pepper and tomato (*Solanum lycopersicum*; Davide 1988).

Plant resistance is considered by many as the best option for nematode management because of its cost effectiveness, compatibility with other management practices and benefit to environment (Starr and Mercer 2009). The first step consists of the identification of one or multiple sources of resistance which can be achieved by the evaluation of the host response of candidate cultivars, breeding lines, among others. This evaluation can lead to the identification of cultivars which can be grown in nematode-infested fields whether as the main crop or as a rotation crop. In addition, natural sources of resistance can also be used in breeding programs.

Although just a few sources of gene resistance especially single, against root-knot nematodes in vegetables are known (Hallmann and Meressa 2018), resistance to *Meloidogyne* spp. was reported in several temperate or semi-temperate vegetables. In carrots, the cultivars Brasilia and population derived from a cross between the European cultivars Scarlet Fancy and Favourite were found to be resistant to *M. incognita* (Parsons et al. 2015). Inbred lines derived from the open-pollinated cultivar Brasilia were used to produce progenies resistant to both *M. javanica* and *M. incognita* (Boiteux et al. 2000; Simon et al. 2000; Vieira et al. 2003). Two bell pepper (*C. annuum*) cultivars, Carolina Wonder and Charleston Belle, with high levels of resistance to *M. incognita* were approved for release by the Agricultural Research Service of the US Department of Agriculture (Fery et al. 1998). Potential resistance was found in five out of 19 carrot cultivars evaluated (Wesemael and Moens 2008). In sweet pepper, several dominant *R*-genes (the *Me* genes and the *N* gene) with resistance to root-knot nematodes have been characterised. Three of these genes (*Me1*, *Me3* and *N*) are routinely used in breeding pepper cultivars resistant to *M. arenaria*, *M. incognita* and *M. javanica* such as Carolina Wonder (Fery et al. 1998; for a summary see Barbary et al. 2016). Some Huacle and Serrano pepper lines were resistant to *M. incognita* (Gómez-Rodríguez et al. 2017). The *M. incognita* resistance locus Me7 has been mapped in a pepper population from a cross between the susceptible parent ECW30R and the -resistant parent CM334 (Changkwian et al. 2019). In celery, sources or mechanisms of resistance were not yet described (Bruznican et al. 2020).

In the Philippines, screening of some vegetables for natural resistance to *Meloidogyne* spp. was carried out by nematologists at Los Baños during the late 1960's and 1970's (Valdez 1968; Dela Rosa and Davide 1969; Toledo and Davide 1969; Ducusin and Davide 1972; Castillo 1976). However, screening for resistance to *Meloidogyne* spp. was mostly limited to lowlands crops and did not include semi-temperate vegetables which are only grown in the highlands of northern part of Luzon.

The study sought to evaluate the host response (resistance and tolerance) of three semi-temperate vegetables, *viz*. carrot, celery and sweet pepper, commonly grown today in the highlands of Benguet Province, to *M. incognita* infection. During a survey of the plant-parasitic nematodes associated with semi-temperate vegetables in the highlands of Benguet Province (Pedroche et al. 2012), this root-knot nematode species was identified as one of the predominant plant-parasitic nematode species infecting these crops.

MATERIALS AND METHODS

Plant material. The most common cultivars of carrot, celery and sweet pepper in Benguet Province were selected for the host response experiments (Table 1). Some have a long history of commercial production in the Cordillera whereas others were recently introduced. The seeds of celery and sweet pepper were pre-germinated in seedling trays containing heat-sterilised soil and after germination transplanted into 8-cm-diameter pots containing a heat-sterilised mixture (2,500 g) of garden soil and sand (1:1). The carrot seeds were directly seeded to 50 cm long pots (8 cm diam.). Plants were fertilized with complete fertilizer (14-14-14) at the recommended rate specific for vegetables at two splits as basal fertilizer and at hilling-up. Urea (46-0-0) (90 N ha^{-1}) and solophos (170 P kg ha^{-1}) were applied at the recommended rate during hilling-up. The plants were maintained at an ambient temperature of 26°C in a greenhouse and watered as needed.

Table 1. Cultivars of carrot, celery and sweet pepper included in the host response experiments.

Plant species	Cultivar
Carrot	Argo Super Kuroda, Caroline,
	Chunhong, Lucky Kuroda, New Kuroda
	New Kurodagosun, Royal Chantenay, Victoria
Celery	Tall Utah Supreme, Utah 52-70 R Imp
Sweet pepper	California Wonder, Smooth Cayene, Yolo Wonder

***Meloidogyne incognita* population.** The *M. incognita* population used in the experiments was originally isolated from galled celery roots cv. 'Tall Utah' grown in a commercial field in La Trinidad in the highlands of Benguet Province. A single egg mass was taken from a single gall and inoculated on a susceptible 4-week-old tomato cv. Apollo seedling potted in a greenhouse. Adult females were collected from the galled roots and identified as *M. incognita* based on the shape of the perineal pattern after 12 weeks (Hunt and Handoo 2009). When the single tomato plant had matured, the egg masses in the roots were isolated and used immediately to inoculate new 4-week-old tomato cv. Apollo seedlings.

Preparation of nematode inoculum. Eight- to 12-week-old galled tomato roots were gently uprooted, washed under running tap water to remove adhering soil particles and cut into 1- to 2-cm-long segments. Eggs were freed from the egg masses by dissolving the gelatinous matrices in 1% hypochlorite (NaOCl). The eggs-hypochlorite suspension was then poured through a series of nested sieves with 150 µm, 74 µm and 25 µm apertures. The eggs retained on the 25-µm-aperture sieve were collected and suspended in distilled water. The number of nematode eggs in the suspension was determined by counting the eggs in 1-ml-aliquots using a stereomicroscope.

Nematode inoculation. Per experiment, nine seedlings of each of the either direct-seeded (carrot) or transplanted (celery, sweet pepper) cultivars were inoculated 2 to 3 weeks after transplanting with 1,500 eggs pipetted into four 5-cm-deep holes made in the rhizosphere soil of the plants. In addition, nine seedlings of each of the cultivars remained uninoculated and served as control plants.

Assessment of root galling and nematode reproduction. The root systems of the plants were gently uprooted, washed under running tap water to remove adhering soil particles and examined for the presence of galls and egg masses at 8 weeks (carrot, celery, sweet pepper) and 16 weeks after inoculation (carrot, celery; WAI). The number of root galls and egg masses were counted using a stereomicroscope. On the basis of these numbers, root gall and egg mass index were determined based on a 0 to 5 score (Taylor and Sasser 1978; Table 2). Root sub-samples of 1 g taken at random per plant were stained with acid fuchsin (Byrd et al. 1983). Staining was only used for assessing the number of the different nematode developmental stages inside the roots while egg masses were counted without staining using a stereomicroscope. Rhizosphere soil sub-samples of 200 ml were taken at random per pot and the nematodes extracted using a modified Baermann tray method (Marais et al. 2017) and counted using a stereomicroscope.

Table 2. Root gall and egg mass index with corresponding assessment of the marketability of the yield of carrot tap roots.

Index	Number of root galls	Number of egg masses	Marketability of yield of carrot tap roots
0	0	0	no stunting: marketable
1	1-2	1-2	visible on the secondary roots with light forking, no stunting: marketable
2	3-10	3-10	none coalesced, forking, no stunting: marketable
3	11-30	11-30	some coalesced, severe forking and moderate stunting: marketable
4	31-100	31-100	many coalesced, severe stunting: unmarketable
5	> 100	> 100	mostly coalesced, severe stunting: unmarketable

Plant growth, yield and nematode damage. At termination of the experiments, plant height, fresh shoot and root mass, and yield were determined. For carrot and celery, data were gathered at 8 and 16 WAI to assess the early and late response to nematode infection; for sweet pepper that matures early, data were gathered at 8 WAI. To measure plant height (in cm), measurements were taken from the base of the plant to the highest leaf tip. To measure the fresh shoot and root mass, and yield (in g), a sensitive balance scale was used. Roots were washed with running tap water to remove the adhering soil particles and blotted dry with paper towels before weighing. Nematode damage was expressed as the percentage change in plant height, fresh shoot and root mass of nematode-infected plants compared with uninfected plants. The edible tap roots of the carrot cultivars were assessed visually for qualitative damage (forking) and their marketability classified as marketable and non-marketable (Bélair 1992; Table 2).

Host response. The evaluation of the host response of the plants to infection by *M. incognita* was based on the Resistance Index (RI; Kouamé et al. 1998; Table 3) which combines severity of root galling and number of egg masses into one single value using the equation: $RI = \sqrt{(\text{gall score}^2 + \text{egg mass score}^2)}$. The assessment at 8 WAI determined the host response at an early stage of infection.

When nematodes successfully established in the roots and reproduce, the late response to infection was assessed at 16 WAI.

Table 3. Host responses to *Meloidogyne incognita* infection based on the resistance index (RI)*

	Host response	RI
IM	immune	0-0.9
HR	highly resistant	1.0-1.9
R	resistant	2.0-2.9
MR	moderately resistant	3.0-3.9
I	intermediate	4.0-4.9
MS	moderately susceptible	5.0-5.9
S	susceptible	6.0-6.9
HS	highly susceptible	RI $\geq$ 7

*Kouamé et al. 1997.

Statistical analysis. There were three replicates per cultivar with three plants per replicate. The experiment was conducted in two trials, with consistent results, the data of the second trial were presented. The plants were arranged randomly in the greenhouse using a completely randomized design (CRD). The data were analysed statistically using Analysis of Variance (ANOVA) (STATISTICA Package Software). Differences among treatments were separated at $P \leq 0.05$ level of significance using Tukey's Honest Significant Difference (HSD) test. If the data had either no normal distribution or homogenous variance, even after log transformation and removal of the outliers, equivalent non-parametric analysis was performed. The Man-Whitney U test was used to compare two independent samples at $P \leq 0.05$ level of significance.

RESULTS AND DISCUSSION

Carrot. Significant differences were observed in the mean number of galls per plant ($P \leq 0.05$) among the cultivars evaluated at 8 WAI (Table 4). New Kurodagosun had the highest number of galls (8) while no galls were observed on Royal Chantenay. The highest number of egg masses was also observed in New Kurodagosun. Significant ($P \leq 0.05$) differences were also observed in the mean number of J2 extracted from the rhizosphere soil among the cultivars evaluated. New Kuroda (72) and Victoria (60) had the highest number of J2 while New Kurodagosun (1) had the lowest number of J2. The number of swollen juveniles and adult females that had penetrated and developed inside the roots did not differ significantly among the cultivars evaluated. During the early stage of infection, at 8 WAI, after 2 generations of nematode life cycle, all carrot cultivars, except Argo Super Kuroda and New Kurodagosun, were classified as immune to *M. incognita* infection based on RI. Argo Super Kuroda and New Kurodagosun were classified as highly resistant. A significant ($P \leq 0.05$) increase in fresh shoot mass was observed in inoculated plants of New Kurodagosun (66.7%) and Victoria (52.1%) compared with uninoculated control plants (Table 5). Similarly, a significant ($P \leq 0.05$) marketable yield increase was observed in inoculated plants of New Kuroda (3.4%), Royal Chantenay (26.4%) and Victoria (33.3%).

Table 4. Average root galling, reproduction of *Meloidogyne incognita*, resistance index (RI) and host response (HoR) of eight carrot cultivars 8 weeks after inoculation with 1,500 nematode eggs.

Carrot cultivar	Galls/ root system	Egg masses/ root system	J2/ 200 ml soil	Swollen juveniles/g roots	Adult females/g roots	RI	HoR
Argo Super Kuroda	6 b	0^+ a	43 bc	0^+ a	6 a	1.2	HR
Caroline	2 ab	0 a	39 bc	0 a	1 a	0.4	IM
Chunhong	4 ab	0^+ a	15 b	1 a	3 a	0.7	IM
Lucky Kuroda	5 b	0^+ a	37 bc	1 a	9 a	0.9	IM
New Kuroda	3 ab	0^+ a	72 c	0 a	4 a	0.8	IM
New Kurodagosun	8 b	1 a	1 a	0^+ a	23 a	1.0	HR
Royal Chantenay	0^+ a	0 a	20 bc	0 a	4 a	0.1	IM
Victoria	2 ab	0 a	60 c	0 a	3 a	0.5	IM

Means in the same column followed by the same letters are not significantly ($P \leq 0.05$) different according to Tukey's HSD test. Host response (HoR) was based on the resistance index (RI) = $\sqrt{(\text{gall score}^2 + \text{egg mass score}^2)}$: IM = immune (RI = 0-0.9); HR = highly resistant (RI = 1-1.9) (Kouamé et al. 1997). $^+$ Value represents an average between 0 and 1. J2 = second-stage juveniles.

Table 5. Effect of *Meloidogyne incognita* on fresh shoot and primary root mass of eight carrot cultivars 8 weeks after inoculation with 1,500 nematode eggs.

Carrot cultivar	Shoot mass (g)			Primary root mass (g)		
	-Mi	+Mi	% change	-Mi	+Mi	% change
Argo Super Kuroda	11.2	12.8	(+)14.3 ns	18.3	16.9	(-) 7.7 ns
Caroline	10.1	12.0	(+)18.8 ns	22.0	18.9	(-)14.1 ns
Chunhong	10.1	11.8	(+)16.8 ns	20.4	21.7	(+) 6.4 ns
Lucky Kuroda	10.6	11.4	(+) 7.5 ns	16.8	14.2	(-) 15.5 ns
New Kuroda	15.0	14.0	(-) 6.7 ns	26.3	27.2	(+) 3.4 ns
New Kurodagosun	10.8	18.0	(+)66.7 *	24.6	29.7	(+)21.1 ns
Royal Chantenay	12.3	12.0	(-) 2.4 ns	24.7	31.2	(+)26.4 *
Victoria	7.1	10.8	(+)52.1 *	27.9	37.2	(+)33.3 *

*indicates the level of significance ($P \leq 0.05$) between uninoculated (-Mi) and inoculated (+Mi) plants according to the Mann-Whitney U test. ns: not significantly different.

At the late stage of infection, significant differences were observed in the mean number of galls and egg masses ($P \leq 0.05$) among the cultivars evaluated at 16 WAI (Table 6). The highest number of galls was observed in New Kuroda (693) which was significantly different ($P \leq 0.05$) from all other cultivars. The lowest number of galls was observed in Chunhong (43). The number of J2 extracted from the rhizosphere soil was significantly different ($P \leq 0.05$) among the cultivars evaluated. Royal Chantenay had the highest number of J2 (28) which was significantly different ($P \leq 0.05$) from all other cultivars evaluated except Argo Super Kuroda and Victoria. The lowest number of J2 extracted from the rhizosphere soil was observed in Caroline (1). The number of vermiform and swollen juveniles, and adult females that had penetrated and developed inside the roots was significantly different ($P \leq 0.05$) among the cultivars evaluated. The highest number of adult females was observed in New Kuroda (798) while the lowest number of adult females was observed in Lucky Kuroda (53). Based on RI, Caroline, Chunhong and Lucky Kuroda were classified as intermediately susceptible to *M. incognita* infection at 16 WAI. Argo Super Kuroda and Royal Chantenay were classified as moderately susceptible and New Kuroda, New Kurodagosun and Victoria as susceptible.

A significant increase ($P \leq 0.05$) in fresh shoot mass was observed in inoculated plants of Royal Chantenay (49.1%) compared with uninoculated control plants (Table 7). In seven out of the eight carrot cultivars tested, marketable yield was significantly reduced ($P \leq 0.05$) to 42.3% in inoculated plants compared with uninoculated control plants (100%).

Table 6. Average root galling, reproduction of *Meloidogyne incognita*, resistance index (RI) and host response (HoR) of eight carrot cultivars at 16 weeks after inoculation with 1,500 nematode eggs.

Carrot cultivar	Galls/ root system	Egg masses/ root system	J2/ 200 ml soil	Vermiform juveniles/ g roots	Swollen juveniles/g roots	Adult females/ g roots	RI	HoR
Argo Super Kuroda	104 bc	65 ab	5 ab	41 bc	20 a	130 ab	5.8	MS
Caroline	113 bc	30 a	1 a	9 abc	154 c	226 ab	4.9	I
Chunhong	43 a	28 a	2 a	2 a	20 a	251 ab	4.5	I
Lucky Kuroda	63 ab	21 a	4 a	20 bc	21 ab	53 a	4.8	I
New Kuroda	693 d	157 b	5 a	16 bc	43 abc	798 c	6.5	S
New Kurodagosun	110 bc	52 ab	3 a	12 abc	60 bc	303 bc	6.2	S
Royal Chantenay	167 c	90 b	28 b	20 c	14 a	316 bc	5.8	MS
Victoria	174 c	65 ab	4 ab	5 ab	87 bc	214 bc	6.1	S

Means in the same column followed by the same letters are not significantly different ($P \leq 0.05$) according to Tukey's HSD test. Host response (HoR) was based on the resistance index (RI) = $\sqrt{(\text{gall score}^2 + \text{egg score}^2)}$: I = intermediate (RI = 4-4.9); MS = moderately susceptible (RI = 5-5.9); S = susceptible (RI = 6-6.9) (Kouamé et al. 1997). J2 = second-stage juveniles.

Significant differences were noted in the number of galls and egg masses among the eight cultivars evaluated. At the onset of disease development, host resistance can be shown in the suppression of nematode entry or nematode establishment in the feeding site. Host response in this study has been determined at 8 WAI to detect any resistance at the early stage of infection. At 8 WAI, only a few of the inoculated nematodes were able to establish feeding sites and formed galls in the roots but nevertheless, failed to reproduce and develop egg masses. At 16 WAI, after four generations of RKN have been produced, development and reproduction of RKN were observed as post-infectional response. The basis for determining the host as resistant is its ability to suppress nematode penetration, growth, development and reproduction. Based on RI, three of the cultivars were classified as intermediate, two as moderately susceptible and three as susceptible to *M. incognita* infection at 16 WAI. Thus, no source of resistance was found among the carrot cultivars evaluated.

Table 7. Effect of *Meloidogyne incognita* on fresh shoot mass and yield of eight carrot cultivars at 16 weeks after inoculation with 1,500 nematode eggs.

Carrot cultivar	Shoot mass (g)			Marketable yield (g)		
	-Mi	+ Mi	% change	-Mi	+Mi	% change
Argo Super Kuroda	19.3	15.8	(-) 18.1 ns	43.5	25.0	(-) 42.3 ns
Caroline	19.2	15.6	(-) 18.8 ns	58.9	35.8	(-) 39.2 ns
Chunhong	16.3	12.3	(-) 24.5 ns	38.2	21.3	(-) 44.2 *
Lucky Kuroda	17.8	16.8	(-) 5.6 ns	35.7	20.1	(-) 43.7 *
New Kuroda	19.0	19.3	(+) 1.6 ns	63.0	6.7	(-) 89.4 *
New Kurodagosun	30.0	23.0	(-) 23.3 ns	58.4	30.3	(-) 48.1 *
Royal Chantenay	10.8	16.1	(+)49.1 *	59.3	0	(-)100 *
Victoria	13.2	12.8	(-) 3.0 ns	62.3	13.9	(-) 77.7 *

*indicates the level of significance ($P \leq 0.05$) between uninoculated (-Mi) and inoculated (+Mi) plants according to the Mann-Whitney U test. ns: not significantly different.

Nematode infection promoted significantly higher primary root mass in carrot cultivars compared to uninoculated plants at 8 WAI. However, at the late stage of infection (16 WAI) differences in the marketable yield became evident. Infection by *M. incognita* significantly reduced the marketable yield of the carrot tap roots in six out of the eight cultivars evaluated at 16 WAI. The quality of the taproot is of major importance for the processing industry. Smooth, long, uncoalesced and unblemished tap roots are the quality requirements for commercial carrot production. Root-knot nematodes usually invade carrots at the root tips and induce (even at low numbers) secondary development resulting in forked and ramified tap roots, in addition to galls. Forking, twisting and cracking were also observed on *M. incognita*-susceptible carrots cultivars (Khan et al. 2018).

Formation of galls was evident around the lenticels of the primary roots with a somewhat light to dark brown discoloration in severe cases, in addition to forking. *M. chitwoodi* can cause severe galling near the lenticels resulting in a rough surface on the carrot taproot (Wesemael and Moens 2008). Lenticels are spongy regions in the periderm of stems and roots that allow gas exchange with the surrounding environment. The discoloration observed in our study may have been caused by secondary infections by fungi. The openings created by J2 of root-knot nematodes after penetration of the roots may serve as entry points for soil-borne microbial pathogens (Manzanilla-Lopez and Starr 2009). In addition to galling and forking of the tap roots, this discoloration further increases damage of these roots, thus decreasing the marketable yield and commercial value of root-knot nematode infected carrots.

Damage caused by *M. incognita* infection to carrots increases with increased duration of plant growth. A significant reduction in marketable root yield was observed in six out of the eight cultivars evaluated at 16 WAI *vs* only three cultivars at 8 WAI. This is consistent with an earlier study which reported that the damage in carrots caused by *M. chitwoodi* (inoculation density 25 J2/100 g soil) increased dramatically from 10 to 70% in a matter of 6 weeks (Wesemael and Moens 2008). Reducing the period that carrots are in the field by advancing the harvest date might significantly reduce the proportion of quality damage caused by *M. chitwoodi*. The life cycle duration of root-knot nematodes takes an average of 25 days at 27°C but may take longer at lower or higher temperatures (Ploeg and Maris 1999). Nematodes were given sufficient time to produce multiple generations creating more damage at 16 WAI (Wesemael and Moens 2008).

Celery. Only a significant difference ($P \leq 0.05$) in the number of J2 extracted from the rhizosphere soil was observed between the two cultivars evaluated at 8 WAI (Table 8). In Tall Utah Supreme, 60 J2/200 ml rhizosphere soil were counted *vs* 27 in Utah 52-70 R Imp. Based on RI, both cultivars were classified as resistant to *M. incognita* during the early stage of infection. No significant reductions in plant height, fresh shoot mass or yield were observed in inoculated plants compared with uninoculated control plants (Table 9).

Tall Utah Supreme had the highest number of galls ($P \leq 0.05$) (220 *vs* 143 in Utah 52-70 R Imp) and the highest number of J2 ($P \leq 0.05$) in the rhizosphere soil (180 *vs* 49 in Utah 52-70 R Imp) at 16 WAI (Table 8). However, the number of egg masses was significantly higher ($P \leq 0.05$) in Utah 52-70 R Imp (45 *vs* 19 in Tall Utah Supreme). Based on RI, Tall Utah Supreme was classified as moderately susceptible to *M. incognita* infection while Utah 52-70 R Imp was classified as susceptible during the late stage of infection. A significant reduction ($P \leq 0.05$) in fresh root mass (82.2%) and yield (72.9%) was observed in inoculated plants of Tall Utah Supreme compared with uninoculated control plants (Table 9).

Table 8. Average root galling and reproduction of *Meloidogyne incognita* on two celery cultivars 8 and 16 weeks after inoculation with 1,500 nematode eggs.

Celery cultivar	Galls/ root system	Egg masses/ root system	J2/ 200 ml soil	Vermiform juveniles/ g roots	Swollen juveniles per g roots	Adult females per g roots	RI	HoR
8 weeks								
Tall Utah Supreme	3 a	1 a	60 b	0 a	0 a	11 a	2.2	R
Utah 52-70 R Imp	4 a	1 a	27 a	0 a	2 a	15 a	2.2	R
16 weeks								
Tall Utah Supreme	220 c	19 b	49 ab	6.3 a	12 a	12 a	5.8	MS
Utah 52-70 R Imp	143 b	45 c	180 c	10 a	11 a	6 a	6.4	S

Means in the same column followed by the same letters are not significantly ($P \leq 0.05$) different according to Tukey's HSD test. Host response (HoR) was based on the resistance index (RI) = $\sqrt{(\text{gall score}^2 + \text{egg score}^2)}$: R = resistant (RI = 2-2.9); MS = moderately susceptible (RI = 5-5.9); S = susceptible (RI = 6-6.9) (Kouamé et al. 1997). J2 = second-stage juveniles.

Table 9. Effect of *Meloidogyne incognita* on plant height, yield, and fresh root mass of two celery cultivars 8 and 16 weeks after inoculation with 1,500 nematode eggs.

Celery cultivar	Plant height (cm)			Fresh root mass (g)			Yield (g)		
	-Mi	+Mi	% change	-Mi	+Mi	% change	-Mi	+Mi	% change
8 weeks									
Tall Utah Supreme	37	39	(+)5.4 ns	24	30	(+)25.0 ns	111	124	(+)11.7 ns
Utah 52-70 R Imp	39	38	(-)2.6 ns	27	34	(+)25.9 ns	136	156	(+)14.7 ns
16 weeks									
Tall Utah Supreme	37	38	(+)2.7 ns	169	30	(-)82.2 *	250	68	(-)72.9 *
Utah 52-70 R Imp	31	31	0 ns	129	106	(-)17.8 ns	241	129	(-)46.5 ns

*indicates the level of significance ($P \leq 0.05$) between uninoculated (-Mi) and inoculated (+Mi) plants according to the Mann-Whitney U test. ns: not significantly different.

Based on RI, both celery cultivars evaluated showed resistance to *M. incognita* at the early stage of infection at 8 WAI. These celery cultivars however became susceptible on extended period of inoculation at 16 WAI. Under the prevailing environmental conditions in Benguet Province, it takes 16 weeks for the celery plants to mature. The significant increase in the number of galls, egg masses, vermiform and swollen juveniles in the roots between 8 and 16 WAI resulted in a significant reduction in fresh root mass and yield of one of the two cultivars evaluated. This observation indicates that damage caused by *M. incognita* infection on celery will increase with increased duration of plant growth. The cultivar Utah 52-70 R Imp included in our study is an improved offspring of Utah 52-70 H which is highly susceptible and sensitive to *M. incognita* infection (Perez and Castillo 1973; Castillo 1976). In our study, in spite of a significant increase in *M. incognita* infection at 16 WAI, plant height, fresh root mass and yield of Utah 52-70 R Imp was not significantly different compared with uninoculated control plants although the yield of Utah 52-70 R Imp reduced to 46% compared with uninoculated control plants. This observation may indicate that this improved celery cultivar has some level of tolerance to *M. incognita* infection but this should be further investigated. None of the infected roots of both cultivars evaluated showed necrosis. Instead, the production of secondary roots was more pronounced where galls were mostly observed.

Sweet pepper. At 8 WAI, nematode reproduction did not differ significantly among the three cultivars tested except the number of adult females in the roots (Table 10). The highest number of adult females in the roots was observed in Yolo Wonder (15) while the lowest number was observed in California Wonder (7). Based on RI, California Wonder was classified as moderately susceptible, Smooth Cayene as susceptible and Yolo Wonder as highly susceptible to *M. incognita* infection. Significant ($P \leq 0.05$) differences were observed among the three cultivars evaluated in plant height, fresh shoot and root mass, and yield between inoculated and uninoculated plants (Table 11). Plant height and fresh root mass were significantly reduced ($P \leq 0.05$) in Smooth Cayene, by 26.6 and 38.3%, respectively. Fresh shoot mass was significantly reduced ($P \leq 0.05$) in all three cultivars evaluated with 16.1 to 27.7%. In all three cultivars evaluated, yield in inoculated plants was reduced by almost 40% compared with uninoculated control plants but this reduction was only significant ($P \leq 0.05$) in Yolo Wonder (39.7%).

Table 10. Average root galling and reproduction of *Meloidogyne incognita* on three sweet pepper cultivars at 8 weeks after inoculation with 1,500 nematode eggs.

Sweet pepper cultivar	Galls/ root system	Egg masses/ root system	J2/ 200 ml soil	Vermiform juveniles/ g roots	Adult females/ g roots	RI	HoR
California Wonder	85 a	33 a	16 a	0.1 a	7 a	5.7	MS
Smooth Cayene	124 a	91 a	33 a	0.3 a	10 ab	6.4	S
Yolo Wonder	211 a	123 a	34 a	0.4 a	15 b	7.1	HS

Means in the same column followed by the same letters are not significantly ($P \leq 0.05$) different according to Tukey's HSD test. Host response (HoR) was based on the resistance index (RI) = √(gall score2 + egg score2): MS = moderately susceptible (RI = 5-5.9); S = susceptible (RI = 6-6.9); HS = highly susceptible (RI ≥ 7) (Kouamé et al. 1997). J2 = second-stage juveniles

Based on RI, the sweet pepper cultivar California Wonder was classified as moderately susceptible to *M. incognita* infection while Smooth Cayene and Yolo Wonder were classified as susceptible and highly susceptible, respectively, at 8 WAI. *M. incognita* J2s were more attracted to sweet pepper cultivars, Yolo Wonder and California Wonder than other sweet pepper cultivars tested and both cultivars were highly susceptible to RKN exhibiting higher galling and egg mass indices (Kihika et al. 2017). Yolo Wonder had gall values similar to the susceptible reference sweet pepper accession and had the lowest marketable yield (Sánchez-Solana et al. 2016). In other reports (see for instance Barbary et al. 2014; Djian-Caporalino et al. 2001; Fazari et al. 2012) the host response of Yolo Wonder to *M. incognita* infection was described as partially resistant but this assessment was based on the low level of root galling induced upon infection and not on nematode reproduction (Barbary et al. 2016). In our study, both root galling and nematode reproduction on Yolo Wonder were very severe. Differences in host response of this cultivar could be attributed to the varying degree of virulence of *M. incognita* populations used in the experiments. In sweet pepper, the emergence of virulent populations of *M. incognita* breaking down the resistance induced by several dominant *R*-genes (*N* and *Me3*) has been reported (Castagnone-Sereno et al. 1996; Thies 2011).

Other nematode taxa were reported previously on semi-temperate vegetable crops in the Philippines. *M. javanica* and *M. hapla* were also abundant on carrot, celery, and sweet pepper (Pedroche et al. 2012). Host response of cultivars to other *Meloidogyne* species could vary from those observed on *M. incognita*. Host response at the time of harvest is important for consumption and marketing purposes.

Table 11. Effect of *Meloidogyne incognita* on plant height, fresh shoot and root mass, and yield of three sweet pepper cultivars 8 weeks after inoculation with 1,500 nematode eggs.

Sweet pepper cultivar	Plant height (cm)			Shoot mass (g)			Root mass (g)			Yield (g)		
	-Mi	+Mi	% change	-Mi	+Mi	% change	-Mi	+Mi	% change	-Mi	+Mi	% change
California Wonder	62.7	56.3	(-)10.2 ns	56.4	40.8	(-)27.7 *	26.3	26.4	(+) 0.8 ns	115.6	70.1	(-)39.4 ns
Smooth Cayene	81.3	59.7	(-)26.6 *	52.2	39.8	(-)23.8 *	28.2	17.4	(-)38.3 *	45.8	28.0	(-)38.9 ns
Yolo Wonder	61.8	58.1	(-) 6.0 ns	52.2	43.8	(-)16.1 *	27.1	20.7	(-)23.6 ns	99.6	60.1	(-)39.7 *

*indicates the level of significance ($P \leq 0.05$) between uninoculated (-Mi) and inoculated (+Mi) plants according to Mann-Whitney U test. ns: not significant.

CONCLUSION

Our results demonstrate the huge impact *M. incognita* can have on carrot, celery, and sweet pepper which are highly-valuable cash crops for food security. Resistance in these crops to this root-knot nematode is scarce. However, where fields are heavily infested with *M. incognita*, a few varieties were identified (carrot cultivars Caroline, Chunhong and Lucky Kuroda; celery cultivars Tall Utah Supreme and Utah 52-70 R Imp of less than 16 weeks old; sweet pepper cultivar California Wonder) which are less susceptible to *M. incognita* infection. Field trials will further validate these promising cultivars which can be recommended to the farmers to help mitigate the damaging effects of this root-knot nematode on semi-temperate crops.

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AGRICULTURAL RESIDUES FOR ORGANIC COMPOST FERTILIZER CATALYZED BY SELECTED MICROBIAL STRAINS

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ABSTRACT

Agricultural wastes become potential sources of greenhouse gas emissions, water eutrophication, soil contaminantion, and pathogen transmittance if it is not treated appropriately. This study sought to evaluate organic fertilizers produced from composting of crop residues and livestock manure catalyzed by selected microbial inoculants. Field surveys on agricultural waste generation were conducted in four northern provinces: Hoa Binh, Ha Noi, Hung Yen and Ha Nam. The pilot experiments for composting of rice straw and pig manure were conducted for 30 days. The calculated results from 4 provinces showed that crop residues generated weighed a total of 3.34 million tons per year, of which rice straw accounts for 551.7 $\pm$ 388.5 thousand tons/year. Manure from pig and poultry in Hanoi were 1,321.2 and 2,107.8 thousand tons/year, respectively. AT-YTB, Bo-Bio 386 containing *Trichoderma spp, Bacillus megaterium, Azotobacter* are optimal for rice straw composting; Bio-MT, Emina and Trichoderma containing *Trichoderma spp., Streptomyces spp., Bacillus spp., Rhodobacter spp., Lactobacillus spp.* are optimal for pig manure composting. Application of these inoculants contributes to an increase in digestible nitrogen content of 32.5-56.6% from rice straw composting and 21.7% from pig manure composting. Further studies on utilization of compost fertilizers from these sources for soil improvement and crop production should be conducted.

Key words: pig manure, microbial inoculants, compost fertilizer, sustainable agriculture

INTRODUCTION

The agricultural sector has contributed a significant generation of wastes including field crop wastes and livestock manure (Kim and Dale 2004, Ottmar et al. 2011). The crop residues are defined as above ground straw or stalks or the remain parts of plant after harvesting (Daioglou et al. 2016, Vo et al. 2018). It was reported that about 140 billion metric tons of biomass are annually generated from agriculture (UNEP 2009). These wastes have been used for animal feed, food, bioenergy, but a significant amount of agricultural biomass discharged or burned freely causing greenhouse gas emissions (Yagi and Minami 1990, Nasaer et al. 2007), environmental pollution (Kaushal and Prashar 2020) and disease dispersion (Kerdraon et al. 2019). In Vietnam, the total amount of agricultural waste is relatively high, reaching 49.83 million tons of dry matter per year, of which rice straw accounts for 85.49%, corresponding to 42.6 million tons of dry matter per year. Meanwhile, large amounts of livestock manures in rural areas are not utilized effectively and discharged into anaerobic digester which remains high concentration of COD effluent (Nguyen et al. 2021).

The nutrient content in agricultural residues is very high. The nutrient concentrations (dry weight) of corn (N 9.2%, P 1.5%, K 6.2%), peanut (N 18.2%, P 1.6%, K 5.8%), rice (N 9.1%, P 1.3%, K 10%) are high (Jia et al. 2018). The content of protein, glucose and cellulose in maize, peanut, and weed in Hoa Binh province was in the range of 26.5% to 36.2% (Vo et al. 2018a). Cellulose is the major component of crop residues followed by protein and lignin. The residues contain a potential carbon source for soil amendment via organic matter supplement. Therefore, reuse of crop residues toward organic fertilizers is essential not only for cleaning and protecting the environment but also returning organic matter for the crops, solving the shortage of organic fertilizer. Using organic fertilizer could contribute to reducing chemical fertilizer use thus enabling the conversion of conventional agriculture toward organic farming. High cellulosic content is difficult to compost and requires long-term degradation. The ideal conditions for organic fertilizer composting require the C:N ratio in the range between 12-20 using effective microorganisms. The current approach is to use microorganisms to shorten composting time, improve quality of compost products, and enrichment of nutrients. Microorganisms in bio-catalytic products accelerate the decomposition of cellulose, lignin, starch, organic phosphorus, pathogenic microorganisms, to shorten the time of incubation, reduce odor, and destroy harmful microorganisms (Abe et al. 2021, Chen et al. 2020).

The application of organic fertilizer often increases soil organic matter and nutrient content such as nitrogen (Mahmood et al. 2017). Organic matter in the soil affects plant growth and productivity through nutrient supply. The changes in soil physical properties contribute to improving root and stimulatory environment for crop development (Liebig and Doran 1999). Crop production based on the use of organic fertilizer is considered to be more sustainable than chemical-based agriculture. Therefore, using organic fertilizers has received the attention of environmentalists, agricultural researchers and consumers in recent years. This research sought to estimate the generation of crop residues and livestock waste in rural areas in northern Vietnam, compost crop residues with livestock manure and different microbial inoculants, and evaluate the quality of composted fertilizer.

MATERIALS AND METHODS

Material and experimental design. The composting experiments of rice straw employed 8 commercial inoculants including AT-YTB, Bo-bio 386, CNX-ABI, Emina, Emic, Fito -Biomix RR, Sagi-Bio, Saccharomyces- Bacillus Trichoderma. The selected inoculants contain microorganisms, *Trichoderma spp, Bacillus megaterium, Azotobacter,* which degrade cellulose, starch, protein, and phosphate and contribute to nitrogen fixation. The density of microorganisms, between 10^6-10^9 CFU/g, was indicated on the label of the commercial inoculant products (Table 1). The field experiment used rice straw at 2000 kg per pile and was designed as randomly complete block design (RCBD) with 3 replicates. The experiments were conducted in Yen Cuong commune, Y Yen district, Nam Dinh province.

Table 1. Treatment of rice straw with bio-catalytic products

No.	Name of Product	Microorganisms	Density *(CFU/g)*	Activity
1	AT-YTB	*Trichoderma spp, Bacillus megaterium, Azotobacter*	$>10^7$	Microorganism for degradation of cellulose, starch, protein and phosphate; nitrogen fixation.
2	Bo-Bio 386	*Trichoderma spp.*	$>5.10^7$	Microorganism for degradation of cellulose, starch and protein.
3	CNX-ABI	*Trichoderma spp.*	$>2.10^9$	Microorganism for degradation of cellulose, starch and protein.
4	EMIC	*Bacillus lacto, Streptomyces,*	$>10^6$	Microoganisms for fermentation; deodorization;

No.	Name of Product	Microorganisms	Density (*CFU/g*)	Activity
		Saccharomyces		degradation of carbohydrate; polysaccharide formation; inhibitor of toxicity.
5	EMINA	*Bacillus lactose, Bacillus subtilis, Bacillus megaterium*	$>10^8$	Microorganism for fermentation and deodorization, degradation for carbohydrate, phosphate and protein; inhibitor of toxicity.
6	Fito-Biomix RR	*Bacillus polyfermenticus, Trichoderma virens, Streptomyces*	$>10^8$	Microorganism for fermentation; degradation of cellulose, starch, protein and Phosphate; inhibitor of toxicity.
7	Sagi-Bio	*Bacillus, Streptomyces*	$>10^8$	Microorganism for fermentation; degradation of cellulose, starch, protein and phosphate; inhibitor of toxicity.
8	S-Trichoderma	*Saccharomyces, Bacillus, Trichoderma spp*	$>10^9$	Microorganism for fermentation; degradation of cellulose, starch, protein and phosphate; inhibitor of toxicity.

Note: the microbial strains and density are provided by the producers as indicated on the label.

The composting experiments of pig manure employed 8 commercial inoculants including Balasa N01, Bio-Mt, Comfort maker, Emina-E, Emuniv, Fito-Bio RR, Saccharomyces-Trichoderma, and Trichoderma- Bacillus (Table 2). The selected inoculants contain microorganisms, *Bacillus subtilis, Saccharomyces cerevisiae, Streptococcus lactis, Thiobacillus spp*., respond to fermentation and deodorization; degradation of cellulose, starch, protein and inhibitor of toxicity. The density of microorganisms in the inoculants was in the range of 10^6-10^9 CFU/g. Composting of manure was conducted on tarpaulins and covered by black plastic. The inoculant rate varies from 1-2% depending on the type of product.

Table 2. Treatment of pig manure with bio-catalytic products

No.	Product	Microorganisms	Density (*CFU/g*)	Activity
1	BALASA N01	- *Bacillus subtilis; Saccharomyces cerevisiae* - *Streptococcus lactis; Thiobacillus spp.*	$>5,8.10^6$ $>3,7.10^6$	Microorganism for fermentation and deodorization; degradation of cellulose, starch, protein and inhibitor of toxicity
2	BIO-MT	- Aerobic microorganism - Protein degradation microorganism - Cellulose degradation microorganism - Starch degradation microorganism	$>10^7$ $>10^6$ $>10^6$ $>10^6$	Microorganism for fermentation; degradation of cellulose, starch, protein and phosphate
3	Compost maker	Cellulose, phosphate, protein degradation microorganisms, fermentation mico-	$>10^8$	Microorganism for fermentation and deodorization; degradation of cellulose, starch, protein and phosphate

No.	Product	Microorganisms	Density (*CFU/g*)	Activity
		organism and deodorize mico-organism		
4	EMINA-E	- *Trichoderma spp.* - *Streptomyces spp.;* *Bacillus spp* - *Rhodobacter spp*; *Lactobacillus spp*	$>10^7$ $>10^8$ $>10^6$	Microorganism for fermentation and deodorization; degradation of cellulose, starch, protein
5	EMUNIV	Cellulose, starch, protein, lipid, pectin degradation microorganisms	$>10^9$	Microorganism for deodorization; degradation of cellulose, starch, pectin and protein
6	FITO-BIOMIX RR	*Bacillus polyfermenticus*; *Streptomyces thermocoprophilus*; *Trichoderma virens*	$>10^8$ $>10^8$	Microorganism for fermentation; degradation of cellulose, starch, protein and phosphate; inhibitor of toxicity
7	S-Trichoderma (Saccharomyces-Bacillus)	Saccharomyces, Bacillus, Lactobacillus, Nitrosomonas, Nitrobacter, Trichoderma	$>10^9$	Microorganism for fermentation and deodorization; degradation of cellulose, lipit, protein and phosphate; ammonization, inhibitor of toxicity
8	Trichoderma – Bacillus.sp	Calcium phosphate (20%), and Trichoderma-Bacillus	$>10^9$	Microorganism for degradation for cellulo, phosphate and protein; inhibitor of toxicity

Note: The microbial strains and density were provided as indicated on the label.

Data collection and analysis. Structured questionnaires interviews were conducted with producers, enterprises and local authorities in intensive growing areas of four northern provinces in 2017 (Hoa Binh, Ha Noi, Hung Yen and Ha Nam provinces). In each province, 9 communes in three districts were selected to collect data on the crop residue and animal manure. In each commune, 15 farm households including 10 farms with mainly crop production and 5 other ones with livestock were interviewed to investigate the sources of raw materials from agricultural activities. The statistical data on production and trading of agricultural products enterprises, pesticides and bio-catalyze products were collected from the Department of Agriculture and Rural Development.

Treatment condition. Rice straw and pig manure were treated separately. The temperature and humidity were measured in each treatment of straw or pig manure 3 days until 30 days after incubation. The thermometer was inserted into the pile with a depth of 30 cm and measured in triplicate.

Composting effectiveness. At 14 days and 30 days after incubation, the straw and pig's manure were randomly sampled for measurement and analysis of physical and biochemical parameters. In each formula, the sample was taken at 3 points and then mixed into one laboratory sample to analyze pH, moisture, organic matter (OM), total nitrogen (N), digestible phosphorus (P) and digestible potassium (K), C/N ratio and microorganism degrade cellulose. Moisture was measured by drying method, pH was measured using a portable pH meter (ISO 10390:2005), organic matter (OM) by Walkley-Black method (TCVN 9294 : 2012), total nitrogen content (%) by Kjeldahl method (TCVN 8557:2010), digestible phosphorus (mg P_2O_5/100g) by Olsen method (TCVN 8559:2010), digestible potassium (mg K_2O/100g) by Mátlova, flame spectrometer (TCVN 8560:2010). Microorganisms including cellulose degradation, *Salmonella* and *E.coli* were analyzed by direct generation on agar and counting the number of micro cells after 48-72 hours.

RESULTS AND DISCUSSION

Crop residues. In this research, we focused on four provinces experiencing high rates of agriculture production in Hoa Binh, Ha Noi, Hung Yen and Ha Nam. The total amount of crops residues (rice, beans, peanut, cassava, sugarcane, and vegetable) were approximately 3.34 million tons per year. The largest amount of residues was generated from agricultural activities in Hanoi with 1.3 million tons per year. Hoa Binh, Hung Yen and Ha Nam accounted for 917 ton/year, 603.8 ton/year and 469 ton/year, respectively (Table 3). On average, rice residues accounted for 551.7±388.5 thousand tons/year, occupied highest yield compared to other crops. Cassava and vegetables accounted for an average of 146.7 and 72.3 thousand tons/year respectively. Hoa Binh province contributed 578.2 thousand tons of cassava per year. These results indicated that residues from agriculture contain high value of biomass having high potential for re-utilization for succeeding seasons. This is a significant contribution of carbon sources if the residues are used in the composting process. To produce organic fertilizer, other sources of nitrogen should also be considered.

Table 3. Residues from major crops in selected provinces in Vietnam.

Province	Rice	Beans	Peanut	Cassava	Sugar cane	Vegetable	Total residues amount
Hoa Binh	215.8	3.4	19.0	578.2	61.1	39.4	916.9
Ha Noi	1,109.5	49.3	19.4	7.9	0.2	167.5	1,353.8
Ha Nam	395.5	0.7	3.1	0.4	34.7	34.7	469.1
Hung Yen	485.9	15.2	7.6	0.1	47.5	47.5	603.8
Average	551.7	17.2	12.3	146.7	35.9	72.3	835.9
SD	*388.5*	*22.3*	*8.2*	*287.7*	*26.1*	*63.7*	*392.9*

Unit: thousand tons/year Sources: field survey, 2018

On the household level, the farmers recognized and classified the main crop residues into rice straws and vegetables. Surveyed households produced a large amount of rice straw in Hanoi, which accounted for 18 tons/year whereas other provinces generated 2.6-3.3 tons/year (Table 4). Farmers in suburban districts invest in planting high yielding varieties and use harvesting machines, therefore, rice straw was not used for any purpose. Recently, burning of rice straw in open spaces in Hanoi posed serious air pollution (Pham et al. 2021) that an alternative treatment of the straw should be initiated.

Table 4. Volume of main crops residues in households in north Vietnam.

Residue source	Hoa Binh	Ha Noi	Ha Nam	Hung Yen
Rice straw	2.6	18.0	3.3	2.7
Vegetables	10.1	7.3	6.4	8.9
Total	**12.7**	**9.1**	**9.7**	**11.6**

Unit: ton/household/year Sources: field survey, 2018

Livestock residues. Livestock is characterized as buffalo, cows, pigs, and poultry raising at household or farm scale in the Red River Delta. Livestock raising in the Red River Delta consisted of 120 thousand buffaloes, 490.7 thousand cows, 7085.5 thousand pigs, and 99.123 thousand poultries (GSO 2018). While the household owned about a few to several hundred heads of pigs or animals, farms raised up to thousand heads. The low treatment technologies applied in livestock production resulted in a large amount of manure. It was reported that effluent from pig production caused a severe impact on water quality, such as increased TSS, nitrate, phosphorus, and coliform (Vo et al. 2018b). The total manure generation in Hoa Binh, Ha Noi, Ha Nam and Hung Yen provinces are shown in Table 5. The highest amount of manure from pig and poultry in Hanoi corresponded to 1,321.2 and 2,107.8 thousand tons/year, respectively.

Table 5. Manure produced from main livestock in some provinces.

Province	Buffalo	Cow	Pig	Poultry
Hoa Binh	591.3	227.4	344.4	340.1
Ha Noi	131.2	495.2	1,321.2	2,107.8
Ha Nam	18.5	101.5	525.0	469.5
Hung Yen	16.9	149.7	459.9	657.0
Total	757.9	973.8	2650.5	3574.4

Unit: thousand tons/year Sources: Field survey, 2018

Residue generation from livestock production generally consisted of feeding materials and manure. The water discharged from cow farming consisted of 8.18-10.49 liters urine/head.day and 84.87-87.53 liters wastewater/head.day, whereas it produced 7,457 kg manure/head for whole life cycle (405 days) (Cao et al. 2018). This indicates a potential for reuse of manure to reduce the load on the environment. Table 6 shows the general types of livestock wastes at household farms in Hung Yen, Hoa Binh, Ha Noi, Ha Nam provinces. For pig production, the solid waste was higher than the amount of liquid wastes, and the highest levels were found in Hanoi and Hung Yen provinces, 4.9 tons and 4.7 tons per household a year, respectively. The waste generated depended on raising density and type of feeding stuff.

Table 6. Livestock waste from the household

Type of waste	Hung Yen	Hoa Binh	Ha Noi	Ha Nam
Pig manure	4.7	4.1	4.9	4.4
Pig urine	4.0	3.5	4.2	3.7
Buffalo/ cattle manure	16.1	9.8	1.4	1.0
Poultry manure	3.8	4.1	4.9	3.9
Total	**28.8**	**21.6**	**15.6**	**13.1**

Unit: ton/household/year Source: field survey, 2018.

It is important to know how much crop residues and livestock waste can be used for composting because crop residues provide sources of carbon whereas livestock manure provides sources of nitrogen and carbon. The ratio C/N impact on the consumption rate of microorganism activities, therefore, impact on composting efficiency. It was reported that pig waste can be used to feed the earthworms (Nguyen et al. 2018, Vo and Tran 2018). In addition, there is the possibility to combine rice straw and pig manure for earthworms in waste treatment and organic fertilization (Nguyen 2018). Table 8 indicates the rate of residue generation on some main crops and solid manure generation from livestock production. For rice production, the residue was generally 50% of total biomass production. Soybean and peanut accounted for the highest rate (70%) while sugarcane, cassava and vegetable produced only 10-20%.

For livestock production, cattle including buffalo and cattle produced 10-15 kg/capita/day, much higher than that of pig and poultry 2.0 and 0.2 kg/capita/day, respectively.

Rice straw compost. The composition of rice straw consisted mainly of 19.64% lignin, 32.15% cellulose, 28.0% hemicellulose, 11.33% ash and others 8.88% (Shawky et al. 2011). Rice straw contained C, 36.88–44.75%; O, 45.99–46.52%; K, 1.43–2.67%; Si, 4.78–13.92% and others (Dinh et al. 2017). Straw are poorly digested by ruminants because of their high cell-wall content. In this research, targeted inoculants of *Trichoderma spp, Bacillus spp,* and *Azotobacter spp* strains were employed to decompose the rice straw. Table 7 shows the pH, OM, N, digestible P and digestible K analysis at 14 and 30 days after incubation and finished treatment of 8 inoculant conditions and control experiment (without presence of microbial). There was no significant difference in total organic matter between treatments and control with an average of 21.17% compared to 22.39%, respectively. For nitrogen content, there were significant difference for AT-YTB and Bo-Bio inoculants at 30 days after treatment compared with the control, 1.10% and 1.30%, respectively. These nitrogen levels contributed to an increase of 32.5-56.6%.

AT-YTB used *Trichoderma spp, Bacillus megaterium, Azotobacter* as main strains and Bo-bio (*Trichoderma spp.*) showed the highest performance of rice straw composting in terms of enhancing organic matter (OM%), total nitrogen (N%), digestible phosphorus (P%) and digestible potassium (K%). It was reported that *Trichoderma spp*. plays a key role for cellulose decomposition, *Azotobacter* for nitrogen fixing, and *Bacillus megaterium* for releasing phosphorus. Azotobacter species play an important role in maintaining soil N status. Azotobacter species were also found to improve their P solubilizing through mutagenesis starting from soil isolates, therefore, the application of these inoculants shows high composting performance (Kumar et al. 2001; Aasfar et al. 2021). Recently, the reduction of lignin, hemicellulose, and cellulose percentage in rice straw was associated with highest observed cellulase activity at 1.5 U/mL by *Trichoderma spp* (Nurul et al. 2018). This finding is consistent with the treatment performance of AT-YTB and Bo-Bio with *Trichoderma spp*. as main strains.

Table 7. Physical and nutrient characteristics in composting samples after 14 days and 30 days

Inoculants	pH		OM (%)		N (%)		Digestible P(%)		Digestible K(%)	
	14	30	14	30	14	30	14	30	14	30
AT-YTB (CT1)	6.40	6.73	21.03	22.91	0.73	1.10*	0.038	0.048	0.16	0.12
Bo-Bio (CT2)	6.59	6.28	21.32	21.69	0.85	1.30*	0.045	0.052	0.14	0.39
CNX-ABI (CT3)	7.11	6.76	22.29	20.20	0.84	0.80	0.047	0.033	0.25	0.13
Emic (CT4)	6.22	6.47	21.17	20.12	0.84	0.98	0.037	0.038	0.05	0.14
EMINA (CT5)	6.31	7.09	20.63	20.84	1.19*	0.95	0.057*	0.039	0.19	0.15
Fito-Biomix (CT6)	6.84	7.42	22.02	20.36	0.81	0.93	0.075	0.059	0.29	0.18
Sagi Bio (CT7)	6.43	6.19	19.69	22.88	0.89*	0.92	0.039*	0.041	0.14	0.07
S-Trichoderma (CT8)	7.27	6.31	21.75	20.31	1.18*	0.75	0.039	0.041	0.20	0.23
Average	6.65	6.66	21.24	21.17	0.89	1.00	0.051	0.048	0.21	0.17
Control (no microbial)	6.42	6.45	21.87	22.39	0.70	0.83	0.029	0.030	0.16	0.13

Note: *: Significant difference at 0.05 by LSD

Livestock manure compost. Physico-chemical composition (%) of pig manure is characterized by pH, moisture (%), total nitrogen (TN%), organic matter (OM%), C:N ratio, total phosphorus (TP%), total potassium (TK%). There were no significant differences in total organic matter between treatments and control with an average of 33.32% compared to 35.20% after 30 days of treatment (Table 8). For nitrogen, there were significant differences for most treatments and higher than the control (without inoculants) after 14 days of treatment, however, the treatment with Trichoderma showed a significant difference with an increase of 21.7% compared to control. Meanwhile, phosphorus and potassium were highest with Bacillus and Trichoderma inoculants. The manure characteristics were affected by the feed diets with 12-14% crude protein in combination with either 2.5 or 8.7% cellulose. Therefore, the biological inoculants such as Balasa, Bio-MT, Compost maker, Emina-E, EMUNIV, FITO-BioRR, Saccharomyces-Trichoderma and Trichoderma-Bacillus containing various effective microbial strains were applied.

The most effective biochemical inoculants for livestock regardless of substitution of inorganic compositions were Bio-MT, Emina and Trichoderma. The advantages of these inoculants include strains of *Trichoderma spp.*, *Streptomyces spp.*, *Bacillus spp.*, *Rhodobacter spp.*, *Lactobacillus spp*. *Trichoderma spp.* is not only suitable for rice straw composting but also good for conversion of solid manure (Islam et al. 2014). The presence of *Streptomyces spp* is considered as biofertilizer to increase plant growth and yield (Buzón-Durán et al. 2020). *Rhodobacter spp.* was reported as most effective for hydrogen sulfide (H_2S) and ammonia (NH_3) removal, contributing to odor reduction (Chen et al. 2018). Inoculation with *Bacillus subtilis* promoted the maturation of compost, enhanced total organic carbon and humic substance and fermentation of the manure (Duan et al. 2020). It was also confirmed that the starch-degrading microorganism was *Bacillus licheniformis* S3, and its highest enzyme activity was estimated at the C/N ratio 0.8% (Wang and Liang 2021).

The coliforms, especially *E. coli* and *Salmonella*, should be removed in the treated manure. *Cellulomonas* bacteria reduced 88.7% in the treatment with Bio-MT (Table 9). In addition, *E. coli* and *Salmonella* bacteria in all the treatments were reduced significantly by 52.2%-88.9% after 30 days of treatment compared to control. There was no significant difference in concentration of Cd and Pb between treatments and control, after 14 days and 30 days. The most effective biochemical inoculants for producing compost fertilizer from animal waste were Emina-E, BioMt and EMUNIV.

CONCLUSION

The agricultural residues were classified into crop residues and livestock manure. The total crop residues in four provinces in Vietnam was estimated at 3.34 million ton/year, with rice residues being the major source. The conventional agriculture practice was burning or freely discharging the crop residues in the field. The livestock manure was sourced from buffalo, cows, pigs and poultry, with pig and poultry as the largest source. Special microorganisms of commercial inoculants revealed good conversion of cellulose, hemicellulose, lignin and starch from rice straw and pig manure. The optimal conversion was obtained from inoculants with *Trichoderma* spp. for rice straw compost. The most effective microbial inoculants for composting pig waste were combined strains within inoculants which enhanced manure composting efficiency by increasing N, P and K content.

Based on the achieved nutrient composition of the compost products, it is recommended for application in vegetables and other crops and compared with the conventional fertilizers.

Table 8. Physical, nutrient characteristics in composting samples from pig manure after 14 and 30 days.

Inoculants	pH_{KCl}		OM (%)		N (%)		Digestible P (%)		Digestible K (%)	
	14	30	14	30	14	30	14	30	14	30
Balasa	8.0	7.0	26.78	35.02	0.83	0.66	0.14	0.24	0.33	0.33
Bio-MT	7.9	6.9	38.54	32.00	0.88*	0.60	0.21*	0.28*	0.36*	0.21
Compost marker	7.9	6.9	34.03	33.51	0.92*	0.62	0.15	0.25	0.37*	0.34
Emina-E	8.0	7.0	35.02	33.08	0.99*	0.64	0.21*	0.29*	0.24	0.30
EMUNIV	8.2	7.2	34.63	32.00	0.97*	0.61	0.22*	0.31*	0.36*	0.39
FITO-Biomix RR	7.8	6.8	28.97	34.87	0.96*	0.67	0.17	0.27	0.24	0.32
S- Bacillus Trichoderma	7.9	6.9	32.78	30.75	0.88	0.73*	0.14	0.33*	0.30	0.36
Tricoderma-Bacillus.sp	8.0	7.0	34.68	33.08	0.95*	0.66	0.12	0.24	0.39*	0.45*
Average	8.0	7.0	32.28	33.32	0.90	0.65	0.17	0.28	0.32	0.34
Control	8.0	8.2	25. 08	35.20	0.82	0.60	0.14	0.23	0.23	0.34

Note: *: Significant difference at 0.05 by LSD

Table 9. Heavy metal and microorganism content in pig manure after biological treatment after 14 and 30 days.

Inoculants	**Cd (%)**		**Pb (%)**		***Cellulomonas microbes* (CFU.10^4/ml)**		***E.coli* (CFU.10^3/ml)**		***Salmonella* (CFU.10^3/ml)**	
	14	**30**	**14**	**30**	**14**	**30**	**14**	**30**	**14**	**30**
Balasa	0.00027	0.00016	0.00028	0.00026	6.17	6.08	7.24	0.87	2.00	0.44
Bio-MT	0.00023	0.00021	0.00019	0.00020	4.81	0.79*	3.83	2.22	1.16	0.63
Compost Marker	0.00013	0.00023	0.00008	0.00019	7.76	1.16	7.66	1.79	0.91	0.31
Emina-E	0.00015	0.00014	0.00011	0.00021	3.44	0.81*	2.74	1.00	0.97	0.41
EMUNIV	0.00022	0.00017	0.00013	0.00016	4.90	1.86	3.32	1.22	0.49	0.62
FITO-Biomix RR	0.00018	0.00020	0.00016	0.00018	7.04	2.07	4.29	1.57	1.23	0.88
S-Trichoderma	0.00020	0.00018	0.00019	0.00018	6.39	3.18	8.55	2.16	1.35	0.81
Tricoderma- Bacillus.sp	0.00015	0.00017	0.00017	0.00019	7.87	2.48	7.85	3.74	1.37	0.58
Average	0.00019	0.00018	0.00020	0.00019	6.05	2.30	5.68	1.57	1.19	0.62
Control	0.00019	0.00017	0.00022	0.00011	5.38	7.01	9.35	7.84	3.34	7.35

*: Significant difference at 0.05 by LSD

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COVID-19 PANDEMIC, REGIONAL STRUCTURAL BREAK AND THE VOLATILITY OF CHICKEN MEAT PRICES IN INDONESIA

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ABSTRACT

The Covid-19 pandemic has shocked the demand and supply of food product leading to large price fluctuations in the market not only at the national level but also at the regional level. This paper examines the existence of structural break and the volatility of chicken meat prices in six main islands in Indonesia by using additive outlier and ARCH-GARCH models. During the Covid-19 outbreak in Indonesia, the structural break in the chicken meat prices occurred in the six islands. There were variations in terms of the timing of the structural break where Kalimantan and Java island experienced the fastest structural break. Further examinations showed that after the structural break time, the prices of chicken meat in Sumatera, Java, Sulawesi and Papua tended to increase. Meanwhile, the prices of chicken meat in Kalimantan and Bali-NT tended to decline after the break time. The Covid-19 pandemic had a significant impact on chicken meat prices in Java and Bali-NT. The results have important implications for local and central governments that are still under the Covid-19 crisis on how to attain price stabilisation of regional chicken meat prices during the Covid-19 pandemic.

Key words: coefficient of variations, demand shock, price stabilization, supply shock, time break

INTRODUCTION

The Covid-19 pandemic exposed unprecedented shocks to demand and supply in all economic activities including in the food products (FAO 2020; Hobbs 2020; Hossain 2020). The demand shock is evident in many countries when many food businesses close their business putting downward impact on food demand. From the supply side, the production, transportation and distribution network are disrupted due to the restriction policies. The combinations of supply and demand shocks contribute to big food price fluctuations.

Food price fluctuations (both decreasing and increasing) will disrupt the livelihood of both consumers and producers (Workie et al. 2020). From consumer side, increasing food prices will limit their access to sufficient and good quality food particularly for poor people. From producer side, decreasing price will reduce farmers' commitment to produce in the next season (Sahara et al. 2015). While increasing output prices might provide spill over effect for farmers since the majority of farmers are consumers as well. Furthermore, big price fluctuations in food markets will threaten food security in many countries including in Indonesia (FAO 2020; Yu et al. 2020).

The first case of the pandemic was announced by the President of Indonesia on 2 March 2020[1], followed by the policy of large-scale social restrictions[2] to limit the spread of virus. Without offering much time for demand and supply to adjust, restriction policy and stay at home orders contributed to food price fluctuations. Indonesia experienced deflation as indicated by negative value of inflation component from volatile food, -0.38% in March, -0.09% in April and -0.5 in May 2020. But, in June 2020, the inflation rate of volatile food jumped to 0.77, then dropped in the period of July and September 2020 before increasing again in the period of October to November 2020 (Statistics Indonesia 2020b).

Ensuring price stability should be the main agenda for Government of Indonesia not only for stapple food (*e.g.*, rice), but also for animal protein sources (Sutawi et al. 2020) including chicken meat, the most popular protein source in Indonesia. Since the chicken meat price is more affordable compared to beef price, it is not surprising that until 2019 the weekly per capita consumption of chicken meat in Indonesia was higher compared to beef, 0.124 kg versus 0.009 kg, respectively (Statistics Indonesia 2020a). From the production side, about 67% of Indonesian meat production consisted of chicken meat (EIBN 2018).

The spread of Covid-19 will undoubtedly affect both the consumption and production of the chicken meat, leading to the changes in the price. Consumers of chicken meat in Indonesia are restaurant and catering sectors; and households. Since many restaurants, caterings and other food stalls are closing their business during the pandemic combined with the reduction of consumer income, the demand for chicken meat reduced significantly leading to price reduction in chicken meat market. On the supply side, the restrictio n policy during Covid-19 impact on reduction in poultry supply at farm level due to input price fluctuations during restriction particularly DOC (Day Old Chicken) and feed. Input price fluctuations might potentially increase the prices of chicken meat in the markets. The restriction policy will also impact on traders since their face difficulty to distribute chicken meat to consumers and inputs to producers which will in turn contribute to the chicken meat price fluctuation in the markets. However, whether the prices of chicken meat in market increases or decreases during the pandemic need to be examined. It is expected that the dynamic in chicken meat prices will still continue considering the spread of Covid-19 is still ongoing and tends to get worse.

The paper examines the dynamic behaviour of chicken meat prices in the main islands in Indonesia (Sumatera, Java, Bali-NT, Kalimantan, Sulawesi and Papua) by using longer time series data on daily prices, the structural change in the chicken meat prices occur s during Covid-19 pandemic, and the impact of Covid 19 pandemic on chicken meat price volatility. To the best of our knowledge, studies focusing on food price behaviours during the pandemic are still limited. This study also focuses on regional level since the price behaviour might vary across islands. The results from this paper will inform policy makers related to the dynamic of chicken meat prices in the six islands and can be used as a base for policy mitigation actions to focus on the islands likely to suffer the most of the price shocks during the pandemic.

METHODS

Secondary data on daily chicken meat prices at retail level in the six islands in Indonesia (Java, Sumatera, Bali-NT, Kalimantan, Sulawesi and Papua) were utilized in this study. The price data was compiled from Strategic Food Pricing Information Center, the official data sources of volatile food

[1] https://www.cnnindonesia.com/nasional/20200302111534-20-479660/jokowi-umumkan-dua-wni-positif-corona-di-indonesia

[2] https://nasional.kompas.com/read/2020/04/15/09375511/pemerintah-psbb-diberlakukan-di-daerah-pusat-penularan-covid-19?page=2

prices produced by Indonesian Central Bank (Bank Indonesia 2020). In order to test the structural break of the chicken meat before and during Covid-19 outbreak, this study used a longer time series data, i.e., daily retail prices for 494 days from 1 January 2019 to 31 December 2020. Data from 1 January 2019 to 2 March 2020 represented to the period before covid outbreak in Indonesia, while the period during covid 19 showed by the data from 3 March 2020 to 31 December 2020.

Descriptive statistic and Coefficient of Variations (CV) were performed in this study. CV was used to examine food price stability (Jati 2014) which the value was calculated by comparing between the standard deviation and the mean of the price. Higher value of CV indicated the fluctuations of chicken meat prices.

This study utilized an additive outlier model to analyze structural break in the time series data of the chicken meat prices in the six islands (Cariappa et al. 2020; Perron and Vogelsang 1992; Baum 2005), There were two steps implemented in this study. The first step was performing the first regression analysis for each island by following equation (1) below.

$$y_t = \mu + \beta . t + \gamma DT_t^* + \tilde{y}_t \ldots (1)$$

Where t was number of time series data (t=1,2..T, in this case T=494), DT_t^* was a dummy variable which the value was equal to one if $t > T_b$ (T_b is the break date) and $\tilde{y}_t$ represented the detrended chicken meat prices.

The next step was performing the second regression analysis by using $\tilde{y}_t$ as dependent variable with dummies and lagged differences as independent variables (equation 2).

$$\tilde{y}_t = \sum_{i=0}^{k} \delta_i D(TB)_{t-1} + \alpha \tilde{y}_{t-1} + \sum_{i=1}^{k} c_i \Delta y_{t-1} + \varepsilon_t \ldots (2)$$

Where $D(TB)_t$ referred to dummy variable which the value is equal to one if $t=T_b+1$, and ε_t is the error term. The null hypothesis of unit root was rejected if the value of t statistic for α=1 was larger than the critical values as given in Perron and Vogelsang (1992), showing the evidence of structural break. The truncation lag parameter was represented by the value of k where the additive outlier model, the value of k was unknown and identified after a grid search for the least t-statistic of unit root hypothesis (α=1) and sequential F tests. This study used 'clemao1' procedure to conduct AO1 test. This procedure captured the sudden changes of chicken meat prices by using the two step procedures as well as testing hypothesises related to the existence of structural break.

In order to test whether the Covid pandemic outbreak had a significant influence on the prices of chicken meat, this research utilized the ARCH-GARCH model. The model had been widely used as a powerful model in the literature in analysing food price volatility since it could capture the heterogeneities in first and second order moment simultaneously (Wang et al. 2020; Yu et al. 2020). This study included daily new infection of Covid 19 as the proxy of pandemic impact in the model (Yu et al. 2020).

$$y_t = u_0 + u y_{t-1} + u_2 Infected + \varepsilon_t + \rho_1 \varepsilon_{t-1} \ldots (3)$$

The variable y_t referred to the daily chicken meat prices in the seven islands during Covid 19 pandemic (March-December 2020). The variable of 'Infected' represented the number of daily new confirmed infections officially reported by the Ministry of Health of Indonesia and ε_t is residual:

$$\varepsilon_t|\psi_t \sim N(0, \sigma_t^2) \quad \ldots(4)$$

Where σ^2 is the variance and followed the equation:

$$\sigma^2 = \omega_0 + \omega_1 Infected + \alpha\sigma_{t-1}^2 + \beta\varepsilon_{t-1}^2 \quad \ldots(5)$$

The parameters estimated were ρ_1, α, and β where α represents the coefficient for GARCH, while β represents ARCH. To capture the impact of Covid 19 pandemic in each island, this study included the variable of dummy interaction by multiplying dummy variable with the number of daily new infections in each island. To avoid dummy trap issues, the study utilized Papua Island as baseline, therefore, the model consists of five dummy interaction variables. The extended model of equation (5) is as follows:

$$\sigma^2 = \omega_0 + \omega_1 Infected + \alpha\sigma_{t-1}^2 + \beta\varepsilon_{t-1}^2 + \theta_1(D_s * Infected_s) + \theta_2(D_j * Infected_j) + \theta_3(D_b * Infected_b) + \theta_4(D_k * Infected_k) + \theta_5(D_w * Infected_w)$$

The variable of D_s, D_j, D_b,D_k, and D_w were dummy variables in the islands of Sumatera, Java, Bali-NT, Kalimantan, and Sulawesi, respectively. The dummy variables for each island were interacted with the number of daily new confirmed infections for the respective island.

RESULTS AND DISCUSSION

Regional price variations. In Indonesia, chicken meat is included as one of the volatile food commodities which contributes to the inflation rate. In order to stabilize the prices of staple food including chicken meat in Indonesia, the Ministry of Trade issued regulation No 07 Year 2020 stating that the maximum prices of chicken meat at consumer lever is Rp 35,000 per kg (Ministry of Trade 2020). In response to Covid-19 pandemic, the Indonesian Ministry of Agriculture through the Directorate General of Livestock and Animal Health issued a Circular Letter 09246T/SE/PK/230./F/08/2020 to stabilize chicken meat price at farm level[3] by controlling chicken meat production. In the circular letter, the supply control is conducted by implemented two strategies: (1) reducing the production of DOC and (2) promoting the utilization of cold storage to increase the shelf life of slaughtered chicken. By implementing these efforts, the prices of chicken meat at farm level is expected to increase and remain stable towards the market reference prices as set by the regulation No 07 Year 2020. The regulation and the circular letters, however, apply to all regions in Indonesia. In fact, the dynamics of chicken meat prices differ among regions in Indonesia.

Descriptive statistic of chicken meat prices in the six islands during the period of analysis (1 January 2019- 31 December 2020) is presented in Table 1. The price of chicken meat per kg in six islands ranged from Rp 29,392 to Rp 38,545 which Papua was the highest, followed by Bali-Nusa Tenggara (Bali-NT), Kalimantan, Java, Sumatera and Sulawesi.

The results from previous studies show food price differ across regions (Chen et al. 2020; Daryanto et al. 2020; Ghosh 2010). This study concludes similar results in which there are differences in chicken meat price among islands. It is expected that production and consumer demand are the

[3] https://ditjennak.pertanian.go.id/kementan-jaga-stabilisasi-harga-ayam-potong

main sources of the price variations of the chicken meat prices. Food prices in low production areas tend be more expensive compared to the high production areas (Kim and Mark 2017; Jati 2014).

Table 1. Descriptive statistic of chicken meat prices in the six islands, 1 January 2019- 31 December 2020

Island	Number of Observations	Mean	Std. Dev.	Min	Max	Coefficient Variation (CV)
Sumatera	494	32,545	2,811	26,040	39,670	8.64
Java	494	33,690	2,310	28,875	41,175	6.86
Bali-NT	494	37,859	3,602	30,817	53,450	9.51
Kalimantan	494	35,182	3,813	27,040	45,380	10.84
Sulawesi	494	29,392	2,334	24,092	35,992	7.94
Papua	494	38,545	1,726	36,000	46,125	4.48

Java is the main production zone of chicken meat in Indonesia, contributing 67.57% to national production in 2019 (2.4 million tonnes), followed by Sumatera Island (443 thousand tonnes or 12.66% of national production), Kalimantan Island (237 thousand tonnes or 6.8% of national production), Bali-NT (139 thousand tonnes or 3.99% of national production), Sulawesi (109 thousand tonnes or 3.14% of national production) and Papua (7.9 thousand tonnes or 0.23% of national production) (Statistics Indonesia 2020c) .

From the production data above, Papua had the lowest chicken meat production. Regional disparities in infrastructure contribute to price variations among islands (Ghosh 2010). Lack of logistic infrastructure provides challenges to distribute food (including chicken meat) from production areas to consumer areas particularly during Covid-19 pandemic (Reardon et.al. 2020). Limited chicken production combined with geographic location Papua - located in Eastern Part of Indonesia which lack infrastructure for food logistic- contribute to high prices of chicken meat in this island.

Bali-NT island had the second highest chicken meat prices. Although production of chicken meat in this island was relatively high and its distance is close to Java Island (the main production zone of chicken meat in Indonesia), demand for chicken meat in this island is not only sourced from local people, but also from tourists visiting this island. It is well known, that Bali-NT is the main tourist destination in Indonesia, particularly before Covid-19 pandemic; therefore, the food prices, including chicken meat are usually relatively high in this island.

Based on results of CV, Kalimantan experienced big price fluctuations of chicken meat compared to other islands as indicated by the highest CV in the island, followed by Bali-NT. In these two islands, the prices of chicken meat were relatively high and above the reference chicken meat price set by Ministry of Trade (Rp 35,000 per kg). Sumatera, Sulawesi and Java also experienced big price fluctuations, but the prices of chicken meat in these islands were below the reference price. Papua has the lowest CV, but the price of chicken meat in this island is relatively high and above the reference price; therefore, more attentions to monitor the price volatility is also needed in this island.

Structural break of chicken meat prices. The results from additive outlier model for unit root testing of sudden (structural) change in the prices of chicken meat are presented in Figure 1-6, which is the summarized in Table 2. All structural break in the six islands occurred during the pandemic, which the times of break varied among islands. Kalimantan experienced the fastest sudden change in chicken meat prices (2 April 2020), followed by Java (19 May 2020), Sumatera (28 May 2020), Sulawesi (27 May 2020), Papua (5 June 2020) and Bali-NT (3 July 2020).

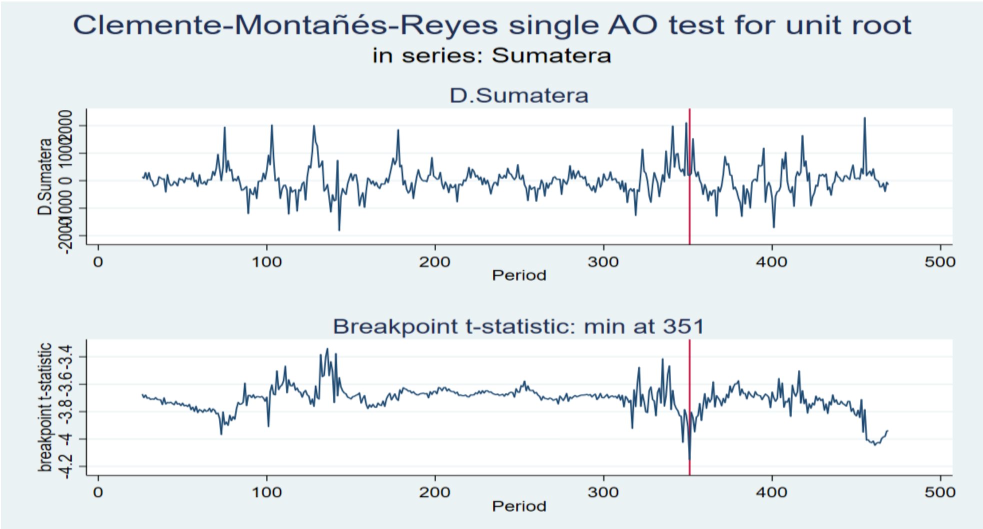


Fig. 1. Structural break of chicken meat prices in Sumatera Island

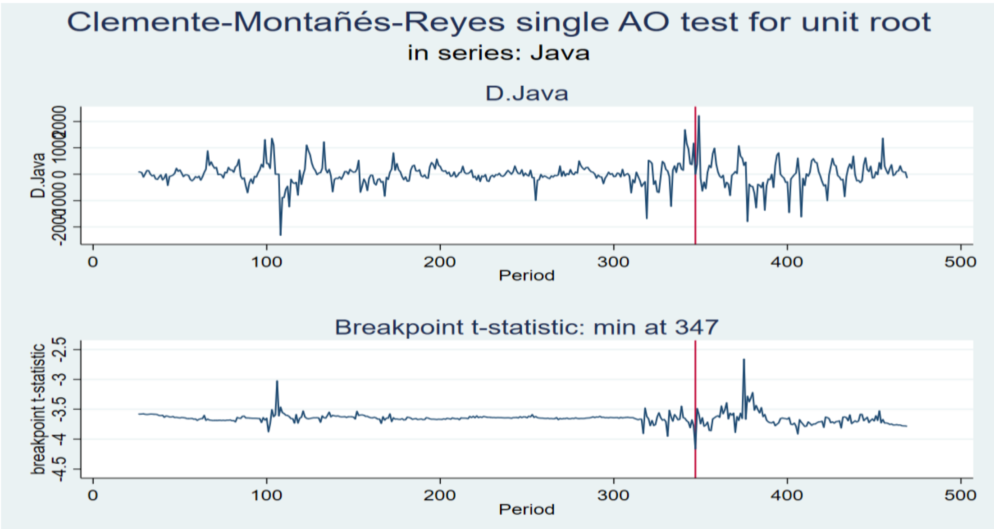


Fig. 2. Structural break of chicken meat prices in Java Island

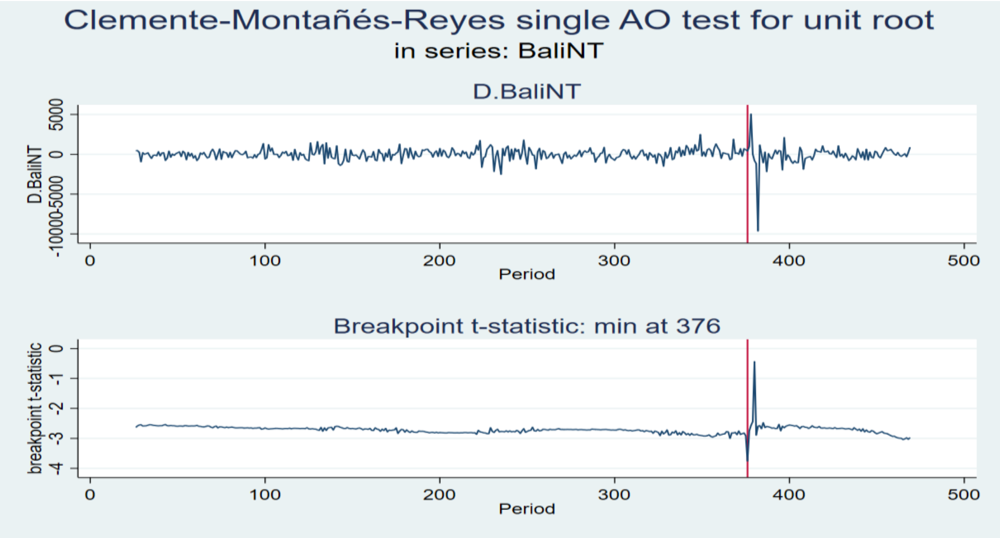


Fig. 3. Structural break of chicken meat prices in Bali-NT Island

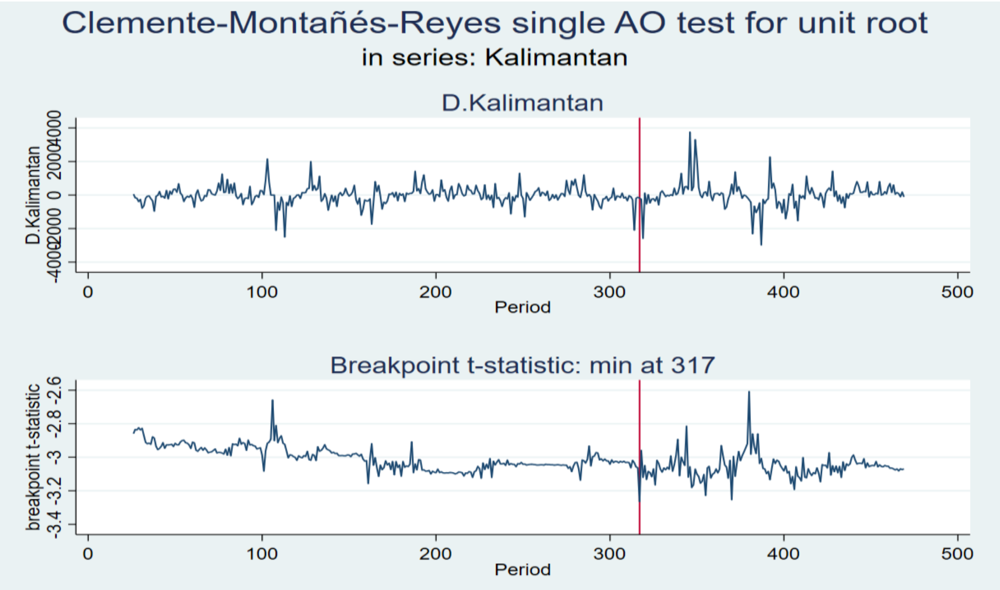


Fig. 4. Structural break of chicken meat prices in Kalimantan Island

During the pandemic, chicken meat prices increased in the four islands, i.e., Sumatera, Java, Sulawesi, and Papua. In Sumatera, the average price before break point was about Rp 32,275 per kg and after the break point the average price increased to Rp 33,209 per kg. In Java and Sulawesi Islands the average chicken meat price after break point increased to Rp 33,949 per kg and Rp 30,737 per kg, respectively. Although these three islands experienced increased chicken meat prices during the pandemic, the average prices were still below the consumer reference prices set by Ministry of Trade.

In Papua, the chicken meat prices before and after break point was always higher compared to the reference prices. Before break time, the average chicken meat price in Papua was Rp 38,060 per kg, increasing to Rp 39,799 per kg after the break point. The fact that food price increased during the pandemic is similar to the previous study by Yu et al. (2020). One possible explanation for this situation is that Covid-19 pandemic has more substantial effect on the chicken meat supply than the reduction in chicken meat demand in the four islands. Restriction policy implemented by Indonesian Government during the pandemic disrupts the transportation and distribution of commodities including inputs for poultry production, leading to the increase in the input prices. This will promote price increase of chicken meat in the four islands.

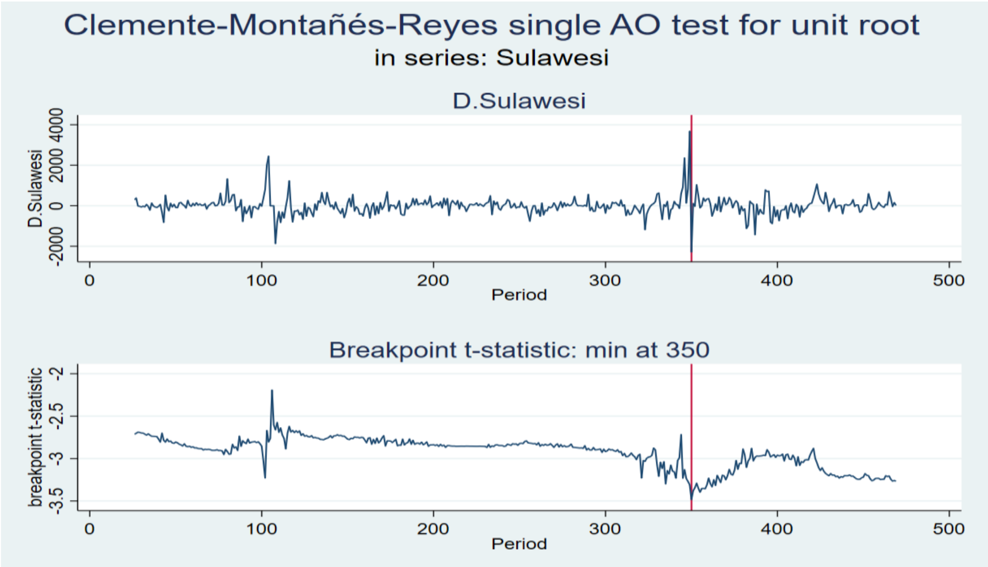


Fig. 5. Structural break of chicken meat prices in Sulawesi Island

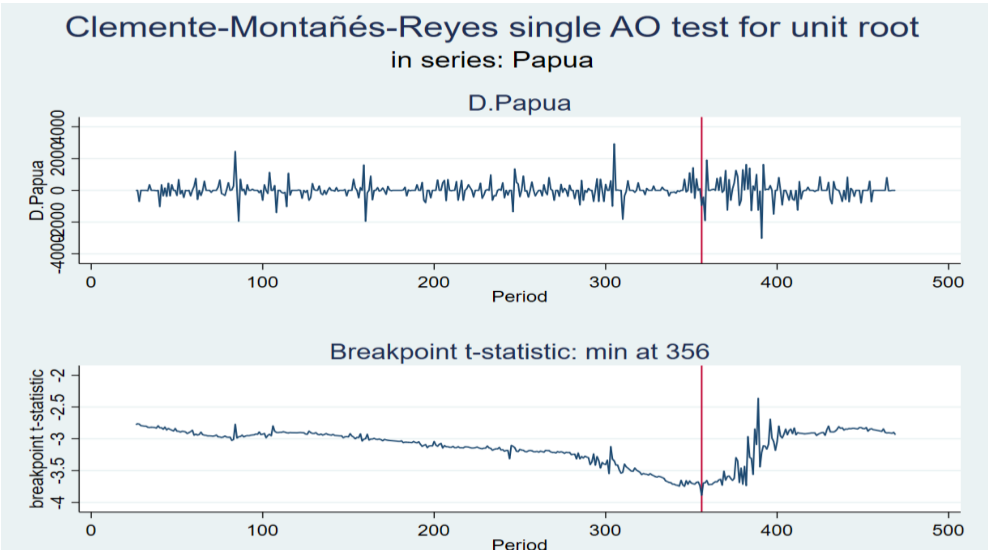


Fig. 6. Structural break of chicken meat prices in Papua Island.

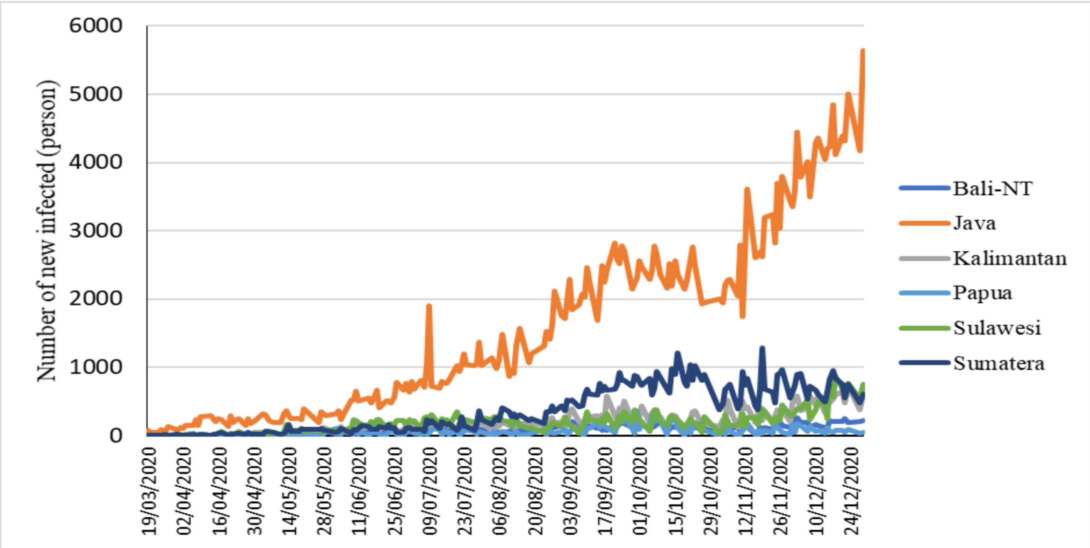
Table 2. Chicken meat prices (Rp/kg) and the coefficient of variation (CV) before and after structural change in the six islands

Island	Before Break Point						After Break Point					
	Period	Mean	Std. Dev.	Min	Max	CV	Period	Mean	Std. Dev.	Min	Max	CV
Sumatera	1/1/19-27/5/20	32,275	2,675	26,040	39,670	8.29	28/5/20-31/12/20	33,209	3,027	27,940	39,650	9.11
Java	1/1/19-18/5/20	33,580	1,746	28,875	38,967	5.20	19/5/20-31/12/20	33,949	3,272	29,325	41,175	9.64
Bali-NT	1/1/19-2/7/20	37,944	3,260	30,817	47,400	8.59	3/7/20-31/12/20	37,591	4,529	31,667	53,450	12.05
Kalimantan	1/1/19-1/4/20	35,464	3,177	28,650	45,380	8.96	2/4/20-31/12/20	34,675	4,712	27,040	43,750	13.59
Sulawesi	1/1/19-26/5/20	28,839	2,152	24,092	35,992	7.46	27/5/20-31/12/20	30,737	2,215	25,450	34,625	7.21
Papua	1/1/19-4/6/20	38,060	1,037	36,000	42,150	2.73	5/6/20-31/12/20	39,799	2,396	36,925	46,125	6.02

The opposite situation occurred in Kalimantan and Bali-NT islands in which during the pandemic, the chicken meat prices tended to decrease. Restrictions implemented by Indonesian Government reduced demand for the chicken meat (Surni et al. 2020). This was noted especially in Bali-NT, the main tourist destination in Indonesia, where demand from restaurants and hotels was reduced reflecting a negative impact on demand for chicken meat. As such the average prices of chicken meat in Bali fell to Rp 37,591 per kg after the time break. It is also important to note that the price of chicken meat in Bali-NT both before and after time break was always higher than the reference price of chicken meat at consumer level set by Ministry of Trade. In Kalimantan, the average price of chicken after the time break was reduced to Rp 34,675 per kg, slightly lower than the reference price. The phenomenon of price reduction during the Covid-19 outbreak is similar to the case of cabbage in China (Yu et al. 2020).

The price fluctuations were more evident after the structural break occurred in the six islands (Table 2). Kalimantan and Bali-NT were the islands registering the highest increase of CV after the break time which the values of CV were above 10. The value of CV of chicken meat in Java doubled from 5.20 to 9.64. A dramatic increase in the CV has also occurred in Papua Island which the value of CV raised from 2.73 to 6.02. A big price fluctuation in the chicken meat products provides a major price risk to consumers and producers.

The impact of Covid 19 pandemic on chicken meat price volatility. Figure 7 shows the trends of newly confirmed infections in each island. The virus has spread to every island with case numbers continuing to increase. Java Island has the highest daily new cases in Indonesia followed by the islands of Bali-NT, Sumatera, Kalimantan, Sulawesi and Papua.



Source: Indonesian Ministry of Health (2021)[4]

Fig. 7. Daily confirmed case of Covid 19 in the six main islands in Indonesia from March-December 2020 (person)

[4] https://infeksiemerging.kemkes.go.id/document/download/cover, downloaded on March 2021

The results of ARCH-GARCH model with the exogenous of daily infected case of Covid-19 pandemic are presented in Table 3. The variable of daily infected case has a significant impact on chicken meat prices.. The regional differences exist shown by dummy interactions are significantly positive in Java Island and negative in Bali-NT Island. The historical chicken meat prices have a significant positive effect on the current prices.

Table 3. The impact of Covid 19 on the Indonesian chicken meat prices.

Variables	Coefficient	Standard error	z	
Prices				
Infected	0.01	0.01	1.92	**
Dummy Sumatera_infected	0.00	0.14	0.01	
Dummy Java_infected	-0.07	0.04	-1.74	*
Dummy BaliNT_infected	2.55	0.71	3.60	***
Dummy Kalimantan_infected	-0.14	0.19	-0.72	
Dummy Sulawesi_infected	-0.12	0.19	-0.63	
Constant	31029.55	1954.68	15.87	***
AR (1)	0.99	0.00	334.87	***
MA (1)	0.19	0.02	7.62	***
ARCH	97.37	25.56	3.81	***
GARCH	0.70	0.05	15.19	***
Constant	-2982.57	2324.305	-1.28	
Observation	1122.00			
Likelihood	-8595.08			
Wald chi2	118810.33***			

Note: *** Significant at 1%, ** Significant at 5%, * Significant at 10%

The present price of chicken meat in Indonesia is influenced by the historical prices and the number of infected cases. The number of new infections have a significant negative effect in Java Island indicating that as the number of confirmed Covid-19 cases increase in Java Island, the chicken meat price experiences a downturn trend compared to the baseline price (in this study the baseline is Papua Island). Java is the epicentre of Covid-19, as such Indonesian government implemented a more strict quarantine policy in this province. This will reduce more demand for the chicken meat than the negative effect of supply.

The outbreak of Covid-19 significantly increased the chicken meat price in Bali-NT than in Papua Island. As outlined previously, Bali-NT depends on Java Island to fulfil the demand for chicken meat. As such, Covid-19 outbreak disrupted the supply of chicken meat in Bali-NT leading to increase in chicken meat prices.

The fact that the pandemic impacted on the food price anomalies does not only appear in the case of chicken meat as examined in this paper, but also for other commodities in several countries such as China (Yu et al. 2020; Chen et al. 2020), Italy (Coluccia et al. 2021) and India (Cariappa et al. 2020). The degree of impact, in terms of changes in food prices, will depend on the magnitude of demand and supply shocks of the commodities in the regions. If reduction in supply due to the pandemic outstrips demand shock, then food prices spike, and vice versa (Barrett et. al. 2020). It is important to note that the attention to the changes in food prices during Covid 19 should focus at the national level as well as the regional level. This is because demand and supply shock due to the pandemic differs among regions, with implications of a regional price gap. This study confirms the existence of regional price gap with different trends among islands.

Learning from the situation, the idea of having daily food price monitoring is no longer seen as a necessity for the Government of Indonesia. Considering that the spread of Covid-19 pandemic is getting worse, particularly after new strain was confirmed, it is expected that price changes will continue in the future and might provide another break. It is worth noting that price fluctuations directly hinder the ability of the producers to invest in the production and consumers' ability to buy food. Reducing access to food, in this case chicken meat, will impact on a reduction of protein intake providing a negative consequence on health of Indonesian people which will in turn increase the risk of infection of the virus.

CONCLUSIONS AND RECOMMENDATIONS

The impacts of Covid-19 on chicken meat prices are heterogeneous in different islands. Structural break of chicken meat prices exists in which the chicken meat prices in Sumatera, Java, Sulawesi and Papua Island increased after the structural break, while in Kalimantan and Bali-NT Islands experienced price reduction. Considering the virus has spread all around the islands of Indonesia, the supply chain of chicken meat will continue to be disrupted. Therefore, the government needs to ensure price stability in the islands since fluctuations in price impact regional food security in chicken commodity.

Local governments, particularly in the islands with higher retail prices above the reference price set by Ministry of Trade, should coordinate more actively with the central government to avoid scarcities and maintain stable chicken meat prices. One of the efforts that can be implemented is by improving infrastructure for safety that can support chicken meat distribution from the production area (Java) to other islands in Indonesia particularly to Papua and Bali-NT. Major reforms are also needed in the marketing system of chicken meat in Indonesia by implementing digital platforms particularly for frozen and processed chicken products to support social distancing policy. Consumers can access chicken meat as well as avoid crowded areas, reducing the probability of being infected by the virus by using these platforms. Local governments should actively release information on the situation of Covid-19 and the supply of chicken meat to meet the price stabilization.

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CONFLICT OF INTEREST

The author reports that there is no competing interest with any institutions and persons in this manuscript.

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EFFECT OF FERTIGROE®N NANOFERTILIZER APPLICATION ON BACTERIAL POPULATION, ENZYMATIC ACTIVITIES, AND MICROBIAL BIOMASS IN TWO SOIL TYPES

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ABSTRACT

Nanomaterials have various beneficial applications, however, studies on their effects on the environment are still lacking and need to be assessed. Microorganisms are considered as the most sensitive indicators of environmental stresses. This study was conducted to assess the application effects of a commercialized Nanofertilizer, FertiGroe®N, on the total culturable soil bacterial population, enzymatic activities namely dehydrogenase (DHA) and urease (UA), and microbial biomass (MCB) using Philippine soils. The factors included the two soil types, Lipa clay loam (LCL) and Sariaya sandy loam (SSL), and four treatments, namely, nanofertilizer, commercial fertilizer (urea), nanocarrier (treated zeolite), and untreated control. The experiment was laid out in split-plot completely randomized design (CRD) over 75 days incubation period under laboratory conditions. Results showed that treatments had no significant effect on the culturable bacterial population in both soil types. FertiGroe®N, urea, and the nanocarrier had no significant effect on the culturable bacterial population and DHA. At 1 day after amendment (DAA), FertiGroe®N significantly increased UA in LCL but was reduced in SSL. Urease activity in SSL was significantly reduced by urea up to 75 DAA. FertiGroe®N had no adverse effect on the microbial biomass of both soil types. On the other hand, urea significantly reduced MCB in both soil types at 35 DAA. The effect of FertiGroe®N and the non-nanomaterials on the parameters measured was influenced by soil type. FertiGroe®N seems to be less detrimental to the microbial biomass than urea. These results indicated that the FertiGroe®N Nanofertilizer can be applied in both soil types to enhance crop production without any adverse effect on soil microorganisms.

Key words: nanotechnology, nanomaterial, soil enzyme, fertilizer

INTRODUCTION

Nanotechnology is the most innovative aspect of the 21st century and has the potential to play a crucial role in food security, safety, and production (Sabir et al. 2014; Servin et al. 2015). Recent focus has been given to the optimization and development of NMs for application in the agricultural industry (Ioannou et al. 2020). Agricultural sector is heeding attention towards nanomaterial-based product in the form of nanofertilizers to resolve the scarcity of food (Pandey et al. 2021). The use of the nanofertilizers in crop production increases the efficiency of fertilizer application through target delivery while reducing the risk of environmental pollution, such as nitrogen leaching, and minimizing the potential negative effects of overdosage as well as the frequency of the application. (Naderi and Danesh-Shahraki 2013; Srilatha 2011; Nbada et al. 2021). A nanoporous zeolite-based N nanofertilizer (nanozeourea) can be used as an alternate strategy to improve fertilizer use efficiency in crop production (Kaushik et al. 2014). Thus, employing these nanoparticles in agriculture will be beneficial to the crops (Srilatha 2011).

Nanotechnology, having an immense potential in various area of research and our daily life, has become a boon to the society (Rai et al. 2012; Ingle et al. 2014) and the production and use of NMs are increasing rapidly (Gottschalk et al. 2013). Since there is an increase in the manufacture, use, and disposal of nanomaterial-based products, their release in the soil and the environment also increases (Rousk et al. 2012; Rizwan et al. 2016; Holden et al. 2014). In fact, as fertilizer, NMs can be directly added to soil (Batley et al. 2011). The extensive production and excessive application of NMs poses a serious threat associated with nanotoxicity, especially the unintentional exposure of non-target environmentally beneficial bacteria, threatening the native soil inhabitants (Santimano and Kowshik 2013; Pandey et al. 2021).

Soil organisms are critically important since they directly influence soil ecosystem processes and soil quality. Thus, they serve as biological indicators and any factor that affect soil microbial biomass, activity, and populations would greatly affect soil quality, sustainability, and plant productivity (Dinesh et al. 2012; Hill et al. 2000). Soil microbial biomass plays an important role in maintaining the soil structure, facilitating microbial metabolic processes, and biogeochemical cycling of essential macro and micronutrients (Zhang et al. 2016; Chaudhary et al. 2021). Strongly associated with the microorganisms are soil enzymes which are closely related to the biophysicochemical characteristics of the soil and are important for the regulation of the formation of soil fertility, including nutrient cycling in nature (Purev et al. 2012; Makoi et al., 2008[CPBJ1]; Shiyin et al. 2004). Soil dehydrogenase, an intracellular enzyme, is highly correlated with the microbial biomass and its activity and can be considered as a good measure of soil microbial oxidative activity (Von Mersi and Schinner 1991; Camiña et al. 1998). Urease catalyzes the hydrolysis of urea into CO_2 and NH_3, hence dictating the fate of urea (Maddela and Venkateswarlu 2018). Research on the effect of nanomaterials on the biological activity in the soil is very important as well as to understand the impact on the environment (Handy et al. 2008).

FertiGroe® is a nanofertilizer containing the macronutrients N, P and K developed by the University of the Philippines Los Baños (Fernando et al. 2017; Fernando et al. 2019). FertiGroe® has been tested in various crops and has shown to increase uptake efficiency of nutrients in banana, rice, corn, sugarcane, coffee, cacao, and vegetables. However, the effects of FertiGroe®N Nanofertilizer on soil microorganisms have not been evaluated. In this study, the impact of using FertiGroe®N Nanofertilizer on the soil bacterial population, microbial biomass, and enzyme activities was investigated. These indicators could serve as important criteria for the commercialization of FertiGroe®N Nanofertilizer.

MATERIALS AND METHODS

Soil collection and characterization. Two soil types were used for the experiments: Lipa Clay Loam (LCL) and Sariaya Sandy Loam (SSL). LCL soil samples were collected from the Central Experiment Station, UPLB. On the other hand, the SSL soil samples were obtained from Brgy. Canda, Sariaya, Quezon. Soil sampling was done by collecting the top 20 cm of the surface soil. Prior to analysis and set-up of experiments, visible debris were removed, and the soil was air-dried and passed through a 2-mm sieve. A portion of the collected soil was characterized chemically by quantitatively determining the pH in water (1:2.5), organic matter (OM) (Walkley and Black 1934), total N (Kjeldahl Method), exchangeable K (Flame Photometer Method), CEC (Ammonium Acetate Method), and available P

(Bray No. 2 Method). Physical characteristics, such as texture (Hydrometer Method) and field capacity (FC) and moisture content (Gravimetric Method), were also determined. Selected physico-chemical characteristics of the two soils are detailed in Table 1.

Table 1. Selected physico-chemical properties of Sariaya and Lipa soil.

	Sariaya Sandy Loam	**Lipa Clay Loam**
pH (1:2.5 soil:water)	5.80	5.40
OM, %	1.40	3.11
CEC, $cmol_c\ kg^{-1}$	14.09	40.06
Total N, %	0.08	0.19
Available P, ppm	348.50	10.50
Exchangeable K, $cmol_c\ kg^{-1}$	1.99	2.05
Texture	6.48% clay, 28.94% silt, 64.58% sand	33.16% clay, 39.61% silt, 27.23% sand
% Field Capacity	33.1	50.8

Experimental design. The experiment was conducted at the Division of Soil Science (DSS)-Agricultural Systems Institute (ASI), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB) (121°14'40.84" E 14°09'34.55" N) from July 2018 to September 2018. The nanofertilizer (FertiGroe®N) and nanocarrier (treated zeolite) were obtained from UPLB-Biotech (National Institute of Molecular Biology and Biotechnology). An incubation experiment was performed in a sterile 10" by 12" polypropylene containers each filled with 500-g sieved moist soil (100% moisture content at field capacity). Moist soil was pre-incubated for a week prior to addition of treatments. A 2 x 4 factorial experiment, which included two types of soils (LCL and SSL) and four types of treatments ((FertiGroe®N, (150 kg N/ha), commercial fertilizer, urea (150 kg N/ha), nanocarrier (150 kg/ha), and untreated soil as control, was carried out in the Soil Microbiology Laboratory of the Division of Soil Science at room temperature. The treatments were replicated three times and laid out in split-plot completely randomized design (CRD) (Gomez and Gomez 1984). Samples were collected at 1, 7, and 14 day(s) after treatments (DAT) for culture-dependent analysis of soil bacteria. Simultaneously, assessment of dehydrogenase and urease (modified non-buffer method) activities, and microbial biomass were also performed at 1, 35, and 75 DAT.

Enumeration of soil culturable bacteria. Indirect viable plate count was performed to determine the culturable soil bacterial population by pour plating in Asparagine Mannitol Agar medium (Thorton 1922) at 10^{-4}, 10^{-5}, and 10^{-6} dilution. Nystatin was added prior to pouring of the medium at a rate of 50 mg/L. Each dilution was plated in duplicate, allowed to solidify, inverted, wrapped with paper, and incubated at room temperature (~29 °C) for 5 days. After 5 days, the bacterial colonies were counted. Valid count is between 25 to 250 colonies for bacteria (Breed and Dotterrer 1916). Bacterial colonies were expressed as colony forming units per gram of dry soil (CFU/g dry soil).

Dehydrogenase activity (DHA) assay. Soil dehydrogenase activity assay was performed according to the modified procedure of Tabatabai (1982). About 1-g soil was placed in a screw-capped tube, to which 1-mL 3% (w/v) 2,3,5-triphenyl tetrazolium chloride (TTC) was added, stirred, and incubated for 96 h at 27 ºC. After incubation, 10-mL ethanol (EtOH) was added to the mixture and vortexed for 30 s. The tube was incubated for 1 h to allow the suspended soil to settle. About 5-mL of the supernatant was transferred to a clean test tube, and the absorbance was measured at 485 nm using a spectrophotometer (MultiSkan™ Go Microplate, Thermo Scientific). The result was reported in μg triphenyl formazan (TF) g^{-1} dry soil 96 h^{-1}.

Urease activity (UA) assay. Urease activity assay was performed using the modified non-buffer method (Kandeler and Gerber, 1988). A 5-g soil sample was mixed with 2.5 mL of 720-mM urea, respectively, and incubated for 2 h at 37 °C (Dash et al.1981). After incubation, the control sample was treated with 2.5-mL of 720 mM urea and all samples with 30-mL of acidified 2 M KCl. The samples were shaken for 30 min on a rotary shaker and filtered using Whatman Filter Paper No. 41. A 1.5-mL aliquot was taken and placed in an Eppendorf tube and centrifuged at 10,000 x g for 5 min. A 1-mL supernatant solution was mixed with 9-mL sterile distilled water, 5-mL sodium salicylate/NaOH solution, and 2-mL dichloroisocyanuric acid. The solution was incubated at room temperature for 30 min and the absorbance determined using a spectrophotometer (MultiSkan™ Go Microplate, Thermo Scientific) at 660 nm. The urease activity was calculated and expressed in μg NH_4–N g^{-1} 2 h^{-1}.

Microbial biomass (MCB) by substrate-induced respiration (SIR). Standardization of the amount of glucose, the substrate to be used, and the maximum duration for the incubation of samples were determined based on the procedure by Swain et al. (1978). Each soil type was standardized prior to laboratory experiments involving nanomaterials. Standardization was performed by mixing 10 mg/1 g to 70 mg/1 g glucose to 10 g of soil. The glass wire, which served as stand, along with plastic cup containing 10-mL of standard 0.04-N NaOH was immediately placed inside the jar, covered, sealed and incubated for 5 h. The amount of carbon dioxide (CO_2) released was determined by titrimetric method using standard 0.04 N of hydrochloric acid (HCl) with barium chloride ($BaCl_2$) and phenolphthalein as indicators. The maximum amount of glucose consumed was used for observing the microbial biomass throughout the experiments involving nanomaterials. The D-glucose used were 500 and 100 mg for every 10 g of soil samples for SSL and LCL, respectively. Using the maximum amount of glucose used, the time for initial biomass emission (z), median biomass emission at first generation (A), and median biomass emission generation at second generation (B) were determined within a 10-h incubation with 1 h of sampling interval. Equations were generated depending on the curve plotted and the value of proportional unit, z, signifying the volume (in mL) of standard 0.04 HCl used for titration. Using the value of z, the value of microbial biomass was computed. For LCL, z=0.714B-1.714A while for SSL, z=4.348B-5.348A. The standardized SIR procedure was used to measure microbial biomass all throughout the experiment. The calculations were performed using the formula below:

$$\mathrm{mg\ CO_2/10\ g\ soil} = \frac{(\mathrm{b} \times \mathrm{N} \times 88)\ \mathrm{mg\ CO_2}}{10\ \mathrm{g\ soil}}$$

$$\mathrm{Microbial\ biomass}\ (\mu\mathrm{g\ C/10\ g\ soil}) = \frac{\mathrm{z} \times \mathrm{N} \times 12 \times 10^6}{10\ \mathrm{g\ soil}}$$

where b is the difference between the delivered volume of HCl of the control and sample; N as the concentration of HCl in normality; and z as the proportional unit.

Statistical analysis. Two-way analysis of variance (ANOVA) (Gomez and Gomez 1984) was performed to assess the effects of treatments on culturable soil bacteria, DHA assay and UA assay, and microbial biomass determination. The Least Significant Difference (LSD) test at α=5% was used to determine whether means differed significantly.

RESULTS AND DISCUSSION

Effect on soil culturable bacteria. The treatments did not show significant effects on the soil culturable bacterial population at α=5% (Table 2). This may suggest that the FertiGroe®N, urea, and nanocarrier did not pose any harm to the culturable bacterial population in both LCL and SSL despite the possibility that nanomaterials may affect the bacteria by interfering in their biological processes such as behavior of cell membranes, biochemical pathways in cells, as well as their genetic code (Klaine et al. 2012). In fact, at 1 DAT, the FertiGroe®N- and urea-treated LCL and the urea-treated SSL had the highest total bacterial counts relative to the control. At 7 DAT, the urea-treated LCL and the FertiGroe®N-treated SSL had the highest total bacterial counts. Perhaps, the increase in the concentration of nitrogen in the soil encouraged luxury consumption of N by soil microorganisms, which might have increased bacterial population (de Mazancourt and Schwartz 2012).

At 14 DAT, the nanocarrier-treated LCL had the highest total bacterial count, and this could be related to the high surface area of the nanocarrier which increased the cation-exchange capacity of the LCL. Addition of porous material in the soil, for example zeolite, can increase the nutrient preserving capacity and water status of the soil (Li et al. 2000; Ippolito et al. 2011).

Table 2. Effects of FertiGroe®N on the culturable bacterial population of Lipa clay loam and Sariaya sandy loam.

Soil Type	Treatment	Total Bacterial Count, log (CFU g^{-1} soil)		
		1 DAT	**7 DAT**	**14 DAT**
Lipa Clay Loam	control	6.04^{ns}	6.15^{ns}	5.40^{ns}
	FertiGroe®N	6.21	6.03	5.46
	urea	6.20	6.32	5.72
	nanocarrier	6.06	5.86	5.98
Sariaya Sandy Loam	control	5.71^{ns}	5.89^{ns}	5.18^{ns}
	FertiGroe®N	6.04	6.27	4.87
	urea	6.48	6.07	5.13
	nanocarrier	5.89	6.02	4.80

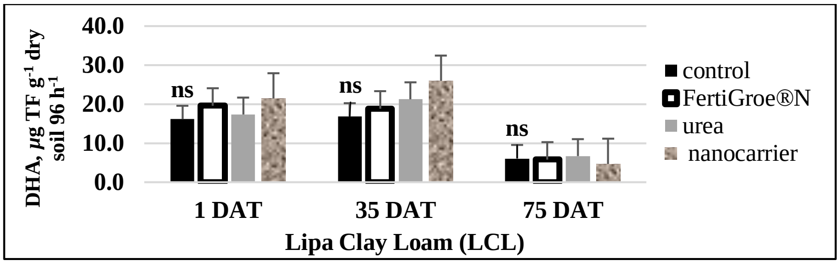
^{ns} not significant

Nanocompounds, such as nanoclay, nanochitosan, and nanozeolite, support microbial population due to high level of phosphorus, organic carbon, and ammoniacal nitrogen (Khati et al. 2017). But, the nanocarrier-treated SSL had the lowest total culturable bacteria which could be due to the physical property of sandy soils. Sandy soils have limited capacity to retain water and nutrients (Baghbani-Arani et al. 2020). However, the addition of nanocarrier could have improved the water retention of SSL since addition of high surface-area and porous nanocarrier can increase the soil moisture content of sandy soil (Ippolito et al. 2011). Zeolite also triggered a microbial mineralization process of soil organic carbon stocks and higher extent of decomposition of the SOM more stable pool.

In SSL, despite the possible increase in the availability of moisture and nutrient, culturable bacteria was still lower than the control. This could be due to the inaccessibility of microorganisms to the nutrient-rich soil solutions as affected by nanocarrier. Instead of the soil solution flowing through the interconnected pores of the sandy loam in a process called mass flow, the soil solution had likewise been retained in the pores and interconnected voids of the nanocarrier. The entrapment of soil solution by nanocarrier cut or reduced the water and nutrient access of bacteria present in the soil macropores, that could otherwise be supplied with growth factors by mass flow. The size and distribution of pores in soil are among the most important factors determining microbial life and water retention (Hattori et al. 1976). Bacteria located in the macropores are therefore subjected to more intense variation between desiccation and wetting (Ranjard and Richaume 2001).

Effect on dehydrogenase activity (DHA). There was no significant interaction effect between soil type; soil type and treatments; and among treatments in both LCL (Fig. 1a) and SSL (Fig. 1b) at α=0.05 except at 35 DAT in SSL in which significant interaction effect among treatments was observed. The nanocarrier-treated SSL had higher DHA while that of the urea-treated SSL had significantly lower DHA relative to the control. Perhaps, the mixing of nanocarrier in sandy loam soil improved its moisture retention, thus, enhanced microbial growth (Ippolito et al. 2011). Nanocompounds (nanoclay, nanochitosan, and nanozeolite) support microbial activity and, hence, the soil health (Khati et al. 2017) . However, the decrease in soil DHA in urea-treated soil might be due to the decrease in soil organic matter (SOM) upon application of urea and that, at 35 DAT, the depleted carbon source was not enough to support microbial growth compared to that of the control. An increase in N concentration in soil accelerated the loss of SOM through its mineralization (Singh 2018).

A significant main effect on soil DHA was observed at 1 and 35 DAT as influenced by the type of soil (Fig. 1c). LCL exhibited higher DHA than SSL which may be attributed to the physicochemical properties, due to its finer particle size, that affected the microbial community (Sessitsch et al. 2001), Thus, a decrease in microbial community structure, as affected by soil texture, might cause a decrease in soil DHA which is related to the quantitative changes in the microorganism population and connected with living microbial cells (Wolińska and Stepniewska 2012).



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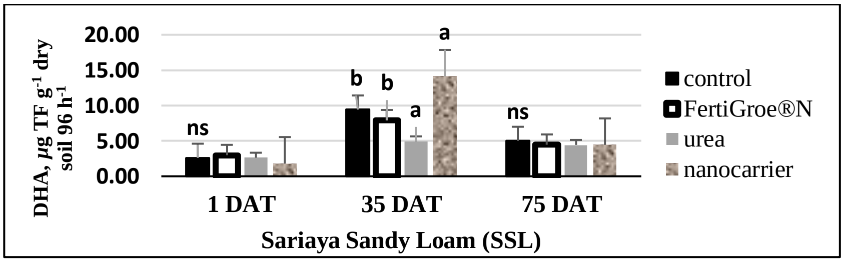

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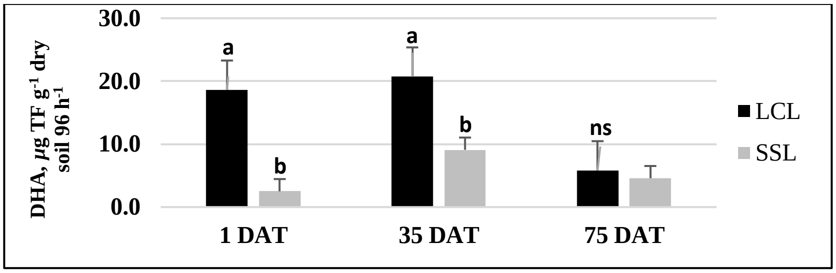


(c)

Fig. 1. Effect of treatments on the dehydrogenase activity of (a) Lipa clay loam and (b) Sariaya sandy loam and (c) interaction effect between soil types . Error bars represent the standard error of the mean (n=3). Different letters indicate significant difference among means ($P<0.05$) according to LSD test.

Dehydrogenase, an intracellular enzyme, can truly reflect microbial activity Mineralization of DHA by other enzymes with respect to the degradation processes of extracellular soil enzymes was possible unless they are either adsorbed by clays or immobilized by humic molecules. Another factor for the higher DHA of LCL than SSL is the level of organic matter. DHA reaches higher values in soils with higher total organic carbon content (Wolińska and Stepniewska 2012).

Effect on urease activity (UA). A significant interaction effect on UA between soil type and treatments at 1, 35, and 75 DAT at 95 % level of significance. Hence, an examination of simple effects of treatments on UA for each soil type is shown in Figure 2. At 1 DAT, significant increase in the UA was observed in LCL treated with FertiGroe®N and nanocarrier compared to control. In contrast, a significant decrease in SSL treated with FertiGroe®N and urea was observed at 1 DAT. At 35 DAT, nanocarrier-treated SSL and LCL were significantly increased compared to other treatments. The UA of nanocarier-treated SSL and LCL, however, had significantly decreased at 75 DAT relative to the control. The increase in the urease activity at 1 DAT might possibly be due to the introduction of porous FertiGroe®N and nanocarrier which caused an increase in the CEC of LCL. UA was found to be strongly correlated with the clay content and CEC, but not significantly correlated with organic C content (Kizilkaya et al. 2004). However, for SSL at 1 DAT, a decrease in UA could be due to the increase in the microbial population considering that nitrogen was introduced to both the LCL treated with FertiGroe®N and urea. SSL contained high phosphate concentration which, along with nitrogen, stimulated the growth of soil microorganisms. However, an increase in the microbial population led to the decrease in the UA of the soil. Previous studies under steady state conditions, showed 79-89 % of the urease activity of the soil was earlier determined to be due to urease adsorbed on soil colloids and an increase in microbial population reduced this percentage temporarily until a new steady state was reached (Paulson and Kurtz 1969). It is possible that the nanocarrier protected the enzyme from degradation by proteases while maintaining the enzyme's function at 35 DAT in both soil types. Surface micropores significantly influenced the protein-nanoporous material interaction (such as the case of nanozeolite). Cyto-c and *Candida antarctica* Lipase B (CALB) showed strong affinity to the porous nZeolite than to nonporous one and larger extent of protein unfolding induced by porous nZeolite was positive for the enzyme activity of Cyto-c while negative for that of CALB (Wu et al. 2013).

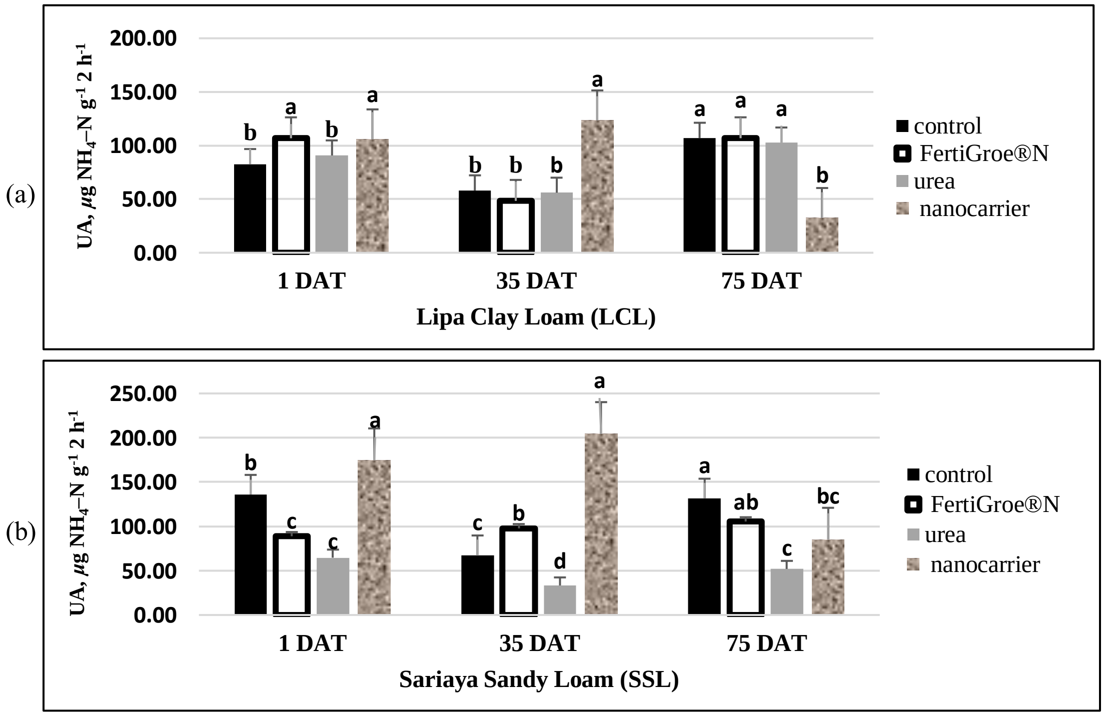
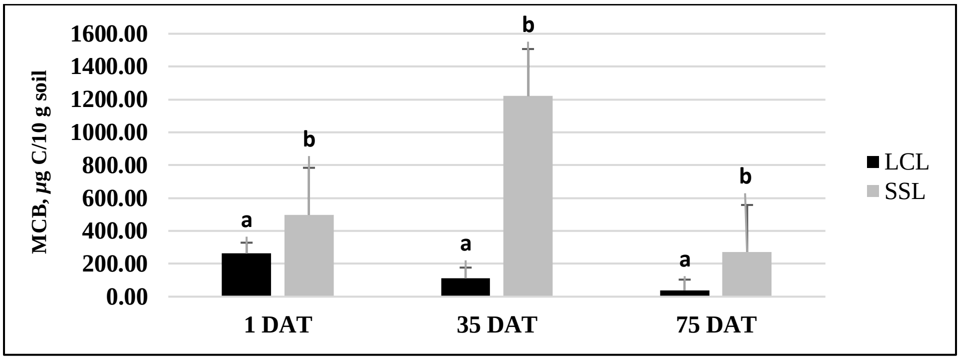


Fig. 2. Effect of treatments on the urease activity of (a) Lipa clay loam and (b) Sariaya sandy loam between soil types. Error bars represent the standard error of the mean (n=3). Different letters indicate significant difference among means (P<0.05) according to LSD test .

FertiGroe®N nanofertilizer effect on soil microbial biomass (MCB). A significant main effect on MCB between soil types at 1, 35 and 75 DAT was observed (Fig. 3a) at 95 % level of significance. The significant main effect among treatments was observed at 1 and 35 DAT for both LCL (Fig. 3b) and SSL (Fig. 3c). At 1 DAT, a significant increase in nanocarrier-treated soil was observed in both LCL and SSL relative to the control. Perhaps, this could be due to the adsorptive capacity of the nanocarrier. Upon addition, the nanocarrier had been immediately inoculated by the microorganisms in the soil and with the nanocarrier's capacity to adsorb nutrients, it was able to sustain the exponential growth of microorganisms.

The microbial activity, and hence the soil health could be improved by the introduction of nanocompounds such as nanoclay, nanochitosan, and nanozeolite (Khati et al. 2017). Nanozeolite, with a 99% purity, was able to support microbial population. However, at 35 DAT, the MCB of urea-treated SSL was significantly reduced compared to the control. This suggests that the introduction of urea increased the utilization of organic matter in the soil between the period of 1 DAT and 35 DAT, thus, reducing the availability of organic carbon at 35 DAT for microbial utilization as growth factor. Similarly, a significant reduction in soil MCB was observed in the nanocarrier-treated SSL. The nanocarrier has been found to decrease the distribution of nutrients, and consequently, the utilization of these nutrients by soil microorganisms by reducing the permeability of water (Tállai et al. 2017).

The amount of macropores was responsible for significant differences in the MCB between LCL and SSL and its interaction effect at 35 DAT. It is possible that the substrate added during the experiment was able to reach the micropores of the LCL which protected it from the utilization of microorganisms present in the macropores. The pore size distribution could give an indication of the accessibility of substrates to decomposition by microorganisms, with finer, clayey soils on average, show slower decomposition rates and higher retention of OM than coarse, sandy soils (Van Veen and Kuikman 1990). The mixing of substrates and microbes as found in the laboratory systems is very much restricted in soil due to the limited water flow and microbial movement. Therefore, the substrates even at distances of micrometers or less might not be readily available for microorganisms. Aside from the entrapment in pores, entrapment in aggregates rendered the substrate inaccessible by microorganisms (Van Veen and Kuikman 1990).



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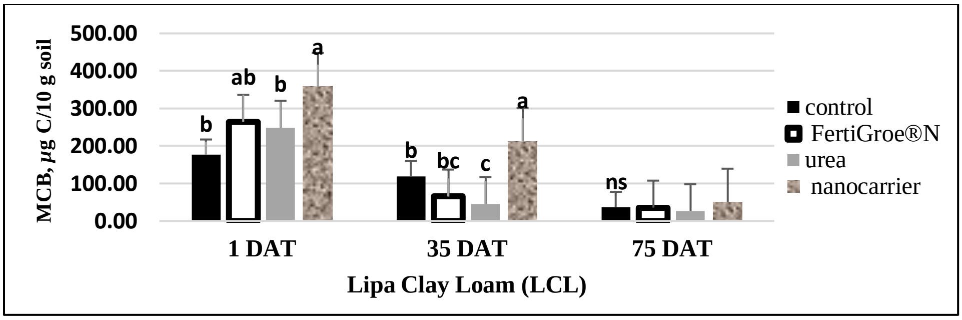

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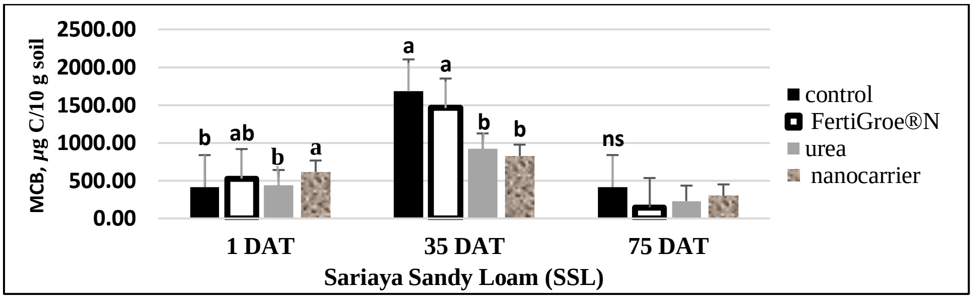

(c)

Fig. 3. (a) Differences in soil MCB between soil types; effect of treatments on soil microbial biomass of (b) Lipa clay loam and (c) Sariaya sandy loam. Error bars represent the standard error of the mean (n=3). Different letters indicate significant difference among means (P<0.05) according to LSD test.

CONCLUSION

An incubation experiment using two soil types was conducted to assess the effects of FertiGroe®N nanofertilizer on the culturable bacterial population, microbial biomass, and dehydrogenase (DHA) and urease activities (UA) prior to its extensive use in crop production and commercialization. The nanofertilizer, FertiGroe®N, was better in increasing both microbial activity and biomass than the commercial fertilizer, urea. FertiGroe®N can be safely applied in crop production. Since the effect of nanofertilizer on soil microorganisms may differ in laboratory conditions and the natural environment, there is a need to investigate the effects of FertiGroe®N on soil microorganisms in pot and field trials.

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AGRICULTURAL TRAMLINE SYSTEM ON THE AGRICULTURAL SUSTAINABILITY OF UPLAND FARMS IN BENGUET, PHILIPPINES

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ABSTRACT

The agricultural tramline system (ATS) is a mechanical conveyance system that works similar to the principle of the cable car and is utilized as a transport system of agricultural products and inputs in hard to reach upland areas inaccessible by road. This paper assessed the influence of ATS on the agricultural sustainability of upland farms in terms of social, economic and environmental dimensions. A survey of 310 upland farmers representing "with ATS" and "without ATS" was conducted in 2018 in 3 major farming municipalities of Benguet province, Philippines to generate information on land use allocations, farming systems, rate of organic fertilizer use, hauling methods, average yields, soil and water conservation practices and socioeconomic data. Soil samples were collected from farmers' fields and analyzed for pH, OM, P, and K to determine the effects of the different levels of fertilizer application on soil nutrients. Data were analyzed using descriptive statistical methods, t-test and partial budget analysis. The ATS improved the working conditions of upland farmers by eliminating the drudgery of manual hauling through mechanizing transport of agricultural products and inputs. ATS also leads to increased productivity through higher crop yields and increased farmers' income by reducing transport costs and thus influenced positively agricultural sustainability on the aspects of social and economic dimensions. This positive influence however, was made possible through intensified use of fertilizers and opening up of forest and idle areas for agricultural land use and contrariwise created undesirable environmental effects which may threaten agricultural sustainability in the long run. The ATS has indirect neutral effect on OM, P and K soil content but has a positive indirect effect on soil pH because farms serviced with ATS used significantly higher amount of organic fertilizer. To maximize the potential of ATS as a sustainable mode of transport while mitigating its undesirable effects, strict land use zoning must be implemented while putting up of ATS adjacent to forest areas and fragile ecosystems should be prohibited. Soil and water conservation innovations in the uplands must also be an integral part of the farming systems.

Key words: agricultural sustainability, land use, transport system, upland farms

INTRODUCTION

In the mountainous regions of the Philippines, access is one of the barriers that until now hindered these regions from reaching its potential productivity. In these areas, road infrastructure and access to market is inadequate because of technical challenges and limitations owing to its rugged terrain, high cost and maintenance of paved road (Dela Cruz et al. 2000; Estigoy 2006; Idago and Ranola 2009; 2012; Tesorero 2017). The traditional method of manual hauling agricultural products remains the predominant mode of transport. In these mountainous regions, hauling cost contributes as high as 30% of the total production cost while postharvest losses due to handling and delay of movement of highly perishable products ranges from 20-50% (Estigoy 2006).

The uplands represent about 55% of the Philippines' total land area (Cruz et al. 1986) which why it is considered by the Philippine government as a latent resource that can significantly contribute to food security and as well as poverty alleviation amidst the challenges of climate change. Social, economic and environmental aspects are central considerations in the design of rural development programs in these mountainous regions, and require efforts to strike a balance amongst the composite dimensions of agricultural sustainability. It is also generally recognized that the uplands play a vital role in the lowlands in terms of the flow of energy, materials, information and other ecosystem services. The uplands is considered a fragile ecosystem, and production in steep slopes would expose the system to environmental problems such as soil erosion, water contamination, sedimentation of river system (Briones 2005; Rola 2004). Hence, any innovation that can alter or influence the different dimensions of economic, social and environmental sustainability must be identified, quantified and projected for the purpose of enhancing the positive impacts while mitigating the unintended and undesirable effects.

One of the popular technological innovations adopted towards the development of the uplands is the agricultural tramline system (ATS) in the past two decades. It is a mechanical conveyance system that works similar to the principle of cable car and is utilized as a transport system of agricultural inputs and products in areas inaccessible by road. The ATS is an offshoot of an old technology that was once applied in the 1970s in mining and logging operations (Idago and Ranola 2009). The closure of these industries for obvious reasons of unsustainability, opened up and cleared areas were devoted for agricultural production while displaced labor force from mining and logging operations adapted the technology for agricultural application. This later paved the way for the development of the ATS technology that is being established in mountainous regions under the lead of the Department of Agriculture. To date more than 200 units of ATS have been established across the mountainous regions engaged in the production of high value crops nationwide (Paz et al., 2017).

A number of studies and published articles on ATS already exist, which looked into its financial and economic viability (Idago and Ranola 2009), farm productivity (Idago and Ranola 2012) performance under different management models (Tesorero 2017) and effects on land use (Idago and Rebancos 2020). There is limited if none on published reference that investigated its contribution and influence on agricultural sustainability, which is central to the design of rural development programs. With the increasing demand for this innovation, information that cut across the dimensions of agricultural sustainability will be useful for crafting of policy direction, programming, planning and allocation of scarce resource. This paper aims to address this information gap by assessing the influence and contribution of ATS on the agricultural sustainability of the uplands at the farm level.

METHODOLOGY

Conceptual framework. The influence of ATS on agricultural sustainability in the farming uplands was established by comparing the "with" and "without" ATS. The conceptual framework of the study shows the system boundary confined at the farm level where the ATS will have its most significant impact (Fig. 1). Agricultural sustainability was assessed based on three dimensions: social, economic and environmental aspects (Yunlong and Smit 1994). Indicators of agricultural sustainability were based on the work of Hayati et al. (2010)

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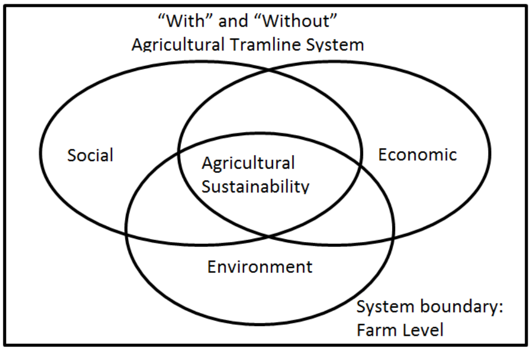

Fig. 1. Conceptual framework of the study.

Time and location of the study. The study was conducted in Benguet province in the municipalities of Kabayan, Tublay and Atok in 2018 (Fig. 2). Benguet province is situated geographically between 16^0 33' N latitude and 120º 34' to 120º 52' E longitude and is bound by Mt. Province on the north, Pangasinan on the south, Ifugao and Nueva Vizcaya on the east and by La Union and Ilocos Sur on the west (PDPFP 2018). Benguet province was selected as the study area as it has the highest number of ATS across the mountainous provinces of the Philippines and is considered one of the first adopters of this transport technology.

Fig. 2. Map of Benguet showing the municipalities of Tubaly, Atok and Kabayan as study sites.

Sampling and data collection. The study used formal survey method with the aid of structured questionnaires to obtain primary data on farmers' socioeconomic profile and land use systems. Two types of farms and farmer-respondents were selected for purposive sampling, representing the "with ATS" and "without ATS" to measure the difference using the pre-identified indicators of the different dimensions of sustainability. The required sample size was determined using Slovin's formula:

$$n = N / (1 + Ne^2)$$

where: n = *sample size*
N = *population size*
e = *error tolerance (0.05)*

The estimated population (N) of farmers "with ATS" is about 250, the sample size for each classification of farmer-respondents was computed as:

$$n = 250/ (1 + 250 (0.05)^2$$
$$=250/ (1 + 0.625)$$
$$= 153.8, \text{ or about 155 farmer-respondents}$$

Soil samples from 30 farms each for "with ATS" and "without ATS", were randomly selected for soil fertility analysis.

Agricultural sustainability assessment. Assessing the ATS' influence on agricultural sustainability in the uplands compels some conceptual framing how this specific innovation contributes towards the broad concept of "agricultural sustainability". "Sustainability in agriculture is a complex concept and there is no common viewpoint among scholars as to its dimension" (Hayati et al. 2010). Moreover, the precise measurement of agricultural sustainability is impossible as it is site-specific and a dynamic concept (Ikerd 1993). Therefore, selection of indicators should be location specific and must be based on the contemporary biophysical and socioeconomic conditions prevailing in the study area (Dumanski and Pieri 1996). Using system theory (Rambo 1983), the boundary of the system under consideration in this study is confined at the farm level but implications were made for the whole landscape.

Recognizing that there is no straightforward or standard indicators to measure agricultural sustainability, the indicators used by the study are those that exhibits the characteristics of being observable, measurable, and exhibits interaction between ATS and upland farms, and can be representative of the different dimensions of agricultural sustainability. Sustainability in agriculture is not a final product but rather a process and direction (Hayati et al. 2010), hence the indicators applied by the study are indices to assess whether or not the introduction of ATS in the uplands is contributing towards agricultural sustainability. To simplify, the study disaggregated agricultural sustainability into three major dimensions: environmental, social and economic aspects (Yunlong and Smit 1994).

Hayati et al. (2010) provided an easy reference for selecting the most relevant indicators in relation to ATS and the upland farms. With these indicators the study can now assess whether certain trends are steady, going up or going down (Pretty 1995) which will determine how ATS is contributing to agricultural sustainability. Table 1 presents the dimensions, indicators and measurement units of agricultural sustainability used in the study.

Table 1. Dimensions, indicators and measurement units of agricultural sustainability used by the study.

Sustainability dimensions	Indicators	Measurement units
1. Social sustainability	• Working condition[a] (manual labor replaced)	• Person-days/ha;
2. Economic sustainability	• Average crop yield[b] • Incremental income[c]	• kg/ha • Php/ha
3. Environmental sustainability	• Usage of organic fertilizer[d] • Land use type conversion • Soil nutrients: pH, OM, P, K • Fossil fuel use[e] • GHG emission[f]	• bags/ha • % • pH level, %, ppm • Li of diesel/ha • CO_2 eq/ha

[a] (Ingels et al. 1997; Van Cauwenbergh et al. 2007)
[b] (Hayati 1995; Nambiar et al. 2001; Rasul and Thapa 2003)
[c] (Herzog and Gotsch 1998; Nijkamp and Vreeker 2000; Pannell and Glenn 2000; Van Cauwenbergh et al. 2007)
[d] (Bosshard 2000; Hayati 1995; Norman et al. 1997; Saltiel et al. 1994)
[e] (Ingels et al. 1997; Nambiar et al. 2001; Norman et al. 1997; Senanayake 1991; Van Cauwenbergh et al. 2007)
[f] (Volenzo et al. 2019)

These indicators were obtained from upland farms representing "with ATS" and "without ATS" and were later compared and analyzed using different statistical analytical methods with the aid of Statistical Package for Social Sciences (SPSS) software.

Measurement of agricultural sustainability indicators

Social sustainability. Improvement in the working condition due to ATS was measured by quantifying the manual labor that was spared from the drudgery of manual hauling by mechanizing transport**.** The number of manual labor force (Lr) replaced by ATS was computed as**:**

$$Lr = (p \times d)/A$$

Where:
Lr= manual labor replaced, in person-days/ha
p= number of persons performing manual hauling
d= days to accomplish hauling @ 8hr per working day, in days
A= farm area, in hectare

Economic sustainability. The computation of average yield and incremental income representing the economic sustainability indicators are presented below:

1. *Average yield (AY)* Average yield refers to the average weight of harvest of specific crop per unit area. The crops considered in the study included the temperate vegetables that are commonly grown in the farmers' field such as cauliflower, potato, cabbage, carrot and chayote. Average yield (AY) was computed using the formula:

$$AY = \frac{C}{A}$$

Where:
AY = average yield of crop, in kg/ha
C = crop yield, in kg
A = farm area, in hectare

2. *Incremental income (I)* Incremental income was computed based on the changes in revenue and costs arising from the use of ATS over the traditional method of hauling. Incremental income (I) was computed using the formula:

$$I = (Ra + Cr) - (Rr + Ca)$$

Where:
I = incremental income, in P/ha
Ra = added revenue, in P/ha
Cr = reduced cost, in P/ha
Rr = reduced revenue, in P/ha
Ca = added cost, in P/ha

Environmental sustainability. The effect of ATS on the environmental indicators was measured by comparing the "with" and "without" ATS. The description and formula used for the measurements of these environmental indicators are presented below.

1.1. Land use-agriculture (LUa)

This refers to areas devoted for growing different crops, mostly temperate vegetables. *LUa* was computed using the formula:

$$LUa = \frac{Aa}{TA} \times 100$$

Where:
LUa = land use for agriculture, in percent
Aa = area devoted for agriculture, in hectare
TA = total land area, in hectare

1.2. Land use-forest (LUf)

Forest land use refers to areas that are under natural cover not engaged by human activity except as a source of water and fiber materials. *LUf* was computed using the formula:

$$LUf = \frac{Af}{TA}\, x\, 100$$

Where:
LUf = Forest land use, in percent
Af = forest area, in hectare
TA = total land area, in hectare

1.3. Land use-idle land (LUi)

This refers to lands that were cultivated before but for a long period of time is now in a state of disuse, abandoned or in a state of fallow. *LUi* was computed using the formula:

$$LUi = \frac{Ia}{TA}\, x\, 100$$

Where: LUi = Land use as idle, in percent
Ia = idle land area, in hectare
TA = total land area, in hectare

2. Organic fertilizer utilizaton (OfU)

Organic fertilizer utilization refers to the average number of bags (50 kg) of organic fertilizer, particularly chicken manure, used per unit area per crop per season. Organic fertilizer utilization (*OfU*) was computed using the formula:

$$OfU = \frac{F}{A}$$

Where:
OfU = fertilizer utilization, in bags per hectare per crop
F = no. of bags of organic fertilizer applied, in bags of 50kg
A= farm area, in hectare

3. Soil nutrients

The study followed the protocol of Bureau of Soils and Water Management (BSWM) for soil analysis. Ten representative farms each from the three project sites were used as sampling points. During soil sampling, approximately a slice of 15 cm depth with 2cm thick and 5cm wide of soil sample were taken from the surface or topsoil from each point using shovel. The levels of OM, P, K and pH were used to determine the difference in soil nutrient levels between farms "with" and "without" ATS (Table 2).

Table 2. Methods used in the measurement of soil pH, organic matter, P, and K.

Soil chemical parameters	Methods used[a]
pH	1:1 Soil: H_2O
Organic matter	Walkley and Black Method
P	(pH<5.5, Olsen Method), (pH>5.5, Bray #1 Method)
K	Cold H_2SO_4 Method

[a] Regional Soils Laboratory of the Bureau of Soils and Water Management of the Department of Agriculture.

4. *Energy use*. Energy used for hauling refers to two sources of energy: human energy for manual hauling and the energy used to power the engine or prime mover of the ATS. The computation of the energy use was based on the average distance of the upland farms to the nearest road, total weight of agricultural inputs and outputs, and the time spent to accomplish the hauling operation. Energy used for both modes of transport is expressed in megajoules *(Mj)* which is computed using the formula:

$$E = P \; x \; t \; x \; 1x10^6 \; s$$

Where: E = energy, in Megajoules
P=power, in watts
t=time, in second

Power (P) is computed using the formula: P= W/t

Where: P=power, in watts
W= work, in newton-m
t= time, in second

Work (W) is computed using the formula: W= f x d

Where: W = work, in newton-m
f = force, in newton
d = distance, in m

5. *Greenhouse gas emission (GHG)*

GHG emission refers to the emission contributed by the internal combustion engine that powers the ATS. GHG is computed using formula (Kramer et al.; 1999) presented below:

$$GHG = GWP \; x \; Mi$$

Where: GHG = greenhouse gas emission in kg CO_2 *eq ha-1*
GWP = global warming potential, 1 for CO_2*;*
Mi = mass of emission gas, in kg

METHODS OF ANALYSES

Descriptive analysis. This analysis was used on socioeconomic characteristics and farm profile of the farmer- respondents. Quantitative data such as rate of inputs utilization, percentage allocation of land uses, size of area, household income, distance of farm to market center, yield per hectare, etc. were presented using mean values. For qualitative data such as types of land uses, vegetable crops grown, land tenure, types of irrigation, etc., frequency distribution was applied.

T-test. Paired t-test was used in this study and the two groups or populations were represented by the "with ATS" and "without ATS". This analysis was used to determine if there is significant difference on the observed changes on selected indicators. The study could have extended further the analysis by running regression analysis to isolate the predictors of some of the indicators of agricultural sustainability. The issue of heteroscedasticity (unequal variance) however in the observed data prevented the use of such analysis and therefore the study was limited to t-test.

Partial budget analysis. The viability of the tramline system from upland farmer's viewpoint was assessed using partial budget analysis. Partial budgeting is an instrument that measures the effects of marginal changes on overall profitability and, in particular, choosing between technologies and enterprises (SEARCA undated) A positive change in income implies that a farmer is better off using the ATS. On the other hand, a negative change in income will imply otherwise suggesting that farmers will be better-off status quo. In the partial budget analysis the change in income (I) is computed using the formula:

$$I = (Ra + Cr) - (Rr + Ca)$$

where: *I = change in income, in PhP*
Ra = added returns
Cr = reduced cost
Rr= reduced returns
Ca= added cost

RESULTS AND DISCUSSION

Study site. Benguet province is geographically mountainous, characterized by rugged, irregularly patterned ridges, canyons and peaks, many of which are above 2,400 meters above sea level (masl) in elevation. The slope classification of Benguet ranges from gently sloping to very steep. Of the total land area, more than half have very steep slopes (slope gradient of 84% and above) while almost one-seventh belong to the steep slope (51-84%) category (PDPFP 2018). There are three major land use systems prevailing in the study areas: a) vegetable-based annual crops, b) perennial crops and c) upland rice-based annual cropping, which are all under agricultural land use. Areas not included in this land use system generally fall under grasslands and other land use types.

Upland farms and farmers characteristics. The average size of upland farms in the study sites is 3500 and 3900 m^2 for “without ATS” and and “with ATS”, respectively. Majority of the farms are irrigated (67%) through sprinkler system tapped from natural spring and more than half of the farms are situated less than 2 km away from the road. Majority of the upland farmers both for “with ATS” and “without ATS” own the land. The average age of upland farmers interviewed is 44 years old. Majority are male (83%), have primary (43%) to secondary (40%) schooling, have been farming for 11 to 20 years (34%) and more than three-quarters of their family income are derived from farming (77%).

Technical description and nature of establishment of ATS. The ATS prime mover is powered by 80 hp internal combustion engine that runs on diesel fuel, the system is built with combinations of pulleys, towers, wire rope cables and a single carrier that can carry 200-300kg load (Fig. 3). The ATS has a hauling capacity of 3 to 5 tons per hour depending on the distance which can range from 200 to 1500 meters between the main station and the farthest loading/unloading stations. Organized group of upland farmers operate, maintain and manage the system.

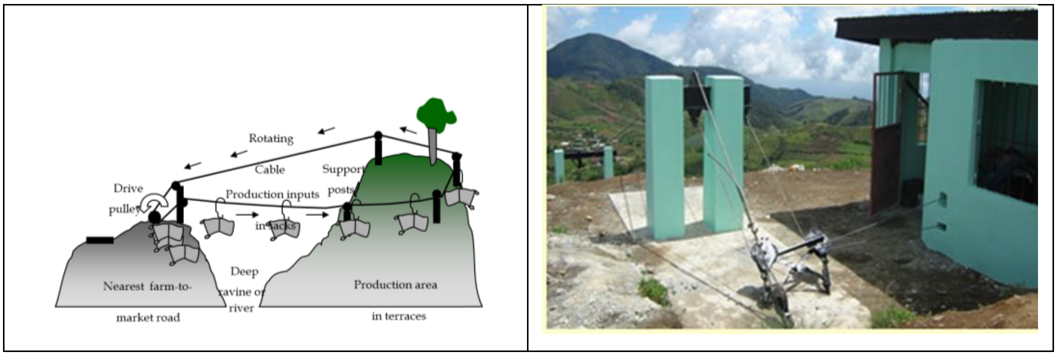

Fig. 3. Schematic diagram of an agricultural tramline system and a template of its structural components (Source: Idago and Ranola 2012; Idago and Rebancos 2020)

About three-quarters (72%) of the existing ATS in Benguet were established by the government as public infrastructure while the remaining by private individuals and farmers group.

The effective service area can range from 3 to 16 hectares. ATS with service areas ranging from 3 to 5 hectares are mostly put up by well-off farmers or group of farmers.

Social sustainability. Apparently the most significant contribution of ATS in relation to social sustainability is its direct contribution to the elimination of the drudgery of manual hauling. This alone contributed directly to improved working conditions in the upland farms. With ATS around 14 person-days are spared from the drudgery of manual hauling of agricultural products in one hectare per season (Table 3) compared to a separate study which had a higher estimate at 17 person-days/ha/season (Dela Cruz et al. 2000). Ninety five percent (95%) of the farmers interviewed claimed that with the ATS they were relieved from the drudgery of transport which they exaggeratedly described as *trabahong kalabaw* (work of a carabao). ATS which is a form of agricultural mechanization contributed to improvement in the working conditions on-farm by eliminating the drudgery of manual hauling, which is one of the benefits of agricultural mechanization (Olaoye and Rotimi 2010; Verma 2008).

Table 3. Hauling duration of vegetables and inputs between manual and ATS.

Item	Mean Hauling Duration (person-days/ha/season)		Mean difference
	ATS	Manual	
1. Inputs	0.84	2.64	-1.8**
2. Vegetables	2.31	14.04	-11.73**
			-13.53

**significant at 1% level of significance

Economic sustainability

Average crop yields. Farms "with ATS" had significantly higher yield compared to farms "without ATS" (Table 4). It can be deduced that higher crop yield is attributable to higher rate of organic fertilizer use particularly chicken manure which is corroborated by the findings of Idago and Ranola (2012). This is also consistent with the study of Ismaeil et al. (2012) that the use of chicken manure has a positive effect on growth attributes and average yield.

Table 4. Average yield of selected crops from farms "with ATS" and "without ATS" Benguet, 2018.

Crops	Average yield, kg/ha		Mean Difference
	With ATS	Without ATS	
1. Cabbage	14,738	18,212	3,474 ns
2. Cauliflower	40,734	28,650	12,084**
3. Carrot	27,595	15,111	12,484ns
4. Chayote	1,918	1,496	422**
5. Potato	14,350	11,089	3,261ns

** significant at 5% level of significance
ns - not significant at 10% level

Incremental income. Replacing traditional manual hauling with ATS resulted in a increase in income of P55,631/ha/yr (Table 5). This suggests that a farmer will be better-off adapting the ATS over the traditional manual hauling practice. Productivity is increased through higher yields and savings from hauling costs.

Table 5. Partial budget analysis (per hectare per year) using ATS against manual hauling; Benguet; 2018.

Added Costs (A)		**Added Returns (B)**	
1. Additional Fertilizer	P20,100	1. Increased yield of vegetables, 10,768kg @	P161,520
– Organic 134 bags @ P150/bag			
– Inorganic 33 bags @ P1,200/bag	P39,600	P15.00/kg	
2. Transportation cost of additional fertilizer, 167 bags @ P50/bag	P8,350		
3. Hauling Cost of additional fertilizer using Tramline, 167 bags @ P25/bag	P4,175		
– Additional yield 216 bags x P50/bag	P10,800		
4. Labor cost of fertilizer application, 137 bags @ P50/bag	P6,850		
5. Labor cost of harvesting additional yield, 0,768 @ P5.00/kg	P53,840		
6. Opportunity cost, 8% of items 1 to 5	P11,497		
Reduced Returns	(nil)	Reduced Costs	
		1. Savings from manual hauling	
		– Fertilizer, 351bags @ P23/bag	P8,073
		– Vegetables, 1218 bags @ P25/bag	P30,450
		2. Time saved from manual hauling	P10,800
		– 36 man-days/yr @ P300/day	
Subtotal A = P155,212		B = P210,843	
Change in income (B-A) = P 55,631/ha/yr or P 27,816/ha/season			

Environmental Sustainability

Usage of organic fertilizer. Farms "with" ATS applied significantly higher amount of organic fertilizer (Table 6). This finding was consistent with the study of Dela Cruz et al. (2000) which revealed that farms serviced with tramline facility increased utilization of production inputs by 30 percent. This was further corroborated by the findings of Idago and Ranola (2012) and Idago and Rebancos (2020) that improving access encourages increase use of production inputs as well as cropping intensity if the production area is complemented with irrigation facility. Based on survey, the cost (94%) and drudgery (95%) of transport are the major barriers that restrict organic fertilizer use.

Table 6. Average organic fertilizer usage in selected crops of farms "with ATS" and "without ATS" Benguet, 2018.

Crops	Fertilizer utilization rate (bags/ha)		Difference
	with ATS	without ATS	
Cauliflower	102	150	48 ^{ns}
Potato	180	90	90**
Cabbage	163	106	57*
Carrot	98	42	56 ^{ns}
Chayote	74	70	4 ^{ns}

** significant at 5% level of significance
* significant at 10% level of significance
ns - not significant at 10% level

Land use type. Based on the study of Idago and Rebancos (2020) areas serviced with ATS had significantly higher land use for agriculture (Table 7). However, areas serviced by ATS have significantly lesser forest area (4%), suggesting that the presence of tramline encouraged expansion of agriculture by converting forested/naturally covered areas for agricultural production. Facilitating the movement of agricultural products provided incentives for land owners to engage more areas for agricultural production by opening up forest lands.

Table 7. Comparison of land use type allocations between farms with and without ATS in the municipalities of Atok, Kabayan and Tublay; Benguet; 2018.

Land use type	Percentage allocation		Mean Difference
	With ATS n=155	Without ATS n=155	
Agriculture	90	82	8***
Forest	5	9	-4***
Idle & other land use types	5	6	-1^{ns}

*** significant at 1% level of significance
ns - not significant at 10% level

Source: Idago and Rebancos (2020)

Soil nutrients. The soil series in the study sites are Paoay loam soil, ambassador silt loam and mountain soil (undifferentiated) for Atok, Tublay and Kabayan, respectively. There were no significant differences in the levels of OM, P and K between farms with and without ATS. The level of pH however on farms without ATS is significantly higher than in farms with ATS (Table 8). This can be attributed to the higher amount of organic fertilizer applied in farms with ATS (Table 6). The results suggest that the presence of ATS has indirect neutral effect on OM, P and K and has a positive effect on the level of pH because of the influence of ATS on fertilizer use. Note that no further analysis was conducted beyond comparison of "with" and "without" ATS because the soil nutrient levels was associated directly to the amount of fertilizer used where ATS has direct influence. Given this limitation, a more in depth study on this aspect can be done to accurately isolate this effect.

Table 8. Soil chemical characteristics of farms with and without ATS in Kabayan, Atok and Tublay, Benguet; 2018.

Soil chemical parameters	With ATS (n=32)	Without ATS (n=30)	Mean difference
pH	5.47	5.02	0.45**
OM (%)	2.08	1.92	0.16^{ns}
P (ppm)	125.99	110.40	15.59^{ns}
K (ppm)	324.53	346.08	-21.55^{ns}

** significant at 1% level of significance
ns - not significant at 10% level

Energy use. The study established that using manual labor to transport 548,950 kg of agricultural inputs and outputs, the average total load per year, at an average distance of 948 m requires 5,153Mj. This is the energy equivalent exerted by manual labor. Using the same volume of agricultural load, the same travel distance and considering the ATS hauling capacity, fuel consumption and energy equivalent of diesel fuel that powers the system, the ATS requires 21,875Mj. This implies that ATS consumed greater amount of energy than human labor to transport the same volume of agricultural load. This shows the need for the design of the system to be further improved to make it more energy efficient.

GHG emission. On the aspect of greenhouse gas emission specifically looking into the CO_2 equivalent, ATS that runs on diesel fuel generates 1,635kg CO_2 per hectare per year. This was established by computing the amount of diesel consumed per year which is 610 Li and multiplied by its CO_2 equivalent which is 2.68kg CO_2/Li. This suggests the need to improve ATS' environmental performance, in terms of GHG mitigation, by using a cleaner source of energy to run the system such as the use of solar power.

CONCLUSION AND RECOMMENDATIONS

ATS positively influenced the social dimension of sustainability as indicated by the improvement in working conditions of farmers by eliminating the drudgery of moving products through mechanization of transport. It also positively contributed to the economic sustainability at the farm level as exhibited by its indirect positive effect on crop yield resulting to higher productivity of upland farms. ATS however has a downside as it encourages conversion of forests and idle areas to agricultural land use resulting in deforestation which can threaten agricultural sustainability in the long run. Also, there is a need for cleaner source of energy to run the ATS. Findings from the study can be used as criteria in selecting specific locations where the potential of the facility can be maximized and sustained. The negative impacts of land use intensification must be seriously addressed to attain agricultural sustainability. The influx of inputs and outputs from the farm would maximize and sustain the tramline operations since underutilized facility leads to poor maintenance and sustainability of its operation. Assuming all dimensions of sustainability to have equal weights, we can say that two out of three aspects of sustainability are influenced positively by ATS. To mitigate its negative effects on the environment, the study recommends strict land use zoning implementation and prohibiting the establishment of ATS adjacent to forest areas and fragile ecosystems. Soil and water conservation innovations in the uplands must also be an integral part of the farming systems. Agricultural sustainability in the uplands can only be attained by striking a balance among the social, economic and environmental aspects of sustainability.

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FINANCIAL ANALYSIS OF DAY-OLD CHICK (DOC) LOGISTICS 4.0 SYSTEM IN SULAWESI, INDONESIA

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ABSTRACT

Day-Old Chicks (DOC) should be distributed from the hatchery to the farm which entails a risk that lowers DOC quality due to stress. The DOC logistics from hatchery to the farm does not have sufficient monitoring, tracking, and tracing systems. In the Industrial 4.0 era, companies have a higher competitive advantage by using existing technology to improve customers' satisfaction, however, this requires huge investment. This study sought to describe the current logistics of DOC, analyze the possibility of implementation of logistics 4.0, and analyze the financial feasibility of implementing logistics 4.0. Observations and direct interviews were conducted in April to August 2020. This study employed investment criteria and evaluation of value added (EVA). The logistics system designed using Internet of Things (IoT) and Web System Networking (WSN) would have an EVA value of IDR261,725,367 and logistics operational costs of DOC 4.0 costs would be lower than conventional logistics. The implementation of DOC logistics system 4.0 would provide an advantage to stakeholders, including the increase of company's credibility, delivery flexibility, route optimization and transparency during logistics process.

Key words: Evaluation Value Added, investment criteria, Internet of Things, Web System Networking

INTRODUCTION

Day-Old Chicks (DOC) logistics undergo a complex and prolonged handling process that is influenced by microclimate changes. This may result in high stress as DOC welfare, growth, development, and performance are strongly affected while in transport from hatchery to the farm (Mitchell and Kettlewell 1998; Yerpes et al. 2021). Unlike other mature livestock that are able to metabolically control body temperature, DOC can not fully manage its body temperature since its organs are not functioning perfectly. As a result, it is sensitive to hot and cold stresses thus requiring technological assistance in the management and maintenance of the ideal environmental state during the distribution process for optimal DOC viability (Mitchell and Ketlwell 2009). According to the observation and information conducted and collected by the authors from the site, transportation for 12 hours could lower the weight of DOC by as much as 10%. Farmers also complained that the birds were often found having dried feet, injury and some even die after transportation.

The process of DOC transportation constitutes the final stage of production for a hatchery company, but it strongly affects DOC quality provided to the farmer. Currently, DOC transportation state does not have any sufficient monitoring, tracking, and tracing systems. Furthermore, a majority of hatchery companies rely on the expedition service in distributing the DOC products to the farmer customer using a modified DOC transport truck. There is no comprehensive standardization for this transportation process, it does not address the possibility of decline of DOC quality and fraud during the DOC distribution process. In addition, the lack of knowledge by employees regarding animal welfare, particularly in DOC, can also influence the decline in DOC quality and performance when it reaches the farmer. Moreover, to improving DOC logistics services, the logistic system must have a control system for environmental conditions during the transportation process to maintain DOC conditions and a sense of security for customers. Control system development which facilitates monitoring, tracking, and traceability, and enables welfare improvement for DOC without disregarding economic aspects to boost advantage in broiler industry should therefore be established.

Control during production and distribution processes from hatchery to customer is needed to decrease and anticipate any potential loss. This may be implemented using 4.0 technology for developing the DOC supply chain (Haji et al. 2020). Logistics 4.0 design for DOC business is a system developed by implementing the technologies today including Internet of Things (IoT) and Web System Networking (WSN) (Jiang et al. 2020). By implementing these technologies, stakeholders of DOC business chain will be able to conduct product monitoring, tracking, and tracing during transportation process so as to boost reliability of the transportation system. Meanwhile, each stakeholder needs to know how much capital must be invested and what benefits they can get from them. This study sought to describe the current logistics of DOC, analyze the possibility of implementation of logistics 4.0 and analyze the financial feasibility of implementing Logistics 4.0.

MATERIALS AND METHODS

This study was conducted at PT. MSP, Maros Regency, South Sulawesi from April to August 2020. Literature study as well as in-depth interviews were conducted with some experts and practitioners of DOC logistics business. The interview was conducted with 2 hatchery managers of different companies, 4 supervisors in the hatchery and DOC transportation services, 5 drivers, 8 farmers, and 3 academics in DOC hatchery industry in a semi-structured manner to gather information on practices and state of logistics process and the implementation of technology 4.0 for DOC logistics. The information gathered such as DOC productions, sales, financial report and logistics cost from 2015 until 2020 were analyzed descriptively for site condition and used as basis for implementation of the logistics DOC 4.0.

Design model framework was made by referring to variable identification of problems and consultation done with several practitioners of DOC for poultry industry in Sulawesi island, then WSN and IoT-based logistics system were designed to facilitate the logistics process and control of DOC transport state. Moreover, further exploration was also conducted in regard with logistics industry today and development of related smart-logistics technology. Therefore, the design of Logistics 4.0 for DOC business was arranged with features which might solve the existing problems.

To decide on whether to invest in logistics system 4.0 or not, three general measures namely Payback Period (PP), Net Present Value (NPV), and Internal Rate Return (IRR) (Mann 2018; Rianto et al. 2018) were used , while performance measurement was conducted using Economic Value Added (EVA) (Septiana 2015). The implementation and accounting adjustment using discounting techniques such as NPV and IRR are required to enhance the implementation and accounting adjustment from the assessment of EVA (Sharma and Kumar 2010). In addition, DOC production in the projected period and delivery frequency to some destination cities was simulated using Monte Carlo with Microsoft Excel (Torok 2015). The simulation result became the basis for calculating the whole delivery distance, total travel in one year, and income from the delivery of each city with the assumption of DOC production transported.

EVA was used to assess how well the company generates operating profit, in accordance with the total capital invested (Chmelikova 2008). EVA concept depicts how efficient or inefficient a company allocated the capital. EVA value was obtained from the difference between Net Operating Profits After Tax (NOPAT) and capital cost (WACC) multiplied by total capital for running the operation (Capital Employed), as identified (Atmadiputra 2019) in the following formula.

$$\mathrm{EVA} = \mathrm{NOPAT} - (\text{Capital Employed x WACC})$$

Where:

NOPAT	= Sales revenue – Operating Cost – Tax
Capital Employed	= Total Initial Investment
WACC	= Capital cost

Cost of capital (*rk*) is the interest from borrowed capital (Stern and Shiely 2001). Calculation of WACC shall be done using the approach of Capital Asset Pricing Model referring to (Benninga 2014) and using the following formula:

$$\mathrm{WACC} = \mathrm{rd}\ (1 - \mathrm{t})\left(\frac{\mathrm{D}}{\mathrm{D+E}}\right) + \mathrm{re}\left(\frac{\mathrm{E}}{\mathrm{D+E}}\right)$$

Where:

rk	= Cost of capital, WACC
rd	= Cost of debt, CoD = $\frac{\text{interest expense x (1+rate intererst)}}{\text{total debt}}$
re	= cost of equity, CoE = $\mathrm{Rf} - \beta(\mathrm{Rm} - \mathrm{Rf})$
D	= Total debt
E	= Total equity
T	= Tax rate

Total debt and total equity from design simulation were determined using Debt to Equity Ratio (DER), which is used to discover debt and equity proportion from simulation cost structure. Debt to Equity is a solvability ratio indicating the extent that a company could comply with the responsibility using capital (equity) possessed by the company (Atmadiputra 2019). The higher DER value, the bigger the company's debt, or it can be perceived that the value of debt is stagnant but the equity declines. This ratio was found out from benchmarking of open logistics company listed in Indonesian Stock Exchange in 2019.

For investment decision-making, if IRR is more than the WACC, then the investment can be accepted. However, in the event that IRR is less than WACC, then the investment is rejected. This criteria was used for the purpose of ensuring that the company at least achieve the desired target in the hope of improving the income and welfare (Auguar 2017).

RESULTS AND DISCUSSION

Current practice of logistics in DOC industry. PT. MSP is a day-old chick hatchery company situated in Maros Regency, South Sulawesi. It has DOC production capacity of up to 50,000 DOC per day. This company serves order for its breeding partners throughout Sulawesi Island and Kalimantan Island. The increase in chicken consumption in Indonesia, triggered PT. MSP to continuously improve its production and product quality. However, the company faces the challenge of maintaining the product's quality during the distribution process particularly while in transport from hatchery to the farm. The DOC are packed in boxes and the total DOC harvested are recorded. The manager adjusts the number of DOC to the existing order and coordinates delivery. The first type of DOC distribution channel in South Sulawesi is from hatchery to plasma farmer, wherein large scale farmers make DOC order through main company, which makes an order to hatchery. The second distribution channel involves independent farmers who order directly to the hatchery.

The hatchery company uses third party logistics for product delivery to the customers. In general, DOCs are transported using trucks with modified compartment. Size, design, number and type of the blower as well the capacity of compartment varied per truck owner. Hence, DOC box compactness within the compartment differs as well because the difference in compartment size and design affects the box arrangement method (Fig. 1) Current loading practice and vehicle design used for DOC logistics. A common issue in the initial phase of distribution (preloading) is the number and grade classification inaccuracy of DOCs in packaging. This is assumed a result of manual packaging method. Loading process is done in the afternoon, around 16.00 p.m., while awaiting transporters' vehicle, the stocks are fed to prevent weight loss during the transportation process. Delivery time is affected by weather condition and absence of routing plan system from the transporting party. One reason for this is that in addition to serving the transportation needs of the PT. MSP's DOC, transporters also provide transportation services to other companies. During transport, there are also DOCs loaded from other hatchery companies for delivery to different destinations, resulting to frequent delays in delivery. Therefore, in a container, DOCs are from at least three different companies experiencing the same problems.

Fig. 1 Current DOC transportation and handling practice

In addition, several trucks with GPS installed enable monitoring of the transport movement and speed. This is important in the estimation of arrival time in the farm and anticipate the risk of any fraud during the transportation. There are no built-in equipment with sensors to monitor temperature, humidity, wind velocity, gas and sound for optimal DOC environment within the transport container. There are two national standards (SNI) regulating logistics governance regarding DOC: SNI 2043:2011 concerning packaging and SNI 2044:2011 concerning transport equipment. The neglected matter during DOC distribution process to date is driver competence on DOC welfare and handling. This becomes the main factor to be mastered by the drivers because it has significant impact on the stability of DOC quality on reaching the farmers customers. During transport, drivers have the full responsibility to maintain DOC state from the hatchery to the farm. Table 1 shows the problems dealt by business actors in the practice of DOC logistics at present.

Table 1. Problems in conventional DOC logistics.

	Stakeholder	Problems
1)	Hatchery	1. Inaccuracy of DOC number within box 2. Inaccuracy of DOC grade type during packing process 3. Prolonged and conventional coordination of information and goods delivery 4. Improper data archive system 5. Prolonged delivery time (delivery only at afternoon and night)
2)	Farmer	1. Decrease in DOC body weight 2. Decline in DOC growth performance 3. Lack of DOC volume received 4. Inaccurate goods arrival time 5. No direct information or access to product specification and traceability
3)	Transporter	1. Unable to monitor and maintain the condition of container in optimum state for DOC 2. Manual delivery planning system 3. No data archive 4. No monitoring system while in transit 5. Challenging road conditions to farm location 6. Delivery can only be performed in the afternoon and night 7. No detailed information on product delivered 8. Penalty agreement has not been systematized well 9. No vehicle standard 10. Losses during transportation

Possibility of implementing Logistics 4.0 in DOC business. The fundamental function of logistics is to carry the right items with the right quantity and quality, at the right time, to the right place and with the right pricing (Rushton et al. 2014). Logistics 4.0 in 4.0 industry is an integration of various kinds of technologies in order to maximize supply chain efficiency and effectiveness which shifts the focus of an organization toward value chain and also maximize the value provided to the customers by improving competitiveness (Amr et al. 2019). This idea can be achieved by improving transparency and decentralization levels between parties through digitalization.

Logistics 4.0 system for DOC allows for maintenance of ideal DOC conditions by means of a monitoring system called web system networking (WSN) from IoT which records microclimate parameters such as temperature, humidity, gas, vibration, slope, as well as vehicle's speed, position and loading status during the logistics process. It can also improve transparency, real-time data processing and real-time supply chain re-planning. The design of Logistics 4.0 system for DOC business cannot be separated from the improvement of the existing vehicle design. The main consideration in the designing process of DOC transportation vehicle is the capability of minimizing harmful stresses that could later on affect the production performance of the DOCs once they are transferred to the care of the farmers. When compared, it was found that the values of body weight reduction had a significant increment along with the higher transportation duration (Bergoug et al. 2013; Jacobs et al. 2016; Yerpes et al. 2021). Even though the performance of transportation with current vehicle condition did not affect significantly the performance of chicken growth, vehicle improvement is needed to improve customer service. Therefore, improving vehicle design in the Logistics 4.0 design for DOC business should be implemented by installing air-conditioners to maintain the stable condition within the containers, as well as adding some sensors to vehicles and DOC containers (Nazareno et al. 2020).

The information system built should be equipped with special features such as identification, searching, location tracking, monitoring, responsive, and distance and time optimizing features. Product identification will provide the system with information about the right item while the searching feature would enable the system to detect missing items as well as ensure the item quantity. Position monitoring could be performed by the information system via vehicle location tracking. Monitoring toward product condition should also be performed to ensure that product quality is maintained. All the data could be monitored real-time and in detail in the system. Thus, responsiveness toward unexpected events during the logistics process could eventually be handled on time. In addition, the provision of such data can be used as a reference in optimizing logistics process. This process can be conducted with the help of artificial-intelligence which is important in supporting and optimizing all the features.

Shipping is the last process in producing DOC and entails moving it from the hatchery to the customer-farms. Today, the shipping services industry has become much more accurate with the use of improved tracking technology. Accurate tracking services, such as GPS technology, are used to detect the location of the consumers or purchasers while delivering products to the sellers or consumers. This also enables communicating with the driver and alerting him/her if there is a need to check the container for DOC quality maintenance, and sending a notification if the driver is out of the route. Even better, by logging this diagnostic data, the compliance with key DOC transportation safety standards can be shown if needed. Technologies such as geographic information systems (GIS) and remote sensing (RS) are used for transportation services and precision analysis, through the collection and integration of data remotely, and for mapping geospatial variability to highlight the movement of goods throughout the entire shipping process (Bosona and Gebresenbet 2013). Other real-time tracking technologies such as the IoT, Radio Frequency Identification (RFID), and blockchain allow easier updates to the shipping process while transporting large volume of products from the manufacturing firms to the distributors (Rivero-Garcia et al. 2019).

RFID is employed to identify vehicle location during delivery so the delivering fee can be optimized by maximizing vehicle operation (Kim et al. 2008). Furthermore, RFID tagging is implemented in many food industries in order to automate their warehouses and distribution centers so errors can be eliminated by ensuring that the right items are being delivered with the right quantity to the right place. The RFID technology is important for the optimization of various supply chains, so that customers can easily access the products (Kelepouris et al. 2017).

The IoT and RFID technology is attached sensors to identifying, finding, and tracking the status of an asset product in real-time via cloud and internet technologies. A technology which applies IoT system will allow data collection, storage, and data analysis which can be connected to the entire supply chains (Tian 2018). This is important for tracking DOCs during delivery due to their vulnerability toward changes in environmental condition that tend to vary with length of time traveled. DOC quality and safety standards can be achieved via the new system because the IoT and cloud technologies can be applied in every stage of value chain for better data collection, and faster as well as more accurate decision making (Verdouw et al. 2016). In addition, the blockchain technology can also be implemented in this system as in the food supply chain to provide the right information about the food demanded (Tse et al. 2017). Logistics 4.0 system model for DOC business is shown in Fig. 2.

- QR code scanner: QR code scanner or smartphone with QR code scanning app is required to read package information.

- Asset creation: After the product (DOC box) is first entered into the warehouse, a new asset data is created in the system. All product information is stored in a QR code.

- Sensors (IoT): These sensors are required in the DOC box and in the container in order to monitor temperature, RH, and when the DOC box was opened. This sensor is required when the product is in transit, and after the consumer (farmer) receives the product, the sensor QR code is scanned by the courier, and if there is death, injury or the quality received does not match the agreed specs, the consumer can refuse and return the goods.

- Product visibility: All stakeholders can see product details in the supply chain that have been inputted in the mobile application. After the user scans the QR code of an existing product or sensor, the user will get all the information about the product in the DOC supply chain via a normal smartphone by using a mobile application or web application.

To synchronise and optimise the inventory, the data and information are inputted to an intelligent inventory management engine to handle order change and picking problems, among which data clustering and some machine learning methods as well as fuzzy inference system are applied for information processing in decision-support. The output is transmitted back to the host application and shares the results with the mobile apps. Hence, the staff involved in this IoT-based can receive the corresponding action information.

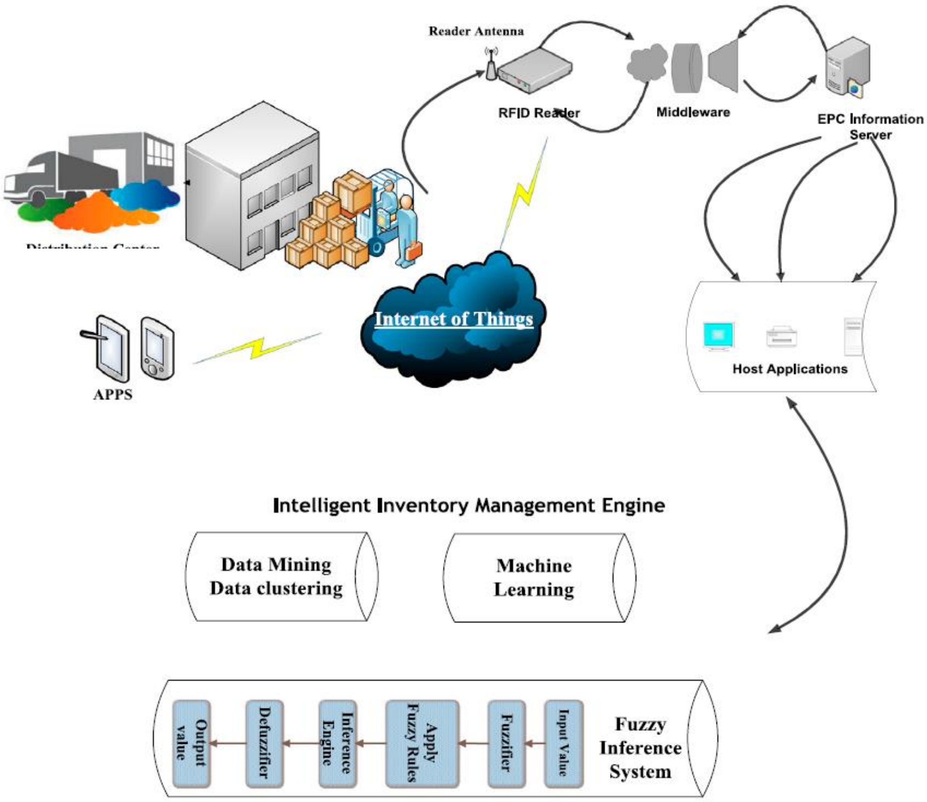

Fig. 2. Integrated Iot and WSN on Logistics 4.0 System

Financial analysis and benefit of Logistics 4.0 for DOC business implementation. The evaluation of the planned Logistic 4.0 System was conducted by creating financial simulation. There will be changes in the cost structure because the adoption of logistics 4.0 requires initial investment as presented in Table 2. Initial Investment, which comprises installation of IoT sensor (Pang et al. 2012), installation of temperature control system, installation of IoT server (Kurniawan 2015), the purchase of IoT sensor for scanning and data input process, bandwidth, installation of server services, as well as labour training will be a total of IDR1,308,061,624.

Table 2. Initial investment for Logistic 4.0 System adoption.

Description	Unit	Amount	Economic age
IoT sensor installation	IDR	446,453,700	10
Temperature controlling system	IDR	596,607,924	20
IoT server installation	IDR	25,000,000	20
IoT installation	IDR	30,000,000	10
Bandwidth	IDR	90,000,000	10
Installation of service from server	IDR	120,000,000	10
Total initial investment	IDR	1,308,061,624	-

Investment simulation was conducted for a period of 10 years (t = 10) and the amount of delivered DOC was also simulated using Monte Carlo to determine distance travelled and frequency of trips per year in a 10-year period. There was an increment in the delivery cost, truck maintenance cost, employee's salary, delivery price, and cost savings in the allocation of cost for risks during transport (Table 3). In the simulation of Model Logistics 4.0 system, the delivery fee increased due to the addition of sensors and temperature controlling systems which consumed more fuel, with an assumption that the increment of fuel consumption in every truck would be 2% of the fuel consumption prior to Logistics 4.0 simulation. The fuel needed in every transportation fleet was based on Nurhayati (2017) which was IDR4,383.- km^{-1}, while the needed fuel in every transportation fleet during the adoption of Logistics 4.0 system was assumed to increase by 2% which was computed to be IDR4,471 km^{-1}. The increment in delivery cost could be interpreted as the cost of quality improvement provided to farmers and hatchery companies as the customers. Optimization of route which was promoted by the Machine Learning and the IoT of transportation fleet tracking affected the cost of truck maintenance. The truck maintenance fee was projected to decline generating savings of IDR1,113,514 for every truck per year in the Logistic 4.0 system, indicating a huge value-added in the customer service in DOC delivery fleets.

The adoption of Logistics 4.0 System also affected management, via the centralized and integrated data management, which could reduce administrative necessities. The simulation of this system assumed that it will only require one person to complete all the administration works due to IoT and IoT server enabling the downsizing of staff. Such reduction in the number of staff employed would decrease labor cost and increase operating profit. Nonetheless, profit was not the only objective of this system. The investment was also focused on the human resources being involved with the system, and these resources are employees. Thus, workforce training must continue to be carried out periodically to improve their ability to operate the new system.

Based on interviews conducted, fraud is often carried out by corrupt couriers during the shipping by taking DOC or exchanging DOC with low quality during the journey. This can occur due to the lack of welfare of couriers so that an increase in employee salaries is expected to improve employee welfare and have an impact on improving performance. The most important employee motivation is salary (Wiley 1997). Companies can increase profits and productivity of their logistics services by focusing on improving employee skills by training, increasing salaries, and improving workplace conditions (Jhawar et al. 2016). Thus, workers will have a good commitment to the company so that fraud that often occurs can be reduced.

The DOC delivery fees varied according to the destination. The simulation used five destination cities/regencies, namely, Maros Regency, Palu City, Bulukumba Regency, Luwuk Regency, and Kendari City, with an assumption that the DOCs were delivered from Maros Regency. The delivery fee increased by IDR 2,044 for each box (Table 3). It was applied due to the upgrade of the services being offered which can provide added values for the customers, improve customer's loyalty, and attract new customers so the trips could possibly be done in full capacities. It was found that the change in the fee in for the 10-year period simulation, as well as the increment in employee's salary, were not harmful to the employment of this system. Instead, the efficiency generated by the Logistics 4.0 system improved the operational profit by IDR 321,763,955 compared with the system before the adoption of Logistics 4.0 .

Table 3. Changes in the cost components before and after simulation of Logistics 4.0 design adoption

Description	Unit	Before Adoption of Logistics 4.0	After Adoption of Logistics 4.0	Increment	Benefit
Expedition					
Avg. of delivery fee	IDR km^{-1}	4,690	4,847	156	Service upgrade
Avg. of maintenance fee	IDR $truck^{-1}$ $year^{-1}$	6,156,493	5,042,979	(1,113,514)	Route optimization
Avg. of risk fee during transport	IDR rit^{-1}	26,631	8,586	(18,045)	Savings
Required number of admin staff	Person	7	1	(6)	HR efficiency
Avg. of employee's salary	IDR	49,820,109	65,655,787	15,835,678	Increment of HR welfare
Avg. of delivery fee	IDR box^{-1}	51,100	53,144	2,044	Increment of price
Avg. of operating profit	IDR	253,235,977	574,999,932	321,763,955	Increment of income
Hatchery					
Required number of admin staff	Person	7	5	(2)	HR efficiency
Avg. of employee salary	IDR	21,137,961	19,002,813	(2,135,148)	Savings
Number of DOC mortality	Box $year^{-1}$	1,286	219	(1,068)	Quality increment
Fee of claim on DOC mortality	IDR $year^{-1}$	1,783,100	303,127	(1,479,973)	Reduction of risk in terms of DOC mortality claim

The decision to invest in the adoption of Logistics 4.0 system, financial analysis using the return period (payback period, PP), NPV, IRR, was performed and evaluation of system performance was calculated by EVA in order to strengthen the implementation of system (Sharma and Kumar 2010).

The payback period of the system would be 3.09 years, suggesting that the cash flow resulting from the adoption of the Logistics 4.0 system could pay back the initial investment in an amount of IDR1,308,061,624 within 3.09 years or 3 years and 1 month (Table 4). A consideration on the lifetime of this system was 10 years in minimum (Table 1), and it was assumed that the investment would be accepted with the return level of less than 4 years. Thus, such return period of 3.09 years was deemed to be acceptable and worth continuing.

The NPV value gained from project's cash flow was multiplied by the rate of WACC discount (8%) and then compared to the rate of the prevailing commercial bank interest. The calculated NPV value of the Logistics 4.0 system indicated a positive nominal amount of IDR 1,207,221,305 indicating that the capital invested in Logistics 4.0 system gave more benefits when not being saved with the rate of return of 3.75% (based on BI 7 days reverse repo rate). The NPV value of more than zero indicates that the amount generated from the investment decision is feasible. The NPV value was then multiplied by the discount rate until it generated zero in number, to arrive at the internal rate of return (IRR). IRR is one of the key variables for investment feasibility which depicts the level of return toward the entire investment (Berutu, 2017). The investment is feasible based on the IRR value of the simulated Logistics 4.0 system investment which was 27% higher than the level of minimum return which was 7.75%.

Table 4. Assessment toward the adequacy of Logistics 4.0 system simulation

Description	Unit	Amount
Initial investment	IDR	1,308,061,624
Payback period	Year	3.09
NPV	IDR	1.228.389.872
IRR	%	28
WACC	%	8
EVA	IDR	261,725,367

CONCLUSION

The existing DOC logistics from the hatchery to the farm is not equipped with adequate monitoring, tracking, and tracing systems, resulting in losses for the stakeholders. Therefore, the Logistics 4.0 System was designed using IoT and WSN and was simulated for a period of 10 years. Under the declared assumptions, the simulated Logistics 4.0 system has value-based benefit potential for the stakeholders in the DOC business which results in a reduction in the workforce for administrative activities by 85%. Thus the company can focus on increasing skills and wages to improve employee welfare. Therefore, the company may take the implementation of Logistics 4.0 system into consideration because the increments of added value to be offered can improve the company's credibility, delivery flexibility, and route optimization which can enhance customer satisfaction. To ensure a committed transport staff the company needs to provide professional training including the chick delivery training not only for the new technology but also on the needs and behaviour of day-old chicks. The implementation Logistic 4.0 in DOC business to ensure its traceability still needs standardization of the equipment, processes and interfaces, joint platform and independent governance as the key boundary conditions for successful implementation of day-old chicks logistics.

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IMPACT OF ORGANIC AGRICULTURE INFORMATION SHARING ON MAIN ACTORS IN LAGUNA, PHILIPPINES

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ABSTRACT

The adoption rate of organic agriculture is still low despite the efforts of the Philippine government and the enactment of the Republic Act No. 10068 or the Philippine Organic Agriculture Act in 2010. This study attempted to clarify information sharing-related factors affecting the low adoption rate, as information links all agricultural sectors, activities, and operations. This qualitative study specifically determined how information sharing influences different actors' decisions to share and adopt organic agriculture technologies in Laguna, Philippines. There were five institutions, 30 farmers, and six family members who were interviewed at least twice between 2017 and 2020 using Historically Structured Inviting as a sampling method. This study also utilized different data collection methods and diverse data sources accessed over time for data triangulation to ensure the validity and reliability of the research. Life History Approach, Trajectory Equifinality Approach, and Grounded Theory revealed needs, importance, sustainability, pressure from others, availability, and easiness to adopt as factors affecting actors' attitudes toward learning, sharing, and adopting organic agriculture technologies. Moreover, the degree of the economic, well-being, and environmental impacts varied. Although personal information sources had the strongest interaction and impact, improvement in the accessibility and availability of other sources (e.g. mass media sources) might increase the degree of the impact. Furthermore, farmers showed interest in technologies that benefited them and their children, who would eventually manage their farms.

Key words: grounded theory, life history, needs, technology adoption, trajectory equifinality

INTRODUCTION

Organic agriculture (OA) has changed unsustainable habits around the globe by inspiring producers and consumers. Concerned organizations and institutions are continuously working to meet the principles of health, ecology, fairness, and care at the core of the organic philosophy (Arbenz et al. 2016). In the Philippines, OA was given priority as one of the essential technologies and farm practices to address current issues on sustainable agriculture, environmental degradation, and climate change. Republic Act No. 10068 (more commonly known as the Philippine Organic Agriculture Act) was enacted on 6 April 2010 to devote five percent (5%) of the agricultural land to organic farming (del Rosario 2018). Despite the efforts of the Philippine government to promote and adopt the technology, the OA adoption rate is still low. The Department of Agriculture (DA) has set a target of 200,000 ha to be devoted to OA, equivalent to five percent (5%) of the estimated 4 million ha cropland (Pantoja et al.

2016). However, the reported 17,156 ha of OA managed land (0.17%) in 2017 by Willer and Lernoud (2019) conveys that the situation is still far from reaching the government target. Various challenges such as policy gaps, lack of production support, promotion, and awareness activities, fragmented and inadequate research and development, extension and capability building activities, and poor market systems may have attributed to low adoption rate of OA (del Rosario 2018; Pantoja et al. 2016).

Research and extension are critical to attaining and achieving the desired outcomes on agricultural development in the Philippines (Aquino et al. 2011). However, in reality, the weak research and extension connections restrict the full implementation of successful agricultural development efforts. Upon reviewing past literature, most studies focused on the economic aspect and production (Piadozo et al. 2016; Shimoguchi and Mojica 2016), while only a few studies about farmers' attitudes and behavior affecting information sharing. Furthermore, there are few in-depth studies of the non-monetary effects of information sharing on farmers. Thus, research models are necessary for developing countries, especially those adequately emphasize the impact of knowledge and information sharing by individuals, organizations, and their respective intentions to technology adoption (Kettinger et al. 2015).

Information is critical in agricultural development as it connects all components, activities, and operations in a value chain network and is a tool for communication and coordination among stakeholders. It also serves as a channel for assessing trends and shaping decisions (Chisita 2012 and Lotfi et al. 2013). Agricultural information sharing has two main actor groups: the institutions as the major sources of information and the farmers as the main receivers or beneficiaries. However, having this kind of conventional major actor group, certain studies acknowledged the vital role of farmers in experimenting and knowledge sharing, especially information dissemination by farmer-to-farmer that has been used significantly in the Philippines and Central America since the 1950s and 1970s, respectively (Chambers et al. 1989; Franzel et al. 2015). In fact, almost all traditional agriculture practices result from the spontaneous spread of innovation from one farmer to another, from one village to another, and even clear across continents (Bunch, 1989). Hence, focusing on the effect or impact of knowledge and information sharing to different actors in agriculture, specifically in developing countries, is needed (Kettinger et al. 2015). Therefore, this study utilizes three main actor groups on OA information: the institution as the main source, the farmers as the primary receivers and main beneficiaries, and the farmer's family members who directly affect farmer's decision and as the secondary receivers or indirect beneficiaries.

To address the problems in mismatched technologies, policies, and programs, farmers should be adequately heard and understood. This initiative can result in effective information dissemination that will eventually lead to adopting the technologies they need. In addition, farmers sharing their motivation and aspirations with younger generations may persuade them to engage in agriculture. Furthermore, promotion and adoption of OA are vital initiatives to uplift the lives of the poorest populations and attain agricultural sustainability, specifically in Asia; however, empirical evidence on its impacts on poor organic farmers is limited, particularly in developing countries (Setboonsarng 2015). Thus, this study attempts to clarify one of the possible solutions to achieve agricultural sustainability through assessing the impact of information sharing to farmers and other actors in the community. This study specifically sought to explore different factors affecting actors' attitudes that affect farmers' decisions, distinguish differences among actors on the value of factors, and assess the degree and interaction of impact of information sharing to farmers.

MATERIALS AND METHODS

Study area. Laguna Province was chosen as a research study area because Los Baños is one of its cities designated and declared through the Proclamation Order No. 349 in 2000 as a Special Science and Nature City of the Philippines catering to different agricultural institutions (Fig. 1). Los Baños has remained the country's hub of science and nature with the presence of national and international research institutions such as the University of the Philippines (UPLB), Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development (PCAARRD), Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA), International Rice Research Institute (IRRI), and Bureau of Plant Industry (BPI). In addition, the province is also the location for various agriculture-related regional offices. OA information is available in most of these institutions and regional offices. Thus, farmers have an advantage point, and OA has considerable potential for growth and development in the area. However, more efforts seemed to be required to disseminate and adopt OA. Furthermore, according to the DA-Agricultural Training Institute (DA-ATI), as of December 2017, there were only 2 out of 85 OA Farmer-Scientist in Laguna.

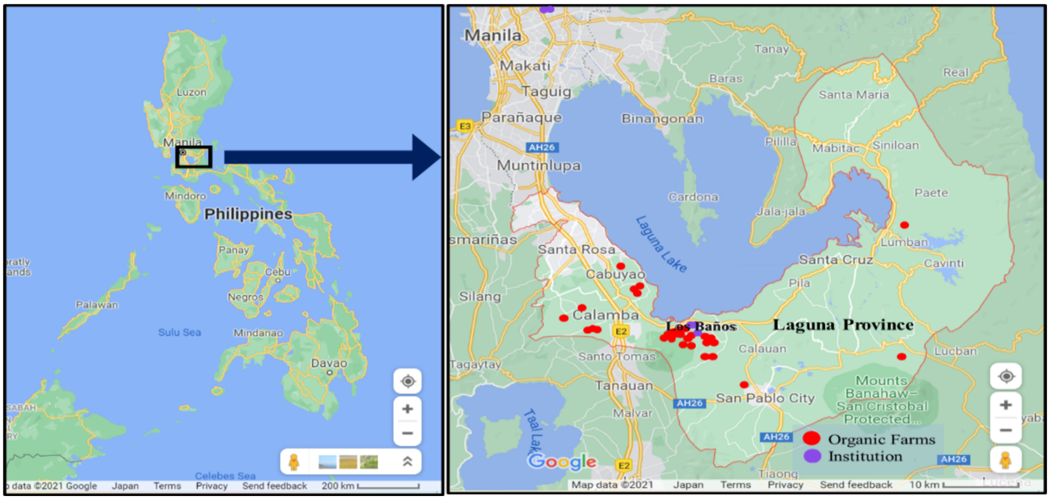

Fig. 1. The Philippine map and locations of organic farms and institutions
Source: Google maps, 2021

Interviews and sampling. Primary data sources were semi-structured interviews to gain good access to people's perceptions, meanings, and definitions of situations to understand and record what is happening on the ground. The purpose of the interviews was to confirm the existence of information systems, record its details and compare what information and technology are being delivered and received by the farmer-participants from the information and technologies offered by different sources. For this reason, this study utilized purposive sampling and the Historically Structured Inviting (HSI) of Valsiner and Sato (2006) and Sato et al. (2007) to arrange participants who satisfied the requirements for the appropriate sample and cases. The primary requirements of farmer-participants were 1) practicing organic agriculture, 2) residing in Laguna, 3) receiving (received) OA training, 4) sharing OA information to others, and 5) being willing to cooperate as a research participant. This study collected different factors from the series of interviews and observations conducted with five (5) organic farmer-participants on July to August 2017, March 2018, and July to August 2018.

From the initial data collected, researchers prepared a questionnaire focusing on information sharing and the adoption of OA technology. Since initial interviews showed that there were farmer-participants with no formal OA training, the primary requirement on "receiving OA training" was changed to "received OA information" in order not to limit the farmer-participants to those who received formal training only. This study then organized HSI and conducted continuous interviews, visits, and observations with 17 farmer-participants from 17 July 2019 to 2 September 2019. Refinement of questionnaires was performed based on the encoded data and observation memos. This study made life histories and plotted the life paths of each farmer using Trajectory Equifinality Models (TEM). Before compiling all the life histories into one TEM, each farmer verified their respective life history (path). It should be noted the TEM results will not be presented and discussed in this paper.

Continuous encoding and comparison of data were executed. From March to August 2020, four (4) more farmer-participants were interviewed in-person and/or through phone. Although this study accomplished data comparison of 21 farmer-participants, 14 complete validated life histories resulted in data saturation. This study distributed at least 35 more questionnaires to other areas in Laguna. Unfortunately, they did not allow farm visits or face-to-face interviews due to the COVID-19 pandemic. As a result, only nine (9) questionnaires were returned, which made 30 farmer-participants.

On the other hand, institution staff-participants consisted of 11 staff from five different institutions and organizations providing OA information were interviewed in 2017, 2018, 2019, and 2020. Staff-participant criteria include affiliation to an institution/ organization that farmer-participants mentioned, direct involvement in OA information sharing to farmers, and willingness to cooperate as a research participant. Six farmers' family members were also interviewed and observed following the criteria: currently living with the farmer-participant, having experience of any organic farm work and information sharing-related activities, and being willing to cooperate as a research participant. It is noteworthy that this study attained consent from both parents and children regarding the interviews and observation of children below 18 years old (including expected results). Moreover, parents or older family member/s were present during the interview.

Study approach and data collection. Qualitative research requires validity and reliability; thus, this study conducted data triangulation to ensure validity and reliability. Triangulation included the use of different data collection methods (e.g., interviews, observation, and surveys), different sources (e.g., information from farmers, institution staff, family members, memos, field notes, books, journals, training modules, websites, and other printed materials) accessed over time between 2017, 2018, 2019, and 2020. In addition, this study conducted constant data comparison and respondent verification.

The Life History Approach (LHA), Trajectory Equifinality Approach (TEA), and Grounded Theory Approach (GTA) were utilized in this study. Equifinality Points (EFP) and Obligatory Passage Points (OPP) were set as a guide in HSI which was one method of sampling selection. The life history of each farmer was plotted with the aid of the TEM. According to GTA as adopted from Locke (2002) and Glaser (2001), data collection was conducted using continuous interviews, observations, and memoing with different actors in the information flow. Data transcription gathered was continuously coded and categorized using the constant comparative method. This method allowed careful comparison of all new data to previous data gathered until achieving data saturation.

Observations included different settings on how actors (farmers, institution staff, and some family members) receive, share, and practice the OA information and technology. OA practices were noted and double-checked during the interviews, while notetaking was also done for particular words or concepts that the participants emphasized. Total participants consisted of eleven staff from five institutions, 30 farmers, and six family members were interviewed, and all mentioned impacts were noted, grouped, and categorized. In addition, this study asked each participant to specify the source, frequency usage, and effectivity ranking of each information they are receiving to clarify the impact of information sharing to the farmer and other actors in the community. The categories of information sources were electronic sources, printed materials, and face-to-face information sharing. All interviews were recorded using IEC digital recorder to capture the voice from the ground.

Data analysis. Qualitative research is interpretative, very dynamic, and free-flowing process. It is used to understand and discover how meanings are formed and transformed, uncover relevant variables that later can be tested through quantitative research, and take a holistic and comprehensive approach to study phenomena. (Babbie 2014; Corbin and Strauss 2015)

LHA helped in the explanation of what happened to the farmer-participants and what caused certain decisions. LHA aided in exploring and identifying the dominant narratives of people's lives within events and situations. These narratives were contextualized and described how a specific event came to be significant and how opinions and decisions changed over time (Hagemaster, 1992); TEM as one of the three major components of TEA (Sato, et al. 2016) was generated following TEA aid in the discussion of LHA; and GTA which was used in categorizing and linking data. Following the analytical method for the grounded theory of Locke (2002) and Glaser (2001), there were constant comparison and categorization of data using GTA with the Archive of Technology, Lifeworld and Everyday Language text interpretation (ATLAS.ti) application until achieving data saturation.

Research participants were asked to specify and rank the factors or reasons they individually considered when choosing a technology to learn, adopt, and share. Main actors were also asked about the influences or changes to their lives after receiving OA information. All answers were noted, encoded, and grouped using Atlast.ti. Codes were set based on varied quotations from the interviews with main actors. These quotations linked to a code of each valued factor were referred to as "groundedness" or the code frequency. For this study, the "groundedness" was divided into three groups: High: 67%-100%; Moderate: 34%-66%; Low: 33% and below, based on the percentage of occurrences of codes per actor group.

RESULTS AND DISCUSSION

Factors affecting actors' attitudes that affect farmers' decisions must be explored to understand and assess the impact of information sharing on farmers and other actors in the community. Afterward, the differences among actors on the value of factors can be distinguished. Subsequently, the degree and interaction of impact of information sharing to different actors can be assessed.

Profile of Participants. Main actors in this study included 11 staff-participants from five institutions (as the main information sources), 30 farmer-participants (as the main receiver or beneficiaries of information and innovation), and six family member-participants (who were the main factor or reason affecting farmer's decision in adopting specific innovation) referring to children and spouses of some farmer-participants. Staff-participants were composed of seven female and four male respondents ages 35 to 62 years old with an average age of 48 years old. Years engaging in formal OA information sharing for staff-participants ranged from 5 to 19 years with an average of 11 years. On the other hand, farmer-participants consisted of 21 male and nine female respondents ages from 30 to 72 years old with an average age of 52 years old. Their farming experiences ranged from 4 to 50 years, with an average of 29 years, while organic farming experience seemed shorter with an average of 10 years. It should be noted that organic farming experience ranged from 1 year (those farmers who tried OA but went back to conventional) to 30 years.

Factors affecting actors' attitude and the differences of valued impacts. Different factors affecting actors' attitudes were identified from the study. These factors were grouped into two parts: the *extrinsic variables* (e.g. characteristics of the farmers, external environment, and innovation) and the *intrinsic variables* (e.g. attitude, knowledge, perception, motivation- that will be understood through participants' life histories). For this study, only intrinsic variables are utilized.

Staff-participants from institutions valued highly the importance of innovation, including OA's safeness for farmers, consumers, and the environment (Table 1). The sustainability of OA and the easiness to learn and adopt were ranked second and third, respectively. For the staff-participants of five institutions, OA is a sustainable agriculture method and the adoption of the innovation itself can be sustained because OA is site-specific which also leads to the easiness to learn and adopt as all inputs are available in the farm. Likewise, farmer-participants and their families tend to value their needs and interests more or the beneficial or positive influences that they will be getting from the innovation. Positive impacts include improved livelihood and family relationships and gained trust from their consumers and neighbors. These results are in line with the study conducted by Aquino (2019), which presented that aside from increased income and profit, small-scale organic farmers in the Philippines had different reasons and needs in adopting specific technology that included the safeness of the family and the environment.

Farmers' attitudes towards receiving and adopting OA technology also tended to be affected by the pressure from neighbor farmers and their family members, followed by the availability of information about the technology and its easiness to adopt. As farm successors, farmers' families tend to value the sustainability of innovation more than easiness to adopt. Thus, these three main actors had different but connected reasons or factors affecting their attitude in sharing or receiving information and adopting OA innovation.

Table 1. Rank of different factors affecting actors' attitude in information sharing and adoption of innovation

Rank	Institutions	Farmers	Farmers' Families
1st	importance of innovation	needs, interest (benefits)	needs, interest (benefits)
2nd	sustainability of innovation	pressure from neighbor-farmers and family members	sustainability of innovation
3rd	easiness to learn and adopt	availability and easiness to adopt	easiness to adopt

Sources: Survey data in 2018 and 2020

Impact of information sharing to different actors in the community.

Based on the main actors' quotations from the validated transcribed interviews, codes were set and categorized, GTA analysis revealed that there were three major categories or themes, with respective sub-categories consisting of different codes. Groups included economic (monetary and non-monetary influences or positive changes from information sharing), well-being (enhanced relationship and improved mental health), and environmental (ecological benefits gained) impacts (Fig.2).

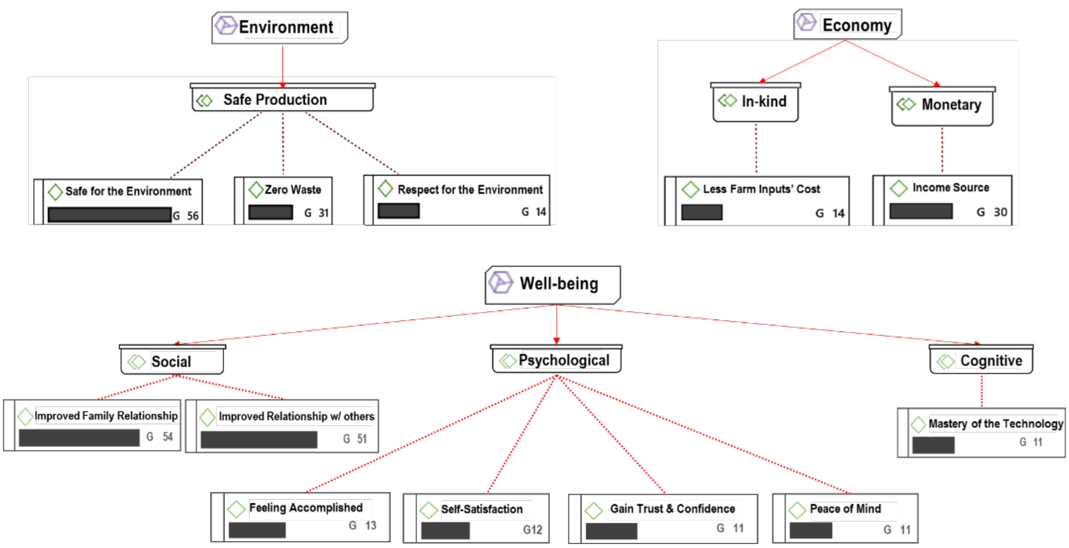

Fig. 2. Categories, codes, and "groundedness" of codes from the impact of OA to different actors in Laguna, Philippines derived using GTA analysis in Atlas.ti

The economic impacts are the positive effects or gains of different actors financially, whether in-cash or not, while practicing and sharing OA. It has two sub-categories: “monetary benefits” and “in-kind”. Economic benefits such as additional income, improved profit, and livelihood through less cost of input were noted. Some had enough savings to send their children to college or buy tricycle or small piece of land. Economic benefits consisted of two sub-categories: “in-kind” and “monetary” with each code of ‘less farm inputs’ cost’ (with 14 linked quotations) and ‘income source’ (or improved income with 30 linked quotations), respectively. “Groundedness” or code frequency refers to the number of times each quotation is linked to specific factor’s code valued by the participants. Thus, the higher the code frequency, the higher the participant values the specific factor’s code.

The environmental impact had a sub-category of “safe production” with three codes: ‘safe for the environment,’ ‘zero-waste,’ and ‘respect for the environment’ with 56, 31, and 14 linked quotations, respectively. “Safe production” contained the tag of quotations from farmers related to producing food that is ‘safe for the environment,’ such as using natural fertilizers and pesticides or farming without synthetic chemicals. In addition, ‘zero-waste’ referred to tags on the process of recycling nutrients and ‘respect for the environment’ representing tags on their efforts or contribution in saving the environment were also included.

On the other hand, the well-being impact is composed of three sub-categories and seven codes. The “social” sub-category considered the participants’ desire to have a role in and help their family and community. The “cognitive” sub-category focused on how participants positively processed the information, knowledge, and learnings they were getting to be more effective and successful in their farm and information sharing activities. The “psychological” sub-category covered farmers’ positive sense of purpose or self-satisfaction.

Positive feelings brought by OA to their lives became their reasons to continue and eventually adopt the OA technologies. Codes belonging to this category were ‘improved family relationship,’ ‘improved relationship with others,’ ‘mastery of the technology,’ ‘feeling accomplished,’ ‘self-satisfaction,’ ‘gain trust and confidence,’ and having ‘peace-of-mind.’ Quotations such as *“This changed my life as the feeling of fulfillment that I now have another role for my family”; “through OA, I was able to be an important family member”; “trust and a good farmer-consumer relationship are also created”; “I am not worried if they [his children] do farm activities with me because I am using natural pesticide and fertilizer, so I am sure that it is safe even for my kids”* were tagged under these codes and category.

Participants were able to get positive benefits specifically on their well-being, knowledge on environmental protection, and economic advantage from the OA information received, practiced, and shared with others. Valued impacts of information sharing on different actors (Table 2) revealed the differences of these impacts to different actors in the study area. All the interviewed staff-participants valued highly the “well-being” impact, and the code impact included ‘gain trust and confidence,’ ‘self-satisfaction,’ ‘peace-of-mind,’ ‘feeling accomplished,’ and ‘mastery of the technology.’ In addition, they also valued ‘safe food and production’ that they shared and imparted to their beneficiaries. They emphasized the importance of the ecological or “environmental impacts” of practicing OA.

The economic impact to staff-participants was lowest among all actors because it did not matter if there is an honorarium or additional pay for extending knowledge and information about OA. They thought that receiving tokens of appreciation from farmers, or local government units are just additional benefits, but they treasured more the trust and self-satisfaction they are getting through imparting the OA innovation to others.

Likewise, the 30 farmer-participants valued well-being and environmental impact greatly on social (improved family and neighbor relationship), psychological (boost confidence, feeling valued), and safety for the environment. Some farmer-participants increased their yield, which led to increased income, but some farmers who could not improve their income believed that other positive impacts (e.g., improved family and neighbor relationships and environment protection) were more important than having a better income. On the other hand, family members-participants, especially the children who were doing gardening in school, valued well-being and environmental impacts the most (e.g., enriched school social relationship, feeling valued). They even often boasted to their other classmates that OA was easy and beneficial. Surprisingly, even though some farmer-participants were not making a high profit from practicing OA, farmer-participants valued well-being and environmental impacts of knowing and understanding OA through information sharing more than its economic impact.

Table 2. Valued impacts of information sharing on different actors

Impacts	Farmers Gr=76	Impact to Farmers Gr (%)	Institution Staff Gr=28	Impact to Institution Staff Gr (%)	Farmers' Family Members Gr=16	Impact to Farmers' Family Members Gr (%)	Total
Economy Gr=44; GS=2	35	Moderate 46	3	Low 11	6	Moderate 38	44
Environment Gr=101; GS=3	67	High 88	23	High 82	11	High 69	101
Well-being Gr=103; GS=7	72	High 95	20	High 71	11	High 69	103
Total	174		46		28		248

Sources: Survey data in 2018 and 2020

Notes: Gr- Groundedness of codes (number of quotations coded by a code) or documents (quotations created in a document); Gs- Number of codes in a code group. Impacts were based on the "groundedness" of each valued factor from Atlas.ti (High: 67%-100%; Moderate: 34%-66%; Low: 33% and below)

Effective information service delivery or dissemination, specifically to the grassroots, could be achieved when adequate attention is focused on how the information is received, processed, communicated, and used positively for the benefit of all the actors. Ogar et al. (2018) stated that information delivery with good quality is the right step towards the growth of agriculture through good communication with the rural population that will enable access to relevant information. In addition, Heeks (2018) pointed out that information communication technology positively impacts poverty alleviation concerning rural development and food security.

Impact on the institutions' staffs as main information sources. Resource speakers tended to try first the different OA technologies before they share these. From the interviews conducted, extension agents, heads, and project staff from the institutions seemed to continuously check the technology for its effectiveness and easiness of application. Also, they seemed to adopt and apply each OA technology to their backyard gardens because they believed in OA's health benefits. The staff-participant from Institution 1 mentioned: *"We want that if we are healthy, they [farmers] should also be [healthy]. I always try and apply the technology in my garden. I want to verify because sometimes it is not doable…"*. In addition, staff-participant from institution 2 declared: *"I do not have a large area, but I can use recycled materials. When persuading other people, it should start with oneself. Also, with this [experience], one can be assured that own crops are free of chemicals and safe to eat while [having the opportunity] to save money. Working eight hours [a day] and five days a week in front of the computer, this [home gardening] is [also] a good physical activity"*. Furthermore, the staff-participant (division head responsible for information sharing) from Institution 4 stated: *"Aba, I also need to have my garden wherein I can adopt technologies that we are disseminating. How can we encourage others if we are* *not practicing it?".* Hence, for this study, staff-participants emphasized on importance of sharing their own experiences to others.

Information providers essentially do, try, and practice the technology first in order for them to understand quickly what the farmers or beneficiaries will be experiencing and be ready for all the questions and challenges the farmers or beneficiaries will ask. These convey that institutions' staffs were able to have peace of mind, mastery of the technology, and confidence in what they are sharing that leads to gaining beneficiaries' trust, feeling accomplished, and self-satisfaction.

Impact on farmers. Farmer-participants seemed to value well-being and environmental impacts of the OA information more than its economic impacts similar to the staff-participants. Their main reason for OA practicing and sharing was the safeness of the technology. Notably, most farmer-participants stated that OA was safe for them, their family, and the environment, which fell under the "well-being" and "environmental" impacts. Transcripts from farmer-participant 3 and farmer-participant 17 highlighted the impact of OA information on most farmers, specifically for becoming aware of the importance of nutrient cycling and the effect of OA on the health of farmers, their families, consumers, and the environment. Farmer-participant 3 stated: "*Synthetic pesticides negatively affects humans, so why do I still need to use them. [Moreover,] if natural pesticides and fertilizers can be found just around here [within the farm], there is no reason for using synthetic ones? OA is safe for everyone [and] safe for the consumer's health. We can also help our environment by not using synthetic chemicals".* Also, farmer-participant 17 mentioned: *"I am practicing OA because of the flow of nutrients. It is just in a cycle. Everything can just be found within the farm…".*

In addition, farmer-participant 4 stated: *"Yes. [Practicing] organic is very hard, laborious, time-consuming, and tedious. However, I realized that it would be more beneficial in the long run because it is safe for me, my children, consumers, and the environment. I am at ease that my children, unlike me when I was their age, now know the importance of organic. They are even bragging in their school about how to do it. They often say that they saw me doing it, and it is easy to do."* In addition, farmer-participant 18 said: *"I want to change not only their [neighbors'] farming ways but also on how they live. Most of the mothers here spend the whole day chatting and talking about others' lives. [When given the opportunity,] I try to teach them what I know from my experiences and own farm practices".*

Transcripts from farmer-participant 4 and farmer-participant 18 indicated that aside from being aware of the safety of the technology, improving and touching the lives of the people around them matters remarkably. As a parent and concerned neighbor and relative, these farmer-participants were also inclined to share the positive impacts of OA information they are receiving to those they care for who live near them.

On the other hand, some farmer-participants were also able to maximize the economic benefits and impacts of OA information and technology in their lives as they could save some money and even buy a parcel of land. For example, farmer-participant 7 stated: *"Farming is important. During this pandemic, we have something to eat. Unlike others, we do not need to go down [to the market] to buy food. Farming and OA have many advantages. Also, after engaging in OA, my husband and I were able to purchase a small [piece of] land through monthly installments."* These statements also emphasized that their OA farm helped them survive and be less exposed to other people, especially during the pandemic.

Farmers seemed to value more the well-being and environmental impacts of OA information sharing, being knowledgeable about the nutrient cycle and the health benefits or safeness of OA to people, and the opportunity to teach and help their family members and neighbors matters notably for the farmers. Moreover, farmers seemed to be satisfied and happy with having safe and accessible food for their respective families, most especially during the pandemic.

Impact on farmers' family members. Family members of farmers were also interviewed to represent secondary or indirect beneficiaries of OA information sharing. Family member-participants also valued well-being and environmental aspects. The wife and son of farmer-participant 3 were interviewed regarding the effects of information sharing and engaging in OA. His wife shared that after engaging in organic farming and attending several OA training, her husband changed positively in terms of having a better family relationship and gaining self-worth as he can decide on his own. The family member-participant 1 (wife of farmer-participant 3): *"It [Our family situation] is better [than before his OA engagement] now, because he has time for us, especially for our children. He is free to decide on matters regarding his farm. He has no boss because he is the boss. He is always with us, but he can still provide for the needs of the family. I also feel proud that students and other (future) farmers are visiting our farm to learn and experience farming."*

The family member-participant 2 (youngest son of farmer-participant 3) shared his personal feelings and preferences as he said: *"I like it now. I can always see my Papa. He can play with me now."* As a 4-year-old son, he needs his parents' attention and affection, especially his father that he rarely sees and remembers when his father was still working as a security guard. Based on their statements, positive effects on the quality of life were also apparent, specifically in terms of improving family time and relationships, boosting confidence and morale, and providing for family needs.

Children of farmer-participant 4 were also interviewed. Based on the observation during the interview, family member-participant 3 (grade 6 son; 12 years old) was shy at first, but when asked about his mother, OA and the effect of the OA training to her, he expressed positively and courageously his feelings: *"I am proud of my mother. I usually do garden activities in school, [similar to what] she is doing on our farm. I can confidently tell my classmates that organic agriculture can be fun and easy if we do what my mom is doing."* In addition, family member-participant 4 (grade 4 daughter; 10 years old) eagerly answered: *"I am happy that my mother is doing better now. She also has more time for us and can help us in school activities."*

Family time greatly matters for these children who had working parents. Engaging in OA seemed to contribute to having better family relationships, while proud children boosted the morale and confidence of their farmer parents. Therefore, it could be concluded that information sharing and engaging in OA seemed to positively affect the farmer-participants through the impact to their families.

Degree of impact on the effectiveness of information sources. Identification of the different information sources and the problems the receivers were facing showed that most farmer-participants were not receiving the needed printed materials. A pamphlet, brochure, or leaflet on the institution and the disseminated technology are often received. Farmer-participant 30 mentioned: *"We often received a pamphlet or brochure that the institution made for us. During the training, they [the institution conducting training] asked us what we learned. They usually [summarized the discussion and] printed it on paper and gave it to us. We already know what the content is. We want something new that will interest us in reading, like new information, knowledge, or technology. So, for me, the printed materials I am receiving are not that effective. However, I am still getting it for the sake of my neighbor farmers."*

Another farmer stated the need for materials more than a brochure. Farmer-participant 18 shared: *"We received a pamphlet or brochure, but we already know what the content is. If possible, I want a book or manual. I keep requesting that, but until now, there has been none. Books will be effective because everything is already there. You will not be lost because it will be your guide for everything. I need a refreshment."* Farmers keep requesting, but until March 2020, they have not received the printed materials. With the pandemic, further delay of the printed material is expected.

Regarding electronic sources, only 14 out of 30 farmer-participants were using electronic sources. Only four used primarily TV to get information about natural or organic crop production practices and weather updates. farmer-participant 19 affirmed: *"I frequently watch the news on TV about the weather updates. I need to know if there will be a typhoon coming to prepare and adjust farm activities. In the past, I could not harvest anything because I was not aware of an incoming typhoon. I mainly focused on the harvest schedule."* The main electronic information sources were radio (four farmer-participants), cell phone (three farmer-participants communicating about training schedules or orders), and internet (three farmer-participants watching YouTube on OA).

The face-to-face sources seemed to show positive results as all farmer-participants could rank at least two of the most effective means. Specifically, the most effective information sharing method is training for 15 farmer-participants, and family members for 11 farmer-participants (Table 3).

Table 3. The frequency distribution of the face-to-face information sources by rank of effectiveness for farmer-participants (n=30)

Information Source	Rank of Effectiveness			Total
	1st	2nd	3rd	
Training	15	4	4	23
Family member	11	2	4	17
Neighbor-farmers	2	9	5	16
Exhibition	-	2	1	3
Demonstration	-	4	1	5
Field visit	-	5	-	5
Farm shop	-	2	2	4
Others	2	2	1	5
Total	30	30	18	

Source: Survey data in 2020

Notes: Farmer-participants initially answered questions on their source of OA information and innovation. Then, they ranked their answers based on the effectiveness of each source or method. Some farmer-participants only gave two sources of information.

Available information sources seemed to be diverse in the study area (Fig. 3). To further understand the information sharing between different stakeholders, information sources were divided into four major categories: personal information sources, public information sources, private information sources, and mass media sources. The impact of information sharing can be divided into three types: Strong impact (refers to complete, effective and efficient transfer of information to the receiver), Moderate impact (refers to the transfer of information with certain assistance for the receiver to understand) and Less impact (refers to transfer of information with no assistance; often contains shallow information of what the intended receiver is looking for).

The personal information sources, including family members, neighbor farmers, and farmers' organizations or association members, were noted to have strong interaction and impact. Based on the previous transcripts, this result could be associated with farmers' attitudes and interests to share information with people who were close to them and knew them well. It was noteworthy that some farmer-participants in Los Baños had strong interaction and impact with the researchers and Extension Agents (EAs) of Institution 1 and the staff from Institution 3. This result was in line with Toma et al. (2018) research that stated access to technical information and knowledge transfer were among the key influences on adoption behavior and information sharing.

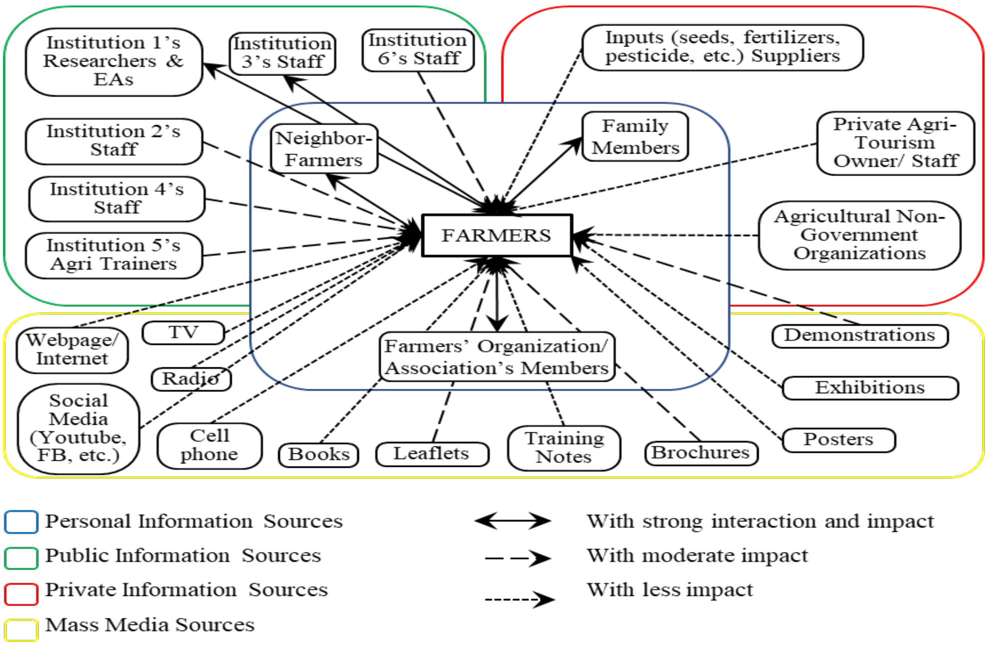

Fig. 3. Information sharing impact to actors by degree and interaction

Source: Survey Data in 2020

Notes: The impact was determined using the frequency percentage of each information source: Strong Interaction and Impact (67%-100%), Moderate (34%-66%), and Less Impact (33% and below). Institutions 1, 2, and 6 are all located in Los Baños, Laguna. Although there was no interview conducted in Institution 6 due to the unavailability of the focal person, this study included Institution 6 for this particular analysis because more than half of the farmer-participants mentioned the effectiveness of their staff.

The remaining public information sources and some mass media sources (e.g., leaflets, brochures, and demonstrations) had moderate impacts, while the remaining mass media sources and private sources had a low impact. Contrary to the expected positive impact of mass media (Ali *et al.* 2016 for Zambian farmers on TV usage), the farmer-participants could not utilize these sources. Notably, the effectiveness of TV, radio, and webpage/internet sources were only 23%, 20%, and 10%, respectively.

Farmer-participants used a cell phone to contact someone about training or availability of produce. This result is similar to the study conducted by Labonne and Chase (2009), which revealed that cell phones provide Filipino farmers more access to information, specifically on various transactions, including selling their goods. However, the cell phone had moderate to low impact since only 10% of the farmer-participants owned one. Moreover, most farmer-participants did not have internet access, and some did not even have electricity. These conditions can be considered reasons for the low usage of electronic sources under mass media. Therefore, improvement in the accessibility and availability of information and sources may increase the degree of impact.

The strong interaction and impact for the farmer-participants also had something to do with the future of their farms. Since farmer-participants usually seemed to want the best for their children, they tended to be interested in the technology that would benefit them and their respective families, especially their children who will inherit their farm in the future.

CONCLUSION

The importance of interpretation of the farmer's attitude and behavior towards OA adoption and how these attitude and behavior affected information sharing and technology adoption were clarified. Life History Approach, Trajectory Equifinality Approach, and Grounded Theory revealed needs, importance, sustainability, pressure from others, availability, accessibility, and easiness to adopt as factors affecting actors' attitudes toward learning, sharing, and adopting OA technologies. The factors affecting the attitude of the three main actors (e.g., farmers, institutions' staff, and farmers' families) in sharing information and adopting OA innovation were different but interconnected. Interaction and degree of impact of OA information sharing were also influenced by and associated with the attitudes and interests of the beneficiaries to accept, share, and adopt information they were receiving. The degree of the economic, well-being, and environmental impacts varied. However, the main actor groups seemed to value OA's well-being and environmental impacts more than economic impacts. Although personal information sources had the strongest interaction and impact, improvement in the accessibility and availability of other sources (e.g. mass media sources) might increase the degree of the impact.

Furthermore, farmer-participants tend to be interested in the technology that will benefit them and their respective families, especially their children who will inherit their farm. Thus, there is a need to plot the life paths of farmers who adopted and know their reasons of continuing or discontinuing OA using the Trajectory Equifinality Model (TEM). This study may formulate new ideas or ways to increase the number of organic farmers and persuade the youth to participate in agriculture.

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STATUS OF VIETNAM'S COFFEE PRODUCTION AND EXPORT IN THE PERIOD OF 2006 – 2015: STRATEGIES AND SOLUTIONS FOR DEVELOPMENT

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ABSTRACT

Vietnam has great potential and favorable conditions for coffee production and ranks as the second largest coffee producer in the world in the 2000 – 2008 period. From 2006 to 2015, the area cultivated, production quantity, and yield increased continuously, with an annual production quantity of 1.18 million tons. Export turnover reached 2.3 million USD per year, which accounts for 2.5% of the annual export turnover for Vietnam. Coffee became one of the most important agricultural products for export and has been exported to over 80 countries worldwide. The Vietnamese coffee sector developed rapidly over the past 30 years, creating jobs for local communities, contributing to hunger elimination, reducing poverty, and generating important foreign exchange earnings. This study sought to analyze Vietnam's coffee production and export, for the period of 2006 - 2015 and propose some solutions for the development of Vietnam's coffee sector. We used descriptive statistics to analyze data from Food and Agriculture Organization of the United Nations Statistics, World Bank Country Reports, and Vietnam Agricultural Census. We performed unstructured interview with farmers, enterprises, and state authorities and used SWOT matrix to identify strategies and solutions for the coffee sector. The results showed that Vietnam's coffee production and export has constantly increased during 2006 – 2013 period but showed signs of slowing down during the 2013 – 2015 period. Major weaknesses of the coffee sector were identified such as underdeveloped processing technologies and weak competitiveness. The study proposed specific solutions to address the shortcomings of the coffee sector, enhance the coffee production, improve its export quality standards and values, and to affirm a stable position in the world market.

Key words: agriculture, competitiveness, SWOT analysis, market.

INTRODUCTION

Coffee is one of the most important agricultural products in the world market and grown in more than 70 countries (Campbell and Ortíz 2011). The economy of several countries, such as Columbia, Brazil, and Central America, depend heavily on coffee production for foreign currency earnings (Sera et al. 2013). In Vietnam, coffee export value has usually occupied around 15% in total agricultural export value, and the share of coffee has always exceeded 10% of agricultural GDP in recent years (ICO 2019). Vietnam has great potential and favorable conditions for coffee production, and is the second largest overall coffee producer in the world after Brazil (Ho et al. 2018). Recently, coffee has expanded beyond the two largest regions of Vietnam – the central highland and southeastern region, to the northern mountain region, which includes Son La, Lai Chau, Ha Giang, and Tuyen Quang provinces. Coffee production has played an important role in economic re-structuring, environmental enhancement, hunger elimination and poverty reduction in the mountainous region (Guingato et al. 2008). In addition, coffee production has created job opportunities in the processing industry. Over the past years, coffee production and export has been prioritized in Vietnam's agricultural development program (Nguyen and Sarker 2018). This facilitated production capability, renovating technology, and strengthening of global competitiveness on coffee quality and price. However, coffee production and export still have a number of shortcomings and challenges to be addressed.

Over the last decade, there was an increasing number of literature regarding Vietnamese coffee issues (Best 2014; Ha and Shively 2008; Ho 2011; Luong and Tauer 2006; Roldán-Pérez et al. 2009). However, most of these studies often focused on micro- and macroeconomic analysis of the coffee sector, with a little focus on solution-oriented analysis. In 2014, the Ministry of Agriculture and Rural Development of Vietnam (MARD) issued a Decision 3471/QD-BNN-TT in 2014 to ratify a proposal to develop Vietnamese coffee sector towards modernism, synchronism, sustainability, strong competitive ability, and high quality added value. Nowadays, in the context of integrated global economy and "open door" policies, especially the participation in the Comprehensive and Progressive Agreement for Trans-Pacific Partnership (CPTPP) and ASEAN Free Trade Agreement (AFTA), Vietnamese coffee sector will have both great opportunities and challenges for development. In order to prepare itself for the integration process, the Vietnamese Prime Minister issued a Decision 1684/QD-TTg in September 30, 2015 to ratify the international integration strategy for the agricultural sector in 2030. Vietnam's Coffee and Cocoa Association (VICOFA) 2014 report also described the overall status of Vietnam and international coffee production, consumption and export during 2011 and 2013, as well as predicted some challenges for the period of 2014 to 2017 (Tam 2013).

Policy makers and coffee producers need more science- and evidence-based approaches for sound policies and solutions. However, contemporary studies and reports show a lack of comprehensive analysis on the status of Vietnam's coffee production and export, its limitations, causes and solutions for the future. Therefore, this study sought to provide an analysis of Vietnam's coffee production and export in the world market and propose solutions for enhancing production and export of Vietnam's coffee.

METHODOLOGY

This study used secondary data collected from both online and offline database from MARD, General Statistical Office of Vietnam (GSO), General Department of Vietnam Customs, and VICOFA from 2006 to 2015. For other countries, data from the International Coffee Organization (ICO), Food and Agriculture Organization (FAO), United Nation Comtrade: United Nations Commodity Trade Statistics Database, and United States Department of Agriculture (USDA) were used.

Unstructured interviews with coffee producers, businessmen and scientists enabled the study to generate a greater degree of trust for exploration of more in-depth information about complex issues arising in coffee production and export. Respondents were selected to represent major stakeholders involved in coffee production and export such as coffee growers, coffee enterprises and associations, government authorities, and scientists. Specifically, two groups of ten coffee producers, five provincial officers in the Central Highlands, four Chief Executive Officers of coffee companies and nine Vietnamese experts from VICOFA, Ministry of Agricultural and Rural Development and Vietnam National Economic University were interviewed. The interviews focused on the following issues: Coffee cultivation techniques; Lessons learnt from unstable coffee productivity and harvest in the period 2006-2015; Strategies for dealing with the areas of old coffee trees and plans for replantation; low prices of Vietnam's coffee export and strategies to improve added value of Vietnamese coffee. Based on the information collected through interviews, strengths, weaknesses, opportunities and threats of Vietnam's coffee production and exports were identified and SWOT analysis was used to determine strategies for the coffee sector. Descriptive statistics were used to analyze secondary data to obtain average, growth rate, and proportion so as to analyze changes in Vietnam's Arabica and Robusta coffee production and export through time in comparison with other countries.

RESULTS AND DISCUSSIONS

Status of Vietnam's coffee production in the period of 2006 – 2015. While there are several species of coffee, Robusta (*Coffea canephora*) and Arabica coffee (*Coffea arabica*) are the two main ones cultivated in Vietnam, with Robusta coffee accounting for 95% of total cultivated area. In 2015, in the central highland alone, the cultivated area reached 580,000 ha, accounting for nearly 90% total coffee area and 92% of total coffee output nationwide. Data on coffee production in Vietnam from 2006 to 2015 are shown in Table 1. Although the government recommended maintaining a stable coffee area of 600,000 ha, the increase in coffee price gave farmers the incentive to expand the area up to 645,200 ha by 2015, with an increase of 21,200 ha per year or 2.9% per year (Mai 2017). Extreme weather conditions have prevailed more during the recent years and have influenced negatively coffee production. Drought reduced productivity from 2006 to 2015, especially in 2007 and 2012, where coffee productivity declined by 7.1% and 1.3%, respectively. Drought and hail caused a total loss of about 5,000 ha in the central highland in 2012. In addition, intensive farming, land degradation and diseases (e.g. *Planococcus kraunhiae Kuwana*, *Ferrisia virgate*, *Cockerell aulacaspis sp.*, *Planococcus linacinus Cockerell*, *Coccus viridis Green*) reduced productivity (Thuy et al. 2011). According to the Department of Crop Production, Ministry of Agricultural and Rural Development, there were about 126,000 ha of old and sanile coffee trees of poor growth and productivity in 2012 (Tam 2013). However, during the 2006 - 2015 period, coffee production still increased by 4.3% per year and productivity increased by 1.8% per year. Specifically, the nationwide coffee production increased from 0.99 million tons with a productivity of 2.04 tons/ha in 2006 to nearly 1.28 million tons with a productivity of 2.35 tons/ha, in 2011. While Robusta accounts for 92.9% of the total coffee growing area, Arabica varieties are responsible for only a few percent. Robusta coffee accounts for about 97% of total coffee production nationwide. The production rate increased 16% compared to the previous year. This is also the highest rate of change in the whole period (FAOSTAT 2016). Although cultivated area slowly increased by less than 1%, the output still reached 1.4 million tons and the productivity was about 2.4 tons per ha in the 2014 and 2015 crop years. These made Vietnam the second largest coffee producer and exporter in the world next to Brazil, and the largest Robusta producer and exporter in the world. Such remarkable results were due to several competitive advantages and policies of Vietnam compared to other countries.

First, Vietnam has the advantage of being located in the tropical northern hemisphere with favorable geography, climate and soil conditions for several industrial crops. The Central Highlands and the southeastern regions have a distinguished advantage of soil types for several industrial crops. The soil of the two regions is divided into 11 main groups (IUSS-Working-Group 2014) of which grey soil (acrisols) and red soil (ferrasols) are the most important. This makes the Central Highlands and the southeastern regions special zones for several agricultural crops, such as coffee, rubber, pepper, cashew, hybrid corn, cotton, tea, vegetables, flowers, and fruit trees. For coffee varieties, Vietnam has two distinctive climate regions. The northern climate is favorable for Arabica, while the southern climate is favorable for Robusta (Schaumburg-Müller and Chuong 2010). Second, Vietnam has a young population which provides an abundant source of labor for economic development. Therefore, the competitive advantage in labor over other coffee-producing countries can help Vietnam reduce production cost and increase net profit. Third, since 2013, Vietnam government has made plans, as well as supporting policies for zoning, creating new coffee varieties, and improving productivity and quality (Ha and Shively 2008). Price subsidy policy has been implemented to support coffee growers when global coffee prices decline.

In addition, due to the increasing demands of the international market and the global development trend, agricultural producers in general and coffee growers in particular have to pay more attention to quality standards and procedures guided by professional bodies. In some provincies in the central highland, number of certified agricultural farms and products have increased in recent years. For example, 34 busineses and nearly 27,000 households in Dak Lak province were certified for sustainable coffee production by 2017, with a total area of 45,000 ha, accounting for 22% of the provincial coffee area. The most commont certification programs are: The Label and Program for Sustainable Farming (UTZ), Common Code for the Coffee Community (4C certification), Rainforest Alliance (RFA), and Fairtrade Labelling Organizations International (FLO International).

Beside the aforementioned achievement and advantages, Vietnamese coffee prodction also had some difficulties. Productivity was already very high in 2015 and it would hardly be higher unless new coffee varieties and production technologies be adopted. Furthermore, Vietnam still had more than 30% land planted to old and senile coffee trees that had to be revived to avoid lowered productivity and quality. Small production scale in the form of household level often leads to high investment costs and uneven output quality in the market. The small production scale is also a barrier to farmers to access advanced sciences and technologies, markets as well as other services such as credit and loans. Eventually, branding and obtaining certificates for coffee products are more difficult.

Thus, to promote sustainable coffee production, the local government tried to control coffee expansion, encouraged farmers to invest in new and drought resistant varieties and to apply Good Agricultural Practice (GAP) procedures. Other crops of high economic value, such as cocoa, could be considered since coffee may not provide any more good output. The development of a coffee processing industry for domestic consumption and export has been prioritized by MARD, specifically increasing the total installed capacity for the coffee processing industry to 125,000 tons per year and stabilizing the coffee area at 600,000 ha by 2020, with 80% of the area applying sustainable coffee production, thus allowing an expected productivity of 2.7 tons per ha and total output of 1.6 million tons per year.

Table 1. Vietnam's coffee production in the period of 2006 – 2015[a].

Year	Area cultivated		Area harvested		Ratio of Area cultivated to Area harvested (%)	Production quantity (bean)		Yield	
	1000 ha	Growth rate (%)	1000 ha	Growth rate (%)		1000 tons	Growth rate (%)	Tons /ha	Growth rate (%)
2006	497.0	-	483.2	-	97.2	985.3	-	2.0	-
2007	509.3	2.5	488.9	1.2	96.0	915.7	-7.1	1.9	-8.1
2008	530.9	4.2	500.2	2.3	94.2	1,055.8	15.3	2.1	12.7
2009	538.5	1.4	507.2	1.4	94.2	1,057.5	0.2	2.1	-1.2
2010	554.8	3.0	511.9	0.9	92.3	1,100.5	4.1	2.1	3.1
2011	586.2	5.7	543.9	6.3	92.8	1,276.6	16.0	2.3	9.2
2012	623.0	6.3	572.6	5.3	91.9	1,260.4	-1.3	2.2	-6.2
2013	637.0	2.2	584.7	2.1	91.8	1,289.8	2.3	2.2	0.2
2014	641.2	0.7	590.2	0.9	92.0	1,408.4	9.2	2.4	8.2
2015	645.2	0.6	602.1	2.0	93.3	1,445.0	2.6	2.4	0.6
Average	**576.3**	**2.9**	**538.5**	**2.5**	**93.4**	**1,179.5**	**4.3**	**2.2**	**1.8**

Source: GSO (2007 – 2016)

[a] Data in this table represents for both Arabica and Robusta coffee varieties with Robusta account for major portion.

Status of Vietnam's coffee export in the period of 2006 – 2015. The average growth of Vietnam's coffee export was 3.1% per year from 2006 to 2015 and the export rate was still fairly high at 8.7% per year. Vietnam exported 980.9 thousand tons of coffee and its value reached 1,217.2 million USD in 2006. By 2012, exports reached 1,735.5 thousand tons, with an increase of 37.7% compared to 2011. Although the average coffee export price per ton in 2012 was 75 USD lower than 2011, export turnover still reached a total of 3,672.8 million USD, three times greater than in 2006 (Table 2).

Table 2. Vietnam's coffee export in the period of 2006 – 2015

Year	Volume of export		Export turnover		Average export price
	1000 tons	Growth rate (%)	Million USD	Growth rate (%)	USD/ton
2006	980.9	-	1,217.2	-	1,241
2007	1,232.1	25.6	1,916.7	57.5	1,556
2008	1,060.9	-13.9	2,113.8	10.3	1,992
2009	1,183.5	11.6	1,730.6	-18.1	1,462
2010	1,218.0	2.9	1,851.4	7.0	1,520
2011	1,260.0	3.4	2,760.2	49.1	2,191
2012	1,735.5	37.7	3,672.8	33.1	2,116
2013	1,301.2	-25	2,717.3	-26	2,088
2014	1,691.1	30	2,557.4	-5.9	1,512
2015	1,293.5	-23.5	2,589.3	1.2	2,002
Average	**1,295.7**	**3.1**	**2,312.7**	**8.7**	**1,785**

Source: GSO (2007 – 2016)

There were three main reasons for high export in 2012. First, suitable commercial structure and temporary storage were favorable for coffee export. Moreover, export price was only reduced by 30 USD/ton, compared to prices in London and New York exchanges. Second, Robusta coffee is more advantageous in terms of processing and cheaper than Arabica; therefore, Robusta is preferred by importers and consumers in a stringent economy around the world as a source of raw material for coffee processing industries. Thirdly, in terms of coffee cultivation technology, various Vietnamese coffee farmers have been applying advanced agricultural production technology to obtain national and international certifications, such as 4C certification (Common Code for the Coffee Community); VietGAP (Vietnamese Good Agricultural Practices); UTZ (UTZ Certified); and RFA (Rainforest Alliance). By the end of 2017, more than 200,000 ha, accounting for more than 30% of the total coffee growing area of Vietnam, were certified by sustainable development initiatives. According to the Department of Crop Production (DCP) of MARD only 10% of the total volume of coffee had such certifications in 2011; however, this had increased to approximately 50% of the total volume by 2014 (ICO 2019). The increased amount of certified coffee also helps Vietnamese coffee sector gains higher export turnover.

In 2013, both product and export turnover reduced by 25% compared to 2012. Export price was by 98.7% compared to the previous year. The reason for a sudden reduction in coffee export was due to the economic crisis in EU and the United States, the two largest coffee export markets of Vietnam, accounting for more than 50% of the total export turnover. Exports to the United States reduced by up to 34.3%, compared to 2012. In Asia, Japan and China are two major coffee export market targets of Vietnam, accounting for 9.7% total export turnover; however, exports to these two markets lowered, compared to 2012, with export to China lower by 26.2% and exports to Japan lower by 2.1%.

In 2014, export volume increased by 30% from 2013; however, export turnover lowered by 5.9%. In 2015, fluctuation in the world economy influenced coffee export in Vietnam. At the start of

2015, coffee price in Vietnam was still low; meanwhile, Real Brazil started to fall making coffee in Brazil competitive and affected coffee export in Vietnam. Export volume of coffee in Vietnam reduced by 23.4% compared to 2014, especially for fresh coffee. Even though coffee price had gone up by the end of 2015, the average export price for the year was only 2000 USD per ton, which increased by 490 USD per ton compared to 2014, and export turnover was 2,589.3 million USD per year, which also increased by 1.2%.

Source: GSO (2007 – 2016)

Fig. 1. Vietnam's annual coffee export turnover and volume from 2006 to 2015

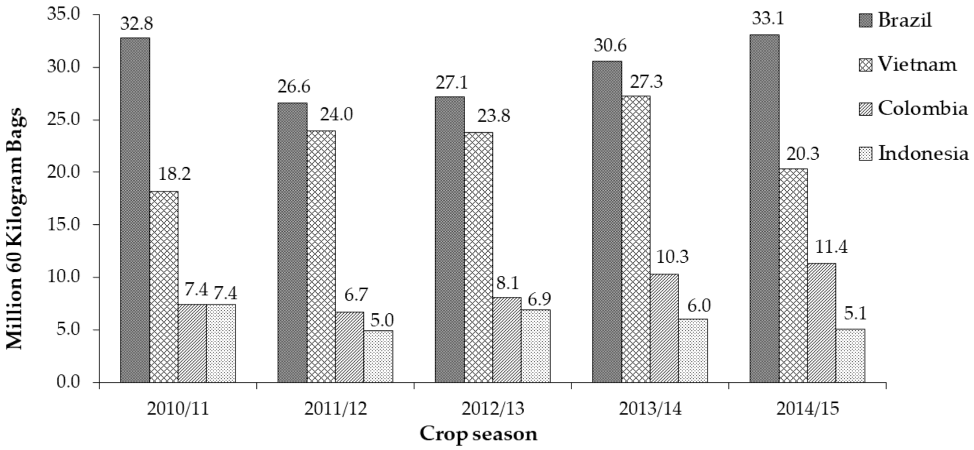
However, considering the whole period of 2006 - 2015, export volume still increased at a rate of 34,700 tons per year, with expo rt turnover increasing to 152.46 million USD per year, accounting for 2.5% of total export turnover of Vietnam products. Fig. 1 showed that 2009, 2013 and 2015 were three difficult years for the coffee export industry in Vietnam. The financial crisis in the world market had a negative impact on coffee export turnover and volume. In 2009, export price was only 1,462 USD per ton, which was lower by 27% compared to 2008. For 2013 and 2015, export turnover has constantly increased. In 2011, there was an average export price peak of 2,191 USD per ton, increasing by 44.1% as compared to 2010. Due to extreme weather and the increase old and low-yield coffee areas in Vietnam and other countries, productivity and output were reduced, allowing an increase in overall export price.

Vietnam has been exporting coffee for 30 years, and export turnover has consistently increased. However, Vietnam exports mainly pre-processed coffee beans, with Robusta being the major variety, resulting in low export turnover (Roldán-Pérez et al. 2009). On the other hand, our analysis showed that Vietnam's export price for coffee was only 54.3% the world average coffee price of 2011 (world average coffee price in 2011 was 4.037 USD per ton) (FAOSTAT, 2016). The price of Robusta coffee in the world market for the last 10 years was 55% of the price of Arabica coffee. The highest export price for Vietnam's coffee was in 2011; however, it is still very low compared to the world market. In 2011, export price of Vietnam's coffee was only 49.1% that of Brazil (FAOSTAT 2016).

There was a decline in the export price of Vietnam's coffee from 2012 to 2014. Vietnam's coffee export has two disadvantages: first, Vietnam's Robusta coffee price is always 30 to 120 USD per ton lower in the London Coffee Exchange (Mai 2017). Second, Vietnam exports mainly pre-processed coffee beans, which are more susceptible to price than processed coffee products. Therefore, it is important for Vietnam to renovate the plant and also introduce policies to restructure the plant, increasing the area planted to coffee Arabica where the land and climatic conditions are suitable (Nhien 2016). Coffee enterprises should focus on improving product quality, processing capacity and competitiveness of Vietnam's coffee in the world market.

Position and market share of Vietnam's coffee in the world market. Coffee production and export plays an important role in the national economy, with coffee becoming the main source of income for about 600,000 farming households, providing a livelihood for about than 2.6 million people (Nguyen and Sarker 2018). More than 80% of coffee area is managed by farmers nationwide. This made an important contribution to the political and social stability in Vietnam Central Highlands and mountainous provinces (Nguyen and Sarker 2018). Coffee export does not only bring a significant source of income, but also contributes in improving Vietnam's position in the world market.

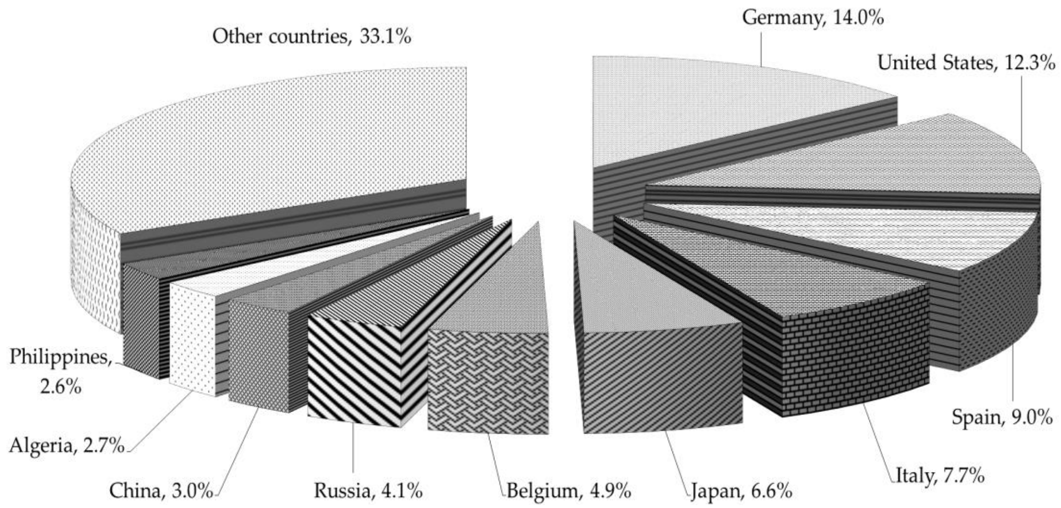
In the world scale, from 2010 to 2015 Vietnam was always in the top four of coffee exporting countries in the world after Brazil (Fig. 2). In 2000, Vietnam exported 0.734 million tons and took over the second position. In 2015, Vietnam exported 1.3 million tons, which was nearly 0.43 million tons lower than 2014, but Vietnam still held second position after Brazil. Moreover, Vietnam is the largest producer and supplier of Robusta in the world. In 2014/2015, Vietnam exported 20.3 million bags, comprising 20.1% the total of world coffee export. Vietnam mainly exports pre-processed coffee bean products; therefore, export turnover was modest, comprising 13% of the world's total export turnover from 2006 to 2015.



Source: USDA, Coffee: World Markets and Trade (2015)

Fig. 2. Coffee export volume of the top four countries in the world.

Vietnam's coffee market started implementing regulations, participating and coordinating with organizations, enterprises and farmers. Recently, the devaluation of bumper crop and the massive sales in coffee upon price decline have been controlled. At the same time, the competition between domestic and foreign companies has initially created an advantageous market for coffee growers. Vietnam's coffee is exported to over 80 countries in the world, with the main importers being EU members (39% of export turnover), the United States (11.1%), and Asia (21.1%) in 2013. Among these countries, Germany and the United States were the two largest importers of Vietnam's coffee. In Asia, Japan is the largest importer of Vietnam's coffee. In 2015, the EU accounted for 44.8%, the United States accounted for 12.3%, and Asia accounting for 21.7% of the total export turnover in Vietnam's coffee. Export turnover of Vietnam's coffee to the ten largest importers already account for 66.9% of total turnover (Fig. 3).



Source: GSO (2016)

Fig. 3. Vietnam's coffee export markets by country of destination in 2015

Currently, processed coffee in Vietnam is still very limited. Vietnam ranks second in the world in terms of exporting pre-processed coffee bean, while its export of processed coffee has not placed into the top 20 coffee-exporting counties in the world. Vietnam has 200 coffee processing enterprises and 140 export enterprises of which there are only four major instant coffee brands and 20 roasted-ground coffee brands for export. Meanwhile Brazil has 20 brands of instant coffee and 3,000 brands of roasted coffee (Nhien 2016). Thus, to increase export, Vietnam needs to improve its coffee processing industry through a good production development plan. Even though Vietnam is one of the leading countries for coffee export, each year it still imports a small amount of coffee beans, roasted coffee, and instant coffee from other countries, such as Lao PDR, Indonesia, and China. From 2006 to 2015, analyses showed Vietnam imported over 7,000 tons per year, with turnover of nearly 24 million USD per year. Compared with export from the same period, imported coffee only accounted for a small portion, at appropriately 0.54% of output or 1.03% of the export turnover. Coffee has been the product of export surplus in Vietnam, with total turnover of more than 23 billion USD from 2006 to 2015.

Recent World Bank data shows that the Vietnamese domestic market can potentially consume about 10% from the total coffee yield. However, the domestic consumption of coffee Vietnam is currently from 6-8%. Meanwhile, there is a big difference in coffee consumption between Vietnam and member countries in the International Coffee Organization, with consumption of International Coffee Organization members reaching 25.16% (Giovannucci et al. 2004; Gonzalez-Perez and Gutierrez-Viana 2012).

SWOT analysis of Vietnam's coffee production and exports

Internal factors

Strengths. First, compare to other countries, Vietnam has many natural and social condition advantages. Especially, the favorable climate and soil conditions in the Central Highland of Vietnam make it the unique region to produce coffee of high quality and productivity. Vietnam also has cheaper labor cost compare to other countries, that make Vietnam coffee price are more competitive. Second, the coffee production of Vietnam is concentrated mainly in the Central Highland region and located near export ports, which is convenient for international trade. Third, the Vietnamese policy system for coffee production and export is open and transparent, creating an equal environment for all agents participating in coffee production, processing and consumption (Nhien 2016). Fourth, Vietnam is the world's second largest coffee producer and exporter, after Brazil. For Robusta coffee, Vietnam is a leading producer and exporter of the world, accounting for half of global Robusta coffee production (Van Long et al. 2015). Moreover, Vietnam is located closer to the northern hemisphere while other coffee producers (Brazil, Colombia, and Indonesia) are all closer to the southern hemisphere. Therefore, Vietnam has a different harvest period, giving the country an edge of being active for six months every year.

Weaknesses. First, coffee areas have increased quickly in recent years, of which large area was spontaneously expanded. There is also a large area of old and sanile coffee trees that need to be replanted. Although Vietnam has already zoned some areas for high quality coffee production for export, the overall production scale is small, with more than 80% of coffee area belonging to households. Therefore, the production which follows national and international quality certificates such as VietGAP standards, 4C certification, UTZ, and RA is still limited. Many small farms and households lack capital to invest in coffee production and processing for export. Second, the organization of production groups and cooperatives are weak and there is lack of good connection between producers and processing companies. Processing companies often buy coffee bean through local collectors. Therefore, the quality control is not secured. Third, quality of exported coffee bean is uneven due to poor cultivation and harvesting techniques. Specifically, facilities for preprocessing and preservation of coffee are backward; In addition, the State's support in terms of capital, processing technology, and training ... is not really satisfactory. Rural infrastructure development is fast but not commensurate with the potential. Management and inspection systems and monitoring of product quality standards are weak and outdated. Finally, Vietnam’s coffee has not been widely recognized in the world market because Vietnam exports mainly Robusta coffee of which more than 95% are pre-processed coffee beans. Thus, Vietnam’s coffee has very weak competitive prices in the world market. Coffee enterprises also lack experience and strategies to participate in international trade and commerce.

External factors

Opportunities. Firstly, international integration, especially in the economic sector, has become a popular trend around the world. Vietnam has signed a number of bilateral and multilateral trade agreements with other countries and associations and become a member of ICO since 1991. Second, the world’s coffee consumption is constantly increasing each year, especially, US and EU markets. Besides, coffee consumption in Asia is also on a strong upward trend, in which Japan and China markets account for nearly 10% of Vietnam's total coffee export turnover. Meanwhile, the domestic consumption market is still full of potential for Vietnamese coffee sector. Third, the Industrial Revolution 4.0 has had a profound influence on the world economy and brought great opportunities to all countries.

Threats. First, climate change and pests across the globe is becoming more complicated and unpredictable, seriously affecting agricultural production in general and coffee in particular. The Central Highlands is one of the areas predicted to be most strongly affected by climate change, specifically on the biological cycle of coffee trees, reducing productivity, and possibly causing other natural disasters such as drought, flood, forest fire ...Second, production input costs such as fertilizer, pesticides are increasing rapidly and adding on coffee prices. The technical barriers in foreign trade activities are more and more stringent, hence the requirements on food safety and quality standards are also higher. There is an intense competition among multinational corporations and leading coffee exporters in the world. Third, Vietnam's agricultural products in general, especially coffee, are heavily influenced by international prices. While the price of roasted and ground coffee still increased significantly, the price of pre-processed coffee beans plummeted. Because of uneven quality, Vietnam’s coffee is often paid lower than coffee of the same kind in London Market (Roldán-Pérez et al. 2009).

Based on combination of pairs of strengths, weaknesses, opportunities, and threats, we developed the following SWOT matrix to propose the most reasonable and effective strategies for Vietnam's coffee production and exports (Table 3).

Table 3. Strategies for boosting Vietnam's coffee production and export.

SWOT	Opportunities	Threats
Strengths	- Coffee growers, coffee enterprises and VICOFA should maximize inherent potentials and advantages of Vietnam's natural and social conditions. - Take advantage of international integration and the Government's supportive policies to accelerate the development of the coffee industry according to international standards and requirements to increase the value, and strengthen the international competitiveness ability. - Consolidate and expand the international market, take good advantages of the trade agreements to boost exports, and efficiently capture the domestic market.	- Scrutinize policies and mechanisms to support coffee growers to scale-up production. - Coffee enterprises should continue to invest in deep processing of roasted and instant coffee in order to improve quality and export value. - Vietnam needs to take good advantages of being a top world coffee producing country, especially Robusta, to regulate a reasonable supply that is beneficial to the selling price. - Establish a price stabilization fund and regular coffee storage mechanism.
Weaknesses	- Promote foreign investment in capital, science and technology for deep processing for export. - Improve the quality of human resources through training and networking within and outside the countries; - Improve coffee production and processing capacity. - Promote exchanges of goods with countries in the region and around the world. - Promote Vietnam's coffee brand into a global product.	- Strictly follow provincial and national agricultural planning, renovating the old coffee area to improve productivity and quality. - Strictly comply with national and international technical processes and quality standards in coffee production and processing, while strengthening the management and inspection system to improve coffee quality and export value. - Promote connection to global value chains to ensure stable coffee consumption and to minimize harsh competition from international markets.

Development solutions for Vietnamese coffee sector. Based on the the status of Vietnam's coffee production and export in the period of 2006-2015, as well as the strategies indentified in the SWOT matrix above, we have set out some specific solutions for coffee sector as follows.

Provinces with large coffee area, such as Dak Lak, Dak Nong, Lam Dong and Gia Lai need to re-evaluate their ecological conditions through a suitable coffee development plan for each province until 2030 and a vision for 2040 based on prevailing environmental conditions. Areas having favorable ecological conditions should be considered for intensive farming, such as grafting, to improve existing varieties or re-planting new coffee varieties. On the other hand, households and local authorities should also consider eliminating old and senile coffee trees. Areas that are not suitable for coffee production should be used and developed according to local land use planning. To ensure the successful development of Arabica coffee in the Northern mountain regions, it is necessary to carefully consider variety, technique, and climate conditions. In addition to supporting coffee production, it is important that the government should also invest and improve infrastructure in coffee-producing regions (Gonzalez-Perez and Gutierrez-Viana 2012).

Coffee quality and production can be improved by applying advanced technologies and increasing farmer awareness on quality and production standards. The State needs to support research in creating new varieties while the coffee sector focuses on transferring advanced coffee production technologies into 4C certification, UTZ Certified, RFA, FLO and GAP Standards (Tran 2014). Agricultural extension needs to be strengthened by transferring advanced technologies, and providing information and training for workers on production and processing, particularly on harvest and preservation. It is important to impart vocational training on farmers, to ensure accessibility of new technology, equipment, and machines for production and consumption (Tam 2013). Agriculture 4.0 should be applied into coffee production from farm to processing, marketing and consumption (Sott et al. 2020). Technologies should be carefully selected based on physical and socio-economic capacity of each households, farms and localities.

Coffee companies need to improve processing, to increase export value through building new processing infrastructures for coffee bean export; install comprehensive, modern and highly-automated production lines; establish coffee processing enterprises toward multi-owners and diversifying products, especially giving priority to building instant coffee processing enterprises with modern technologies; and connect coffee production with processing and consumption (Arnot et al. 2006).

The state needs to improve land use policies, remit taxes, support credits and technologies in accordance with WTO regulations, to provide favorable conditions for coffee growers and enterprises. Each locality needs to have a suitable support policy to speed up coffee replantation or conversion to other crops, encouraging the development of certified coffee productions. It is important to implement supporting policies on post-harvest loss, to encourage enterprise and households to re-invest in processing and preservation facilities (D'haeze et al. 2005). The state likewise, needs to establish strong management, inspection and supervision mechanisms for coffee processing and consumption, in such a way that it strictly follows technical and quality standards. The state also needs to create a clear legal corridor to attract resources from all economic sectors and non-governmental organizations interested in investing in coffee production (Luong and Tauer 2006).

The state needs to strengthen investment cooperation and association, such as strengthening international cooperation in research, production, preservation, processing and consumption of coffee. This would encourage domestic and foreign investors to cooperate in building and upgrading coffee processing enterprises with modern technologies, and diversifying and ensuring high quality safety products. On the other hand, the local government needs to provide households and farms with materials and advanced techniques, and to encourage the establishment of coffee growth, processing and consumption cooperatives. The local government should also extend cooperation among households, farms and enterprises on coffee production, processing, consumption and export (Gonzalez-Perez and Gutierrez-Viana 2012). In particular, exporters need to work closely with coffee growers to establish stable material areas for environmentally friendly and certified coffee.

Vietnamese coffee sector needs to remain a close association in the value chain, from production to consumption. This is to ensure that quality is controlled at every stage of production and a fair benefit for all actors. The linkages among farmers, scientists, entrepreneurs, and state authorities should be promoted and maintained to ensure a stable output for coffee growers and secured input for processing and exporting firms. At present, it is necessary to restructure subject groups in the value chain by establishing key companies with strong financial ability, research, processing and distribution to compete with other foreign coffee brands. Based on coffee production zoning, it is important to establish processing zones, logistic zones for export, especially in Vietnam Central Highlands. The state, coffee associations and companies need to strengthen their international cooperation in building supply chains for coffee companies and helping Vietnam's coffee companies to actively participate in the global distribution system, so as to stabilize price and step-by-step increase export price (Nguyen and Sarker 2018).

Finally, it is necessary to continue re-structuring the coffee sector (coffee varieties, types of product and areas), its production and business to improve its capability and effectiveness. Coffee companies and enterprises need to play an active role in forecasting the market, fostering trade and logistics through trade fairs, and broadcasting trademarks. Moreover, it is necessary to raise awareness and encourage companies to actively build their trademarks, criteria, and quality standards suitable with ICO and importing countries. The coffee terminal market and trading floors should be well-operated, forming effective coffee transaction markets in Vietnam with suitable international practices (Ferris et al. 2014).

CONCLUSION

Vietnam has both great potential and favorable conditions for coffee production. Coffee continues to hold an important position in Vietnam's economy, and economy many farming household in the mountainous areas, especially in the Central Highlands. From the analysis, we pointed out four competitive advantageous strengths of Vietnamese coffee sector. However, coffee production and export in Vietnam over the past years has not been up to par given its potential and strengths. The study also summarized the main weaknesses, such as a weak production system, unstable development, lack of supportive policies and infrastructure, underdeveloped processing, lack of effective production organizations, and low export price compared to the world market. However, Vietnamese coffee sector has a high potential local market, as well as opportunities to upgrade its processing technologies but there are difficulties such as small production scale, unpredictable climate change impact, ambiguous credit policies for replantation, weak competitiveness of enterprises, rising production input costs, and underdeveloped processing technologies. Therefore, the policies and solutions set out for Vietnam's coffee industry in the coming years are: strictly following agricultural planning and zoning for coffee; taking advantages of agriculture 4.0 to improve coffee quality and productivity; training and development of human resources; improving processing capabilities; building suitable mechanism, management and supporting policies; strengthening investments and joint-venture cooperation; promote the linkages among farmers, scientists, entrepreneurs, and state authorities to ensure stable output for coffee growers and secured input for processing and exporting firms; speeding up production and consumption in the value chain; enhancing competitiveness, fostering trademark and market development. These are crucial solutions for Vietnam's coffee production and exports that help to strengthen its position in the world market.

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SPATIAL AND SEASONAL VARIATIONS OF WATER QUALITY ASSESSMENT IN INLE LAKE, MYANMAR

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ABSTRACT

Inle Lake is the second largest freshwater lake located in southern Shan State, Myanmar. Assuming that the lake water quality is becoming degraded because of human activities, including agriculture and tourism, the water samples were monthly collected from eight locations inside the lake for 10 months, and its physiochemical parameters were examined to establish appropriate sampling methods for monitoring lake water quality by observing the spatial and temporal variations. The results showed that there were two clusters according to cluster analysis (CA); cluster 1 included St. 1 and St. 2, and cluster 2 included the rest of the stations (St. 3 to St. 8). The recorded values changed significantly according to the seasons and were generally higher during the agricultural season (March-October) than the tourism season (November-March), suggesting that the agricultural practices, such as fertilization, influenced the temporal variations. The proper sampling locations should probably be St. 1 and/or St. 2 in cluster 1and St. 6 in cluster 2 and that the changes in water quality should be observed twice a year: in April during the agricultural season and in December during the tourism season for monitoring in the future.

Key words: floating garden, human activities, tourism, sedimentation, water pollution

INTRODUCTION

Wetlands provide ecosystem services that benefit both human and non-human living beings, which include a huge range of biodiversification, habitats for plants and animals, water resources, fishery, agricultural activities and tourism opportunities (Dixon and Wood 2003; Jenkins et al. 2010; Zhong et al. 2019). Those benefits depend on the freshwater quality (Filik Iscen et al. 2008), and unfortunately, the degradation of the quality of freshwater in the lake ecosystem have been influenced by intensive human activities such as agricultural and industrial ones and sewage contaminations, as well as the increase of recreation and tourism activities (Barakat et al. 2016; Hemond 1988; Rahmani et al. 2013; Zhong et al. 2019).

Inle Lake is the second largest freshwater lake and located in the Nyaung Shwe Township in Myanmar, and it has been providing numerous ecosystem services by its environmental, touristic, economic, and agricultural values. In addition, it is also the habitat of diverse species of amphibians and fishes, flora and fauna (Lwin and Sharma 2012). The lake also provides fresh water by regulating the water flow and by supporting natural water filtration (Karki et al. 2018). Previous studies reported that the water quality of Inle Lake was threatened by especially sedimentation and anthropogenic activities such as agricultural practices in and around the lake and tourism industry (Akaishi et al. 2006; Lwin et al. 2012).

One of the primary sources of sedimentation of the lake is deforestation in the surrounding mountains and the surface erosion from the watershed areas (Htwe et al. 2015a; Sidle et al. 2007; Thin et al. 2020). Moreover, the expansion of the agriculture industry in and around the lake had a major impact on the water quality of the lake. The local inhabitants have practiced the traditional method of floating agriculture, which is one of the main forms of livelihood, including the cultivation of tomato and other vegetables (Myint and Maung 1996; Than 2007). The residents around the lake also practice on-land cultivation, which consists of crops on the flat areas between the lake and the mountains, comprising of paddy fields and upland fields of other major crops such as sugarcane, potato, groundnut and others (Htwe et al. 2015b). The associated adverse impacts consist of water pollution from the use of fertilizers and pesticides on floating gardens inside the lake and on-land agricultural industry around the watershed areas of the lake (Butkus and Myint 2001; Lwin et al. 2012).

Furthermore, the government officially declared 1996 as the year of tourism, which was also called the “Visit Myanmar Year”, to boost the tourism sector. Since then, the number of visitor arrivals in Myanmar has increased gradually and has contributed to the improvement of the local economy (Akaishi et al. 2006; Ingelmo 2013). According to the Ministry of Hotel and Tourism, the total number of tourists’ arrivals in Myanmar increased from 416,344 in 2000 to 3,551,428 in 2017 (Myanmar Tourism Statistics, MoHT, 2017). Additionally, the total number of tourists’ arrivals in Inle Lake has also risen from 131,102 in 2014 to 249,989 in 2017. Due to the blooming of tourism, the number of hotels in and around the lake and in the township area gradually increased from 36 in 2000 to 103 in 2017.

The urbanization associated with increasing tourism and industrialization in the area was one of the main factors for lake water quality change (Akaishi et al. 2006; Lwin and Sharma 2012; Su and Jassby 2000). Akaishi et al. (2006) indicated that the physiochemical water quality degraded, which was associated with agricultural activities in and around the lake including the floating cultivation, which were supposed to release pollutants such as K^+ and NO_3^- into the lake. It is considered that tourism growth has negative effects on the water quality of the lake. Moreover, other related factors may include sewage disposal, petroleum products from motorboats and waste products from households and hotels, resulting in water pollution and eutrophication (Sidle et al. 2007; Thin et al. 2020).

Thus, monitoring is urgently needed to assess the changes in water quality for sustainable water management. However, to monitor the lake water quality for a long term implies in high labor costs and time-consuming work. Hence, the present study sought to clarify the most appropriate locations and timing for sampling in order to make efficient monitoring of water quality in the lake by studying the changes in water quality of Inle Lake according to the spatial and temporal variations around the lake.

MATERIALS AND METHODS

Site description. Inle Lake is located in the Nyaung Shwe Township, Taunggyi District, Southern Shan State in the Republic of the Union of Myanmar. The Inle Lake is located between 20° 18' and 20° 53' N latitudes and between 96° 50' and 96° 57' E longitude, at an average elevation of 890m, and the drainage area of the lake is 3683 km^2 (Htwe et al. 2015b; Su and Jassby 2000). The lake sits in a tropical monsoon climatic region with an annual rainfall of 1217 mm in 2017 (Fig. 1). Inle Lake has three seasons: dry (March-June), rainy (July-October) and winter (November-February) (Re et al. 2018). The agricultural season occurs during both dry and rainy seasons, from March to October. The tourism period occurs during the winter, from November to March. The tourism period and the non-agriculture season happen at the same times. There are 29 inflows: 17 streams from the East, 11 streams from the West and 1 stream from the North. The only outlet, Nan Pilu stream, is in the south and the lake water flow mainly from North to South.

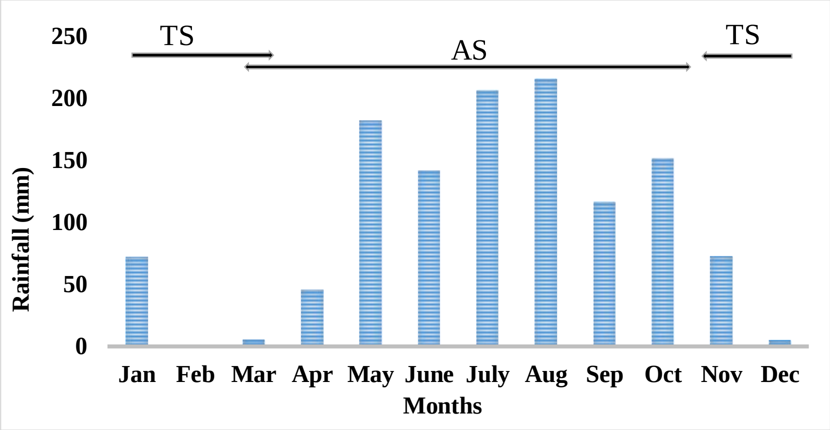

Fig. 1. Monthly rainfall in Nyaung Shwe Township (2017)
AS: Agriculture season, TS: Tourism season

Water sampling. The water samples were monthly monitored at eight locations around the lake (Fig. 2), during the period from August 2017 to May 2018. The water quality samplings were conducted at two inflow streams (St. 2 and 4), one near populated areas (St. 1), one at the center of the lake (St. 3), one near the floating garden areas (St. 5), one at open water area of the lake (St. 6), one near the weaving village (St. 7) and one near the outlet (St. 8). The names and the characteristics of the eight stations are shown in Table 1. A previous study was carried out in 2004 to observe the lake water quality by measuring the physical and chemical parameters in Inle Lake (Akaishi et al. 2006). There were 5 same sampling locations with the present study and those stations are St. 1, St. 2, St. 3, St. 4 and St. 7. Eight water samples were collected from the lake surface with plastic bottles and sent to the Department of Agricultural Research (DAR) in Yezin, Myanmar.

Table 1. Names and the characteristics of the sampling stations in Inle Lake

Station	Name	Characteristics of the location	Geographic coordinate
St.1	Tourism hub	Very populated area (near township area)	20°39'33.18"N 96°55'29.16"E
St.2	Inlet 1	Flows through the tourism hub area	20°38'15.47"N 96°55'17.35"E
St.3	Center	The center of the open water area of the lake	20°34'15.78"N 96°55'04.20"E
St.4	Inlet 2	The inflow is located near the banks of the lake	20°32'15.48"N 96°53'57.61"E
St.5	Floating garden	Inside the floating island (Tomato cultivation area)	20°29'55.70"N 96°55'03.58"E
St.6	Open water	Open water area of the lake	20°29'53.72"N 96°54'59.71"E
St.7	Weaving village	Near the dyeing and textile production area	20°26'43.18"N 96°53'45.30"E
St.8	Outlet	The outflow section of the lake	20°26'15.20"N 96°54'06.62"E

The pH was measured by a pH meter (F-51, HORIBA Ltd.) and the electrical conductivity (EC) by a Conductivity meter (DS-51, HORIBA Ltd.). Major cation concentrations, such as calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) and potassium (K^{+}), and other elements, such as iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn), were analyzed using an atomic absorption spectrophotometer (NovAA 400, Analytik jena Ltd.). The concentrations of Fe, Mn, Cu and Zn were low, and these data were not shown. Carbonate ($CO_3{}^{2-}$), bicarbonate (HCO_3^-) and chloride (Cl^-) were analyzed by the titrimetric method using a titrator. SO_4^{2-} was analyzed by the turbidimetric method with a UV-VIS spectrophotometer (Janway 6305, Keison products Ltd.). Total suspended solids (TSS) and total dissolved solids (TDS) were determined using a water bath and oven. Nitrate (NO_3^-) was analyzed by a devarda's alloy with Kjeldahl distillation unit. Hardness was calculated as the sum of Ca^{2+} and Mg^{2+} concentrations.

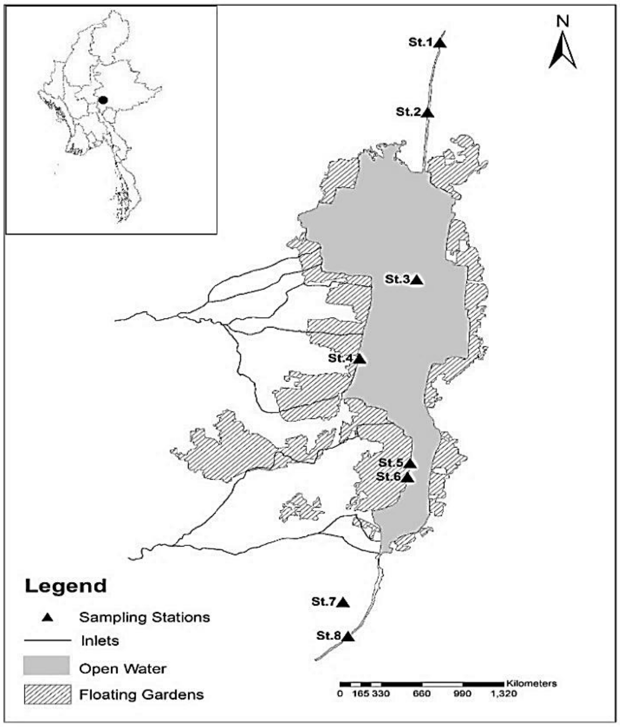

Fig. 2. Map of Inle Lake and the location of sampling stations (St.1-8). The dot in the map of Myanmar (upper left corner) shows the study area.

Regarding the data analysis, the actual data of the water quality (Table 2) were normalized as the data of some parameters, mainly the Cl^- concentrations among all stations were extremely high compared to the other research data of Akaishi et al. (2006) and my own (2019, unpublished) and the cause of this discrepancy is unknown. The latter data in 2019 were those by one time measurement, and the water samples were collected in May. The analytical measurements were done in Kyoto University, Japan and the analytical methods were different from the Department of Agricultural Research (DAR) in Myanmar. Thus, it is considered that the normalized data should be used for the analysis. Normalization was done to restrict the data to a certain range obtained by the formula: x = (x-xmin)/(xmax-xmin), where xmin means the minimum value of each station and xmax refers the maximum value of each station. The analysis of variance (ANOVA) and post hoc test was used to compare the sample means. The mean comparison between agriculture and tourism seasons was done using paired *t*-test. The hierarchical cluster analysis (CA) was applied to identify homogenous groups of stations. RStudio (Version 1.0.153) was used for all the statistical analysis in this study.

Table 2. Comparison of water quality data in 2004, 2017 and 2019 in Inle Lake

Elements	**St.1**			**St.2**			**St.3**			**St.4**		
	2004	**2017**	**2019**	**2004**	**2017**	**2019**	**2004**	**2017**	**2019**	**2004**	**2017**	**2019**
pH	7.20	7.12	7.97	7.20	7.13	8.22	7.00	7.13	8.91	7.00	7.22	8.10
EC (μScm^{-1})	378	343	566	324	338	396	260	257	302	327	263	495
Ca^{2+} (mgl^{-1})	50.20	61.05	58.80	48.80	60.28	42.25	36.10	34.41	16.62	43.80	43.82	50.18
Mg^{2+} (mgl^{-1})	14.60	8.94	30.94	12.00	9.15	20.94	11.80	8.44	26.05	14.50	9.61	31.57
Na^{+} (mgl^{-1})	9.63	8.95	10.95	4.50	8.72	10.26	7.71	6.34	9.59	13.40	6.34	6.79
K^{+} (mgl^{-1})	6.44	3.15	4.43	5.65	3.00	2.99	5.51	4.04	2.68	3.80	2.64	4.43
Cl^{-} (mgl^{-1})	3.67	105.09	14.40	2.51	106.76	14.87	2.26	97.23	13.39	2.55	93.18	10.62
SO_4^{2-} (mgl^{-1})	0.77	14.28	16.97	0.16	15.82	8.53	3.10	14.53	3.00	6.90	15.66	0.91
NO_3^{-} (mgl^{-1})	DL	1.55	9.17	0.01	1.49	7.12	DL	1.19	0.54	DL	1.11	0.00
TA (mgl^{-1})	140.00	295.40	ND	129.00	277.60	ND	100.00	246.90	ND	117.00	274.20	ND
TH (mgl^{-1})	186.00	69.98	ND	171.00	69.42	ND	139.00	42.90	ND	169.00	52.84	ND
pH	ND	7.14	7.69	ND	7.17	7.82	7.00	7.22	ND	ND	7.18	8.11
EC (μScm^{-1})	ND	236	493	ND	295	383	264	259	ND	ND	256	344
Ca^{2+} (mgl^{-1})	ND	39.80	53.31	ND	38.35	31.02	39.80	39.35	ND	ND	39.55	27.03
Mg^{2+} (mgl^{-1})	ND	9.00	33.52	ND	8.99	29.83	24.50	8.66	ND	ND	8.66	26.58
Na^{+} (mgl^{-1})	ND	5.34	3.24	ND	4.49	4.03	12.60	5.40	ND	ND	7.39	5.16
K^{+} (mgl^{-1})	ND	2.94	2.81	ND	2.99	1.77	4.72	1.97	ND	ND	2.56	1.83
Cl^{-} (mgl^{-1})	ND	98.08	5.07	ND	89.01	6.00	2.05	100.19	ND	ND	90.88	7.48
SO_4^{2-} (mgl^{-1})	ND	14.48	0.71	ND	14.38	0.83	0.96	17.20	ND	ND	14.66	2.04
NO_3^{-} (mgl^{-1})	ND	1.37	0.00	ND	1.37	0.00	0.45	1.18	ND	ND	1.45	1.72
TA (mgl^{-1})	ND	262.30	ND	ND	260.10	ND	117.00	258.50	ND	ND	224.10	ND
TH (mgl^{-1})	ND	48.78	ND	ND	47.33	ND	200.00	48.01	ND	ND	48.21	ND

Source: Akaishi et al. 2006 (November 2004), The present study (2017), Unpublished data (2019), ND: No Data.
The 2019 data was one time measurement and collected the samples in May.

RESULTS AND DISCUSSION

Spatial and seasonal variations of water quality in Inle Lake. The hierarchical cluster analysis (CA) was performed for grouping the water monitored stations (Fig. 3). The dendrogram showed that the cluster 1 included St. 1 and 2 that are located near the township area, and it was shown in Table Apd-1 that those 2 stations showed the highest values of most parameters, such as Ca^{2+}, TSS, TDS, and hardness. The cluster 2 was composed by the rest of the stations, including the floating garden area, the station near a weaving village and the outlet of the lake.

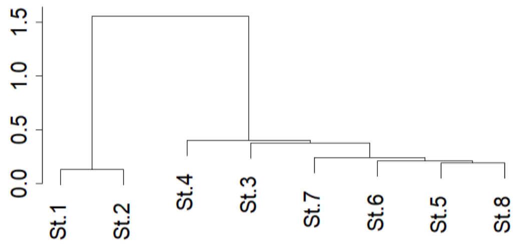

Fig. 3. Dendrogram revealing spatial similarities of the stations of Inle Lake by CA

The main sources of the Ca, Mg and hardness were erosion and sediment loadings from the bedrock, which consists mostly of limestone and calcareous sandstone in the drainage basin (Bhateria and Jain 2016; Thin et al. 2020). The composition of the TSS and TDS may include sand, silt, clay, minerals, sediments and organic matters, as well as sewage effluent (Bhateria and Jain 2016; Butler and Ford 2018). The elements Ca^{2+}, TSS, TDS and hardness were significantly higher in St.1, St.2 and St.4 (Table Apd-1), which are the main inlets of the lake. The sediment loads from the surface erosion, which are transported in through the inlets from the adjacent watershed area of the lake, summed up to the sewage contamination from the populated areas might impact the variation of those elements.

The seasonal variation of some parameters such as pH, Ca^{+}, K^{+}, Cl^{-} and NO_3^{-} were shown and those were significantly different in months with $p < 0.001$ (Fig. 4 to 8). The variations of the other parameters (Mg^{2+}, HCO_3^{-}, SO_4^{2-}, TSS, TDS, hardness and alkalinity) were provided in the Appendix 1. Among the above five elements, only Ca^{+} was significantly different among the stations ($p < 0.001$). The St. 1 and 2 had similar trends, which showed significant differences with other stations (St. 3 to 8), in almost every month of all parameters. The pH values in September and November were higher than those of the other months in all stations. The values in October, January and April were low and these showed significant differences with September and November (Fig. 4). Regarding the Ca^{2+} values, the higher values of St.3, 5, 6, 7 and 8 in September were found and it was significantly different with August, December, February, March, and May (Fig. 5). The values of K^{+} in August and April showed the same trends and were significantly higher than the other months such as November, January to March, and May in all stations. The values in October were significantly lower than August (Fig, 6). The values of Cl^{-} were higher during the period from August to December in all stations than those in the other periods, and lower in January, February and April (Fig. 7). The values of NO_3^{-} of all stations were higher in April and May than those in the other months excluding November (Fig. 8). The highly fluctuated water chemistry was observed in Inle Lake.

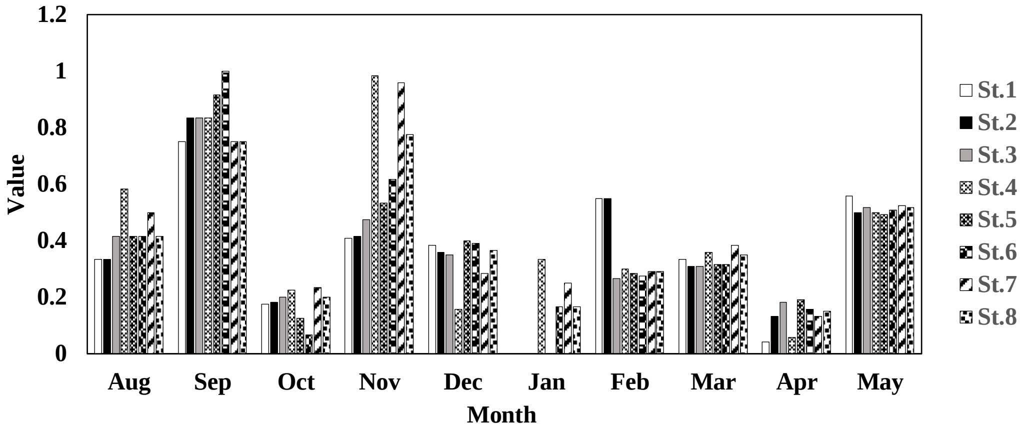

Fig. 4. Seasonal variations of pH among the stations. There are significant differences among some months and some stations, but in order to avoid complexity caused by many small letters, significant relations are shown in the text.

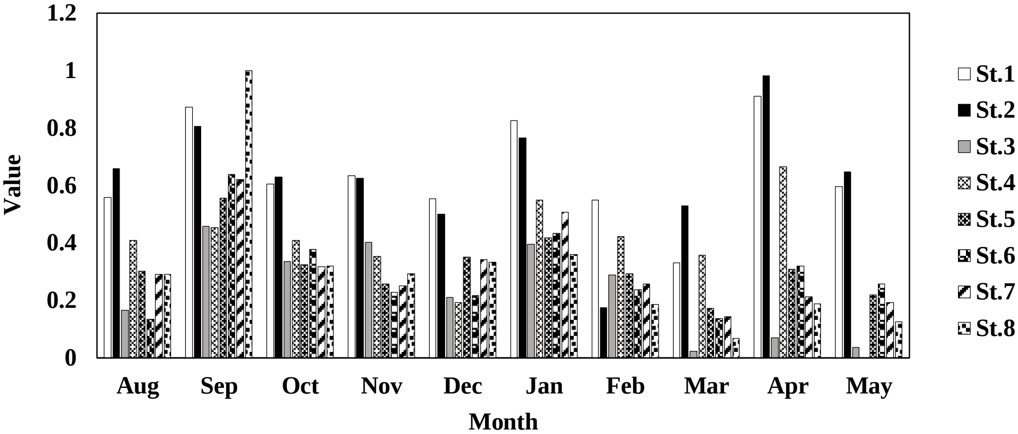

Fig. 5. Seasonal variations of calcium among the stations. There are significant differences among some months and some stations, but in order to avoid complexity caused by many small letters, significant relations are shown in the text.

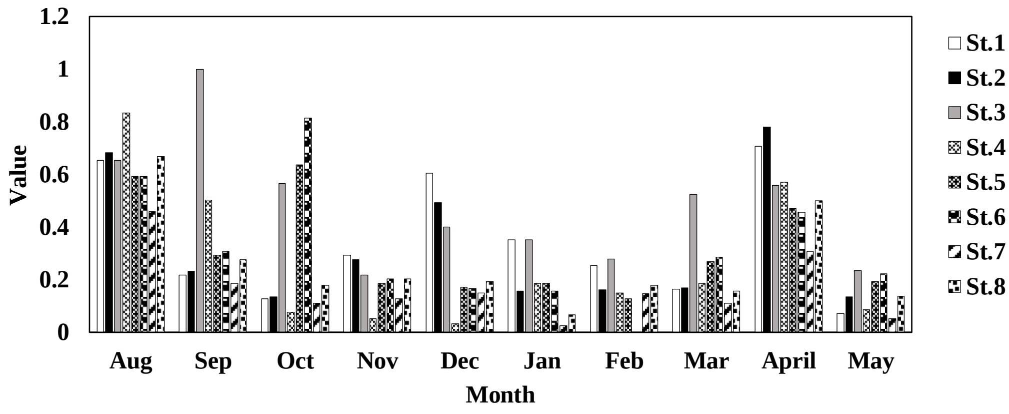

Fig. 6. Seasonal variations of potassium among the stations. There are significant differences among some months and some stations, but in order to avoid complexity caused by many small letters, significant relations are shown in the text.

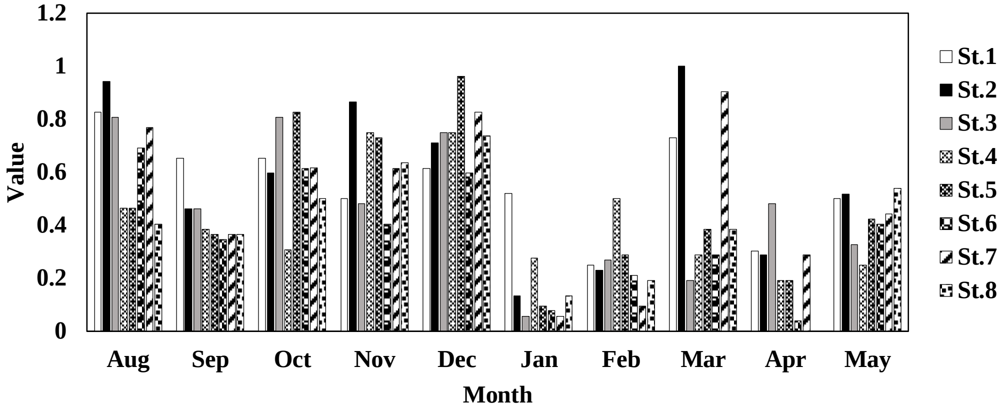

Fig. 7. Seasonal variations of chloride among the stations. There are significant differences among some months and some stations, but in order to avoid complexity caused by many small letters, significant relations are shown in the text.

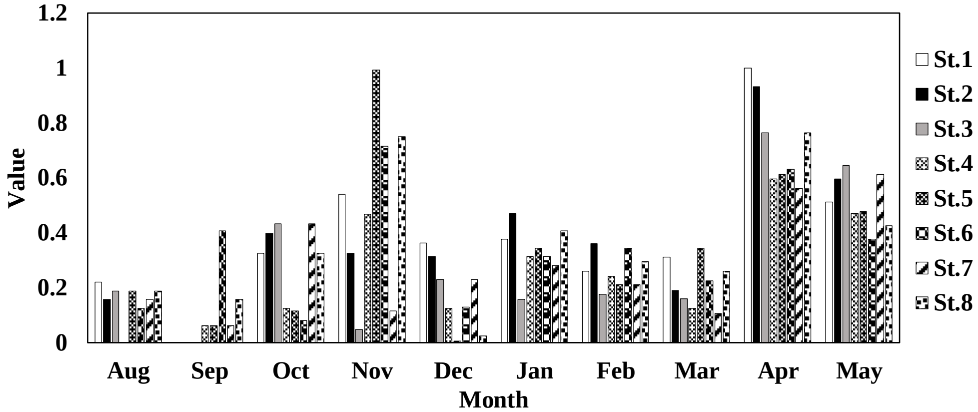

Fig. 8. Seasonal variations of Nitrate among the stations. There are significant differences among some months and some stations, but in order to avoid complexity caused by many small letters, significant relations are shown in the text.

Assuming that the activities of agriculture and tourism are the main factors influencing the lake water quality, the sampling months were divided into two seasons: agriculture and tourism seasons (Table 3). The result revealed that the element values in the agricultural season tended to be higher compared to the tourism season. There was a statistically significant difference between the two seasons with pH and Mg^{2+} ($p < 0.05$), K^+, HCO_3^-, TDS and alkalinity ($p < 0.01$) and SO_4^{2-} ($p < 0.001$).

The fertilization in agriculture areas is also one of major anthropogenic sources of K^+ and NO_3^- in surface water (Petzoldt and Uhlmann 2006; Skowron et al. 2018). The value of K^+ was significantly higher during agricultural season (Table 3), compared to tourism season. However, NO_3^- values were not significantly different between the two seasons due probably to the nutrient uptake by cultivated crops or phytoplankton in the surface lake water. The Cl^- generally originates from anthropogenic sources including agricultural practices, wastewater, and sewage discharges (Ludwikowski and Peterson 2018; Oberhelman and Peterson 2020). Based on the results of Cl^- values, the sewage contamination and waste disposal, agricultural activities, and tourism-oriented activities around the lake might influence the water quality during the whole year. The finding of the present study was consistent with the result of other research, in which water contamination from human activities, including agricultural runoff, affected the Cl^- contents in the Lake Tonle Sap, Cambodia. (Oyagi et al. 2017).

Table 3. The t-test results comparing agriculture and tourism season on water quality.

	Agriculture season		Tourism season		
	Mean	**SE**	**Mean**	**SE**	***t*-test**
pH	0.40	0.01	0.36	0.02	*
EC	0.58	0.03	0.54	0.04	ns
Ca^{2+}	0.40	0.06	0.35	0.05	ns
Mg^{2+}	0.57	0.02	0.49	0.03	*
Na^{+}	0.22	0.03	0.22	0.02	ns
K^{+}	0.38	0.04	0.21	0.03	**
CO_3^{2-}	0.15	0.02	0.15	0.02	ns
HCO_3^{-}	0.51	0.02	0.62	0.02	**
Cl^{-}	0.48	0.04	0.46	0.03	ns
SO_4^{2-}	0.24	0.01	0.31	0.01	***
NO_3^{-}	0.33	0.02	0.30	0.03	ns
TSS	0.18	0.04	0.13	0.05	ns
TDS	0.33	0.03	0.25	0.02	**
Alkalinity	0.59	0.03	0.73	0.03	**
Hardness	0.38	0.07	0.35	0.06	ns

*$p<0.05$, **$p<0.01$, ***$p<0.001$ SE: Standard Error

Representative sampling locations and times for long term monitoring. The representative stations for each cluster and months for each season were selected based on the CA and the variation of the data of pH, Ca^{2+}, K^{+}, Cl^{-} and NO_3^{-} (Fig. 4 to Fig. 8), which showed significant differences and are considered to be agriculturally important.

According to the CA, the two clusters were grouped. The cluster 1 included St. 1 and St. 2, located near the populated tourist's hub, and the highest values of most parameters were found in these two stations. Thus, it is more likely that tourism influences the water quality of these stations. On the other hand, the cluster 2 contained the rest of the stations. The trends of the stations were checked, and then, the one with the largest variations among the stations was selected as the most appropriate station for each season. With the largest variations, it is easy to detect changes. The St. 1 and 2 had the similar trends among the stations regarding the seasonal variations. The St. 6 had the largest variations which had the similar trends with other stations on the pH, Ca^{2+}, and Cl^{-}. Thus, the St.1 and/or St.2 from the cluster 1, and the St. 6 from cluster 2 were considered to be the appropriate stations for monitoring.

Regarding the comparison of the influences of agriculture and tourism, the values of elements such as pH, Mg^{2+}, K^{+}, HCO_3^{-}, TDS, alkalinity and SO_4^{2-} were significantly higher in agricultural season (March to October) than tourism season (November to March) (Table 3). Therefore, the observation of lake water quality should be done twice a year. Regarding the seasonal variations, April and May during the agricultural season and November and December during the tourism season had similar trends among the stations in most of the parameters. The influential elements of agriculture and tourism, i.e., K^{+}, NO_3^{-} and Cl^{-} of those four months were checked again to select the most appropriate months for each season. The values of K^{+} and NO_3^{-} in April were much higher than those in May, and the Cl^{-} values in December were higher than those in November. It is indicated that water quality of April is highly influenced by agricultural activity and that of December can be representative month for tourism. Thus, the changes in water quality should be monitored in April for agricultural season, and in December for tourism season.

CONCLUSIONS

The spatial and temporal variations of the water quality of Inle Lake were clarified to select appropriate sampling locations and time periods for future monitoring in this study. The elements Ca^{2+}, TSS, TDS and hardness were significantly higher in the stations at the main inlets of the lake. It is possible that the sediment loads of the surface erosion summed up to the sewage contamination might impact the variation of those elements. The result also show that the St.1 and St.2 included in the Cluster 1 based on the CA were found to show the highest values of some parameters and thus, the tourism might influence the water quality of these stations. Then, the record values were higher during the agricultural season compared to the tourism season, indicating that the fertilization of agriculture areas might have impact on the lake water quality. Overall, the lake water is mainly contaminated by the impacts of sedimentation and human activities, including agricultural practices and tourism.

Inle Lake needs to be maintained, considering the variations in water quality and for the preservation of the biodiversity of the lake. The present study could contribute to future planning sampling design, which can reduce labor costs, times, and monitoring sites. The continuous monitoring and analysis of the lake water are required to meet the future long-term changes. In order to achieve that the proper sampling locations should probably be St. 1 and/or St. 2 from cluster 1 and St.6 from cluster 2. Moreover, the lake water quality should be measured at least twice a year: in April for agricultural season and in December for tourism season. Then, it is also recommended to adjust the amount and frequency of fertilizer usage following Good Agricultural Practices (GAP), such as application of organic fertilizers, in the agriculture sector in order to preserve the lake water quality.

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Appendix-1

Table Apd-1. Mean and SE of water quality parameters among the stations of Inle Lake (August 2017 – May 2018)

Parameters	St. 1	St. 2	St. 3	St. 4	St. 5	St. 6	St. 7	St. 8
pH	0.35±0.07	0.36±0.07	0.36±0.07	0.43±0.09	0.37±0.08	0.39±0.09	0.43±0.08	0.40±0.207
EC	0.71±0.08	0.70±0.06	0.50±0.208	0.52±0.06	0.45±0.07	0.60±0.07	0.51±0.06	0.50±0.07
Ca^{2+}	0.64±0.06[a]	0.63±0.07[a]	0.24±0.05[b]	0.38±0.06[bc]	0.32±0.03[bd]	0.30±0.05[be]	0.31±0.05[bf]	0.32±0.08[bg]
Mg^{2+}	0.51±0.10	0.54±0.08	0.44±0.08	0.61±0.08	0.52±0.08	0.52±0.07	0.47±0.06	0.47±0.05
Na^{+}	0.32±0.04	0.31±0.05	0.22±0.01	0.22±0.09	0.18±0.04	0.15±0.04	0.18±0.08	0.26±0.08
K^{+}	0.34±0.07	0.32±0.08	0.48±0.08	0.27±0.09	0.31±0.06	0.32±0.08	0.17±0.04	0.26±0.06
CO_3^{2-}	0.18±0.03	0.17±0.03	0.16±0.02	0.20±0.09	0.12±0.02	0.09±0.03	0.14±0.02	0.10±0.03
HCO_3^{-}	0.65±0.05	0.59±0.11	0.50±0.04	0.53±0.08	0.57±0.04	0.62±0.05	0.55±0.06	0.57±0.07
Cl^{-}	0.56±0.06	0.57±0.10	0.46±0.08	0.42±0.06	0.47±0.09	0.37±0.07	0.50±0.09	0.39±0.07
SO_4^{2-}	0.20±0.08	0.22±0.09	0.20±0.08	0.22±0.09	0.20±0.08	0.20±0.08	0.24±0.10	0.20± 0.08
NO_3^{-}	0.39±0.08	0.37±0.08	0.28±0.08	0.25±0.06	0.34±0.09	0.33±0.07	0.28±0.06	0.36±0.08
TSS	0.32±0.08[a]	0.30±0.06[ag]	0.05±0.01[bh]	0.26±0.07[ah]	0.07±0.02[ch]	0.08±0.02[dh]	0.10±0.03[egh]	0.08±0.03[fh]
TDS	0.37±0.03[a]	0.41±0.07[a]	0.22±0.04[b]	0.31±0.03[a]	0.27±0.03[a]	0.28±0.04[a]	0.26±0.04[a]	0.26±0.04[a]
Alkalinity	0.75±0.03	0.70±0.09	0.61±0.06	0.69±0.07	0.66±0.03	0.65±0.03	0.65±0.04	0.55±0.07
Hardness	0.63±0.05[a]	0.69±0.05[a]	0.21±0.05[b]	0.36±0.06[bc]	0.30±0.03[bd]	0.28±0.05[be]	0.29±0.04[bf]	0.29±0.08[bg]

Mean±SE: SE= Standard Error

Different letters indicate statistically significant differences ($p<0.05$) between the stations.

The values were normalized by the formula: x= x-xmin/xmax-xmin.

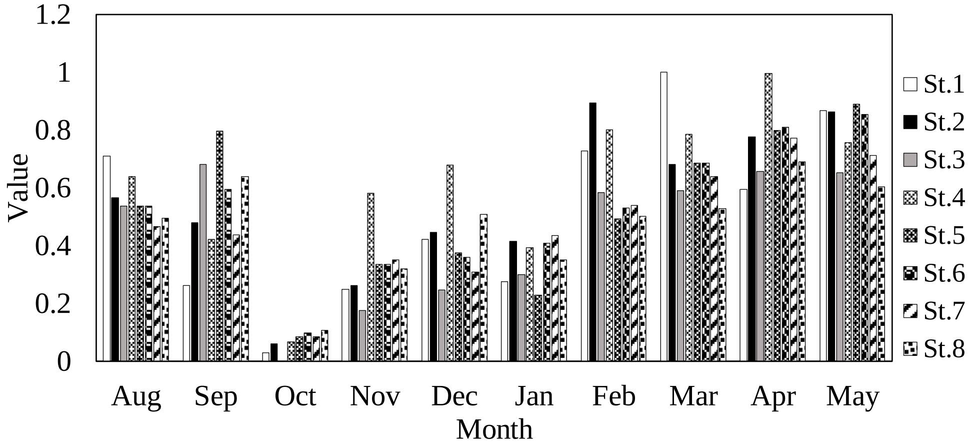

Figure Apd-1. Seasonal variations of Magnesium among the stations

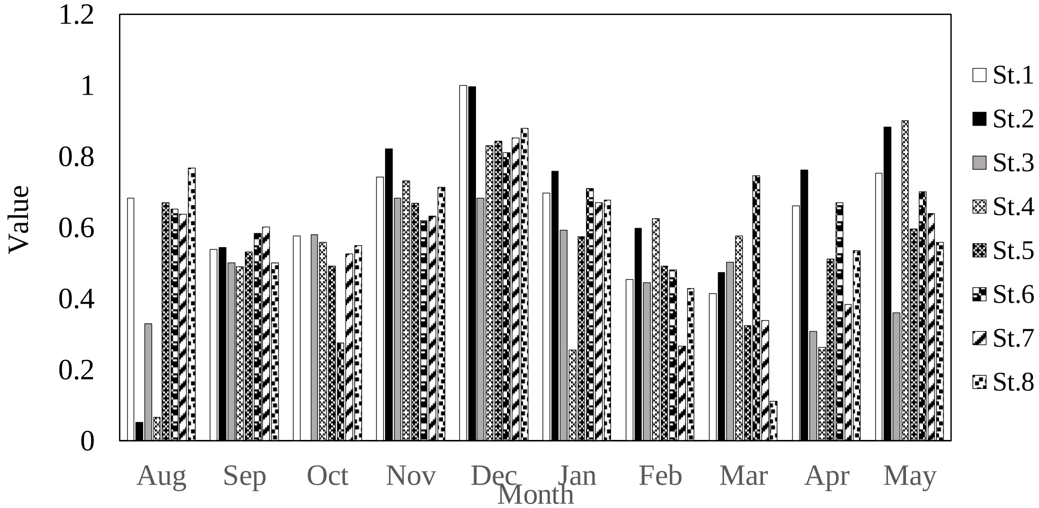

Figure Apd-2. Seasonal variations of bicarbonate among the stations

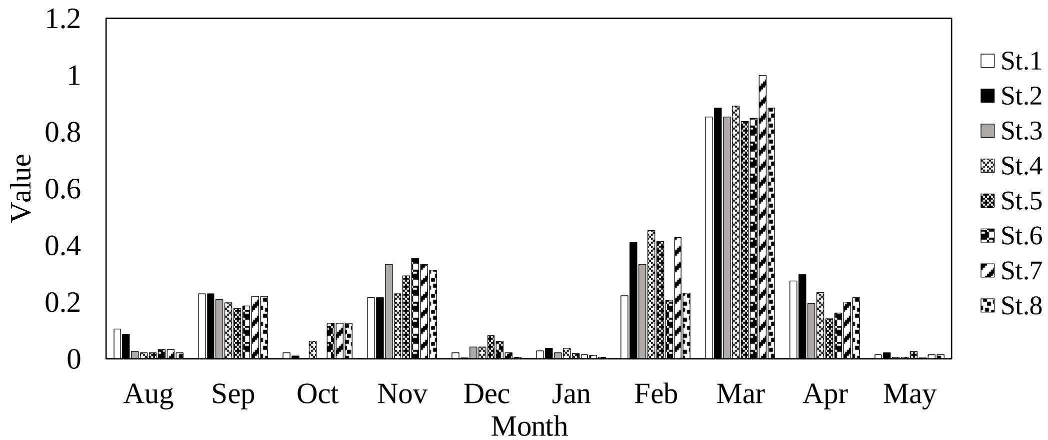

Figure Apd-3. Seasonal variations of Sulfate among the stations

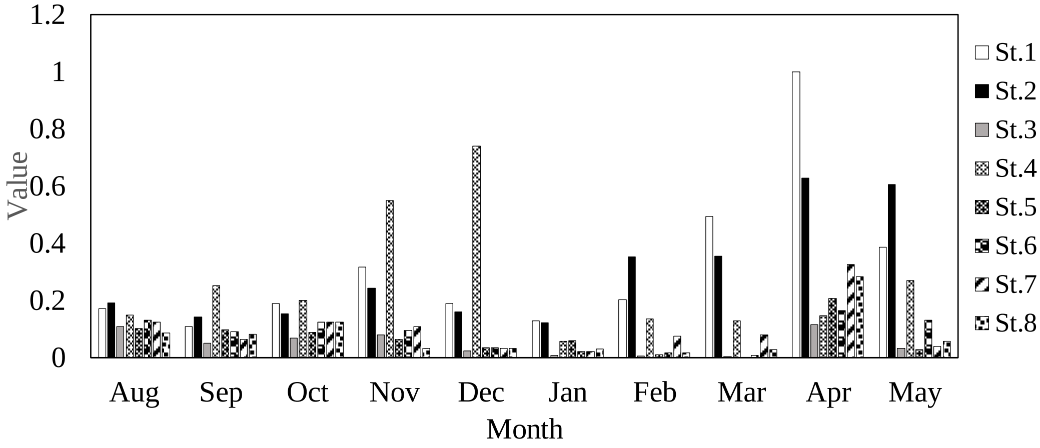

Figure Apd-4. Seasonal variations of Total Suspended Solid among the stations

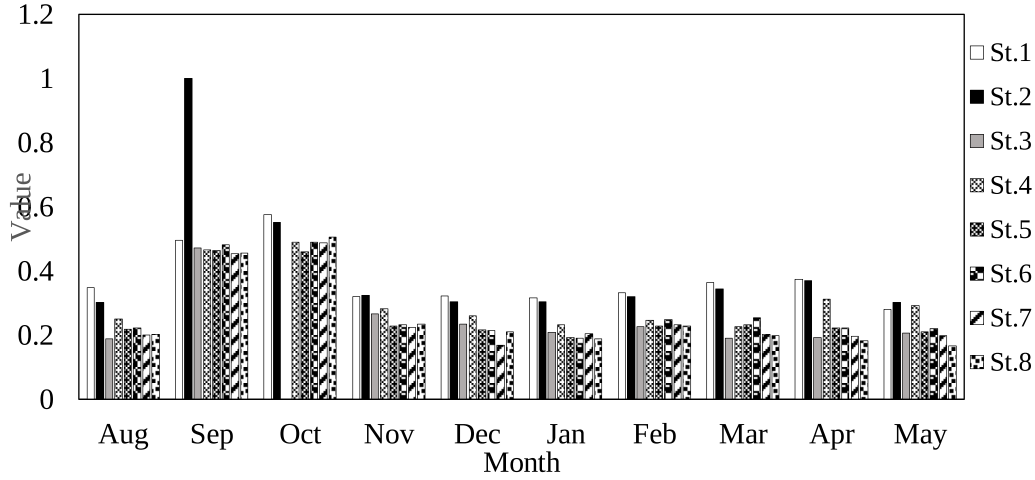

Figure Apd-5. Seasonal variations of Total Dissolved Solid among the stations

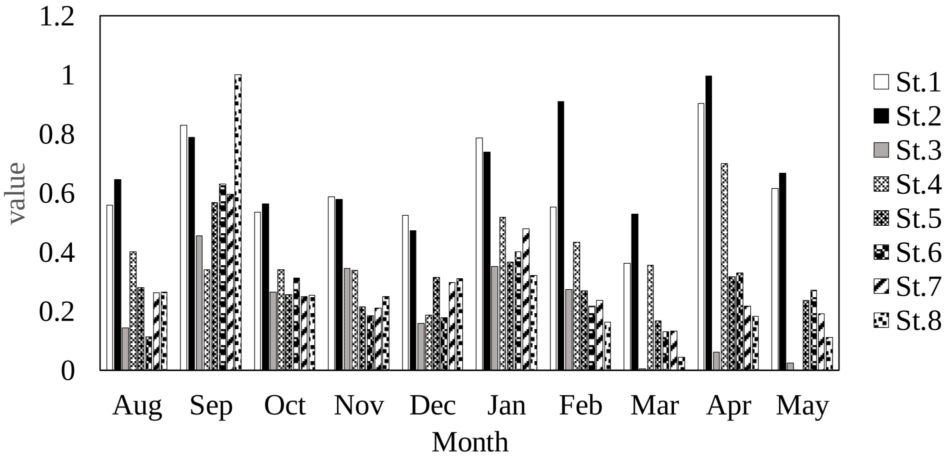

Figure Apd-6. Seasonal variations of Hardness among the stations

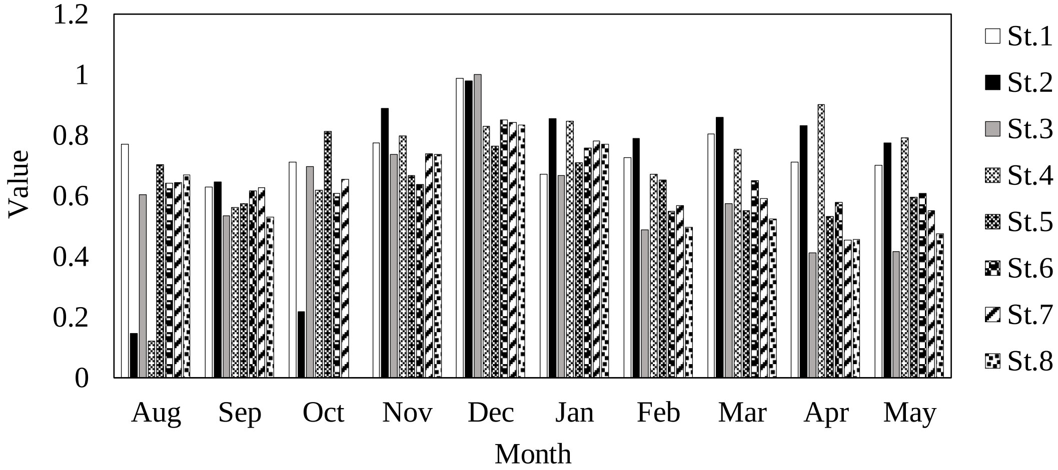

Figure Apd-7. Seasonal variations of Alkalinity among the stations

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PROTECTED CULTIVATION IMPROVES GROWTH OF 'LOLLO ROSSA' LETTUCE UNDER CHILLING CONDITIONS IN BENGUET, PHILIPPINES

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ABSTRACT

A study was conducted in two sites (Paoay, Atok and Balili, La Trinidad) in Benguet Province, Philippines to investigate the growth and development of lettuce variety 'Lollo Rossa' through vegetative, biomass and yield, physiological, and phytochemical parameters using low tunnel and mulch (black polyethylene plastic [PEP], alnus leaves and white PEP) as mitigation strategies against cold stress. The average temperature in Atok and La Trinidad was 15 and 21 °C, respectively. In Atok, *andap* or frost was experienced on February 15 and 16, 2017 with air temperature of 1.8 and 1.5 °C, respectively. Another *andap* occurred on March 8 and 19, 2017 having an ambient temperature of 3.3 and 3.9 °C, respectively. Performance of 'Lollo Rossa' was better with the use of low tunnel compared to those grown without. Shoot length, root and shoot fresh weight, yield, shoot dry weight, and total phenolic content (TPC) of lettuce was significantly higher with the use of plastic mulch. However, the use of alnus mulch against chilling stress was ineffective. In general, white PEP was advantageous when used in Atok, while black PEP was more beneficial when used in La Trinidad. In terms of combination effect, shoot fresh weight, yield, and root dry weight were significantly high in 'Lollo Rossa' grown in Atok under white PEP and low tunnel. 'Lollo Rossa' grown in La Trinidad had significantly high root and shoot dry weight, and yield under black PEP and low tunnel.

Key words: cold stress, secondary metabolites, low tunnel, mulch

INTRODUCTION

Plants, being sessile, are inevitable to experience various abiotic and biotic stresses which include water (drought or waterlogged condition), high or low temperatures, light, air (e.g. ozone), and salinity stresses (Sayyari et al. 2013) as well as pest and pathogens (Fujita et al. 2006). Temperature changes, including diurnal and seasonal changes, can limit respiration, photosynthesis, and growth (Moynihan et al. 1995). Environmental stresses, in general, are the primary causes of crop losses worldwide, reducing about 50% average crop yields for most crops (Bray et al. 2000).

Lettuce (*Lactuca sativa* L.) is the most popular salad vegetable which is consumed in increasing amounts because of peoples' perception of 'healthier foods' (Llorach et al. 2008). The

Philippines produced about 3,780 mt of lettuce harvested from an area of 501 ha during the five-year crop production survey from 2010 - 2014 (BAS 2015). The average lettuce production in Cordillera Administrative Region (CAR), specifically Benguet, was 1,195 mt planted in 145 ha. Limiting factors for the production of lettuce in the northern part of the Philippines are the incidence of pests and diseases as well as various abiotic stresses such as water, nutrients and chilling temperature.

Baguio City and Benguet Province experience temperature drops during the cool season. (Luces 2014). A temperature drop of 10°C in Baguio City experienced on January 16, 2014 was associated with a temperature drop in Benguet ranging from 6.8 to 8.5°C on that day (Galacgac 2014). Frost or *andap* was also reported on December 29 and 30, 2014 with temperatures of 12.8 and 12.7 °C, respectively. The temperature was believed to have occurred below 10°C, considering other factors such as humidity, wind, water supply and overcast skies (Mamaria 2015). With such chilling temperatures, patches of farmlands suffered crop losses from frost or *andap* in some villages in Paoay, Atok, Kibungan villages of Madaymen, Taliboy-oc, Cagam-is and Masala, the village of Cada in Mankayan and in the municipality of Loo, Buguias, all of which are located in Benguet Province (Cabreza 2014). Potato and cabbage were affected mostly by *andap* which are planted during the cool season (Picana 2014). Other major crops in Benguet include cabbage, Chinese cabbage, carrots, chayote, beans, broccoli and lettuce (Batt et al. 2007).

Mitigation strategies have been identified against cold stress such as temperature acclimation or hardening, good planting schedule, proper zone or location, site exposure and slope, availability of water for sprinkler irrigation, chemical or hormone application, and protected cultivation (Decoteau 2005). Protected cultivation is a cropping technique which uses tunnels and mulches to control partially or fully the microenvironment surrounding the plant body during their period of growth to maximize yields and resource saving (Reddy 2015). With available strategies, low temperature-affected lands may be of use for crop production rather than just being idle. There are limited data on how these strategies help lettuce crops cope in the Philippines where chilling temperature occur. Therefore, this study was conducted to investigate the growth and development of lettuce using protected cultivation against chilling stress. Specifically, this study sought to determine the use of protected cultivation on the growth and development of lettuce subjected to chilling stress, and to evaluate the physiological and chemical properties of lettuce subjected to various mitigation strategies against chilling stress. This study investigated the effect of low tunnel and mulch using white and black polyethylene plastic (PEP) and alnus (Japanese alder) mulch.

MATERIALS AND METHODS

The experiment was conducted in Paoay, Atok and Balili La Trinidad, Benguet, Philippines from December 2016 to March 2017.

Plant materials. Red (Lollo Rossa) cultivar of leaf type lettuce was used in this study. 'Lollo Rossa' lettuce has an attractive leaf color from red to brown, with strongly crimpled leaves that makes a compact rosette (Koudela and Petříková 2008). Sowing was done using seedling tray with planting media composed of 1:1 coconut coir dust and carbonized rice hull. The seedling trays were maintained inside a greenhouse nursery dedicated for seedling production.

Experimental layout and statistical analysis. The required area was prepared and divided into three blocks consisting of 30 plots measuring 1 m x 5 m. The seeds were sown in seedling trays prior to transplanting. The seedlings were transplanted in the field one month after sowing with a distance of 30 cm x 30 cm between hills and rows. This spacing allowed 30 seedlings to be planted per plot. Watering after transplanting followed to prevent transplanting shock. Application of fertilizer depended on the recommended rate for the experimental area one week before transplanting. The study was carried out in a 2 x 4 factorial experiment with three replications in RCBD. Data was statistically analyzed using ANOVA and treatment means were compared using the LSD at 5% level of probability.

Setup of low tunnel and mulching materials. *Low tunnel*. This was constructed in a 1 m x 5 m bed using four bamboo strips to make the hoop and transparent polyethylene plastic sheet was kept in place on top of the hoops using twines. The plastic sheets were twined in such a way that it could be adjusted to cover the whole tunnel from 6 pm to 6 am, then folded on one side of the furrow from 6 am to 6 pm.

Synthetic mulches. Black and white (semi- clear) PEP sheets were then placed and stretched onto the beds, covering the edges with soil to keep them in place. Holes were cut at a planting distance of 30 cm x 30 cm.

Alnus leaves as natural mulches. Fallen leaves and small branches were collected from Halsema highway, Atok, Benguet. One week after transplanting, the organic mulch was evenly distributed on the raised bed with at least 2.5 cm thickness.

Control treatment. The control treatment in this study was without mulching material.

Cultural management and harvesting. Cultural management practices like weeding, irrigation, hilling-up and application of pesticides were properly employed throughout the duration of the experiment. Harvesting was done 40 days after transplanting.

Secondary metabolite content determination. Lettuce leaves were oven-dried at 45°C until dry for the determination of total phenolic content (Velioglu et al. 1998), total flavonoid content (Zhishen et al. 1999), anthocyanin content (Wrolstad 1976) and antioxidant activity (Shimada et al. 1992).

Data collection. Data collection were various parameters related to soil and ambient temperature, vegetative growth parameters (number of leaves), biomass and yield (shoot fresh and dry weight, and yield), chilling injury (CI) index and phytochemical content (total phenolic contents [TPC], total flavonoid content [TFC], and antioxidant activity).

Laboratory thermometers were installed in plots while digital thermometer and hygrometer was used for ambient. The soil and ambient temperature and relative humidity at the experimental area were measured at 6 am, 12 noon and 6 pm, while the aerial temperature inside the low tunnel were measured at 6 am. The CI index was taken from 32 plant samples, 40 days after transplanting, using scales from 1 to 5, with 1 being normal and 5 having severe extensive necrotic areas or growth restrictions.

RESULTS AND DISCUSSION

Number of leaves. Both low tunnel and mulch factors affected the number of leaves of 'Lollo Rossa' grown in Atok and La Trinidad (Fig. 1). 'Lollo Rossa' grown in Atok and La Trinidad had more leaves when grown under low tunnel compared to those grown without. In addition, white PEP increased number of leaves in 'Lollo Rossa' grown in Atok, while black plastic mulch was effective in La Trinidad. The average number of leaves of 'Lollo Rossa' grown in Atok and La Trinidad was 11 and 14 leaves, respectively.

The improvement of plant growth and development in terms of number of leaves was observed with the use of low tunnel and mulch. The use of low tunnel was significant in increasing the number of leaves in 'Lollo Rossa'. Protected cultivation improved the number of leaves in lettuce (Santos-Filho et al. 2009). This indicates that low tunnels are advantageous in enhancing growth in lettuce because it can increase soil and tunnel temperature for the promotion of early growth and development (Hochmuth et al. 2009; Jenni et al. 2003). However, the use of alnus leaves as mulching material was not as effective as that of the white PEP mulch in terms of number of leaves. This may be due to the lower soil temperature with the use of alnus mulch (15.7°) compared to that of white PEP (17.7°C). Organic mulches decrease afternoon temperatures which suggests that organic mulches probably act like insulators that slow down daytime conduction of heat, thus, affecting the overall soil temperature (Schonbeck and Evanylo 1998).

The effective mulching material for 'Lollo Rossa' grown in Atok and La Trinidad was the white and black PEP, respectively. The soil mean maximum temperature for Atok and La Trinidad from February to March, 2017 was 20.5 and 24.5°C, respectively. The use of white PEP or in combination with low tunnel in La Trinidad increased maximum temperature data at 27.1 and 28.6°C, respectively, of which is exceeding the upper optimal temperature limit of 25°C (DAFF 2010). However, the use of black PEP only exceeded the upper optimal temperature by 1°C. This probably explains that temperatures above optimum will have a negative effect on the plant (Lament 1993). On the other hand, heat is needed by the plants in Atok due to its cold climate. The use of white PEP alone, or in combination with low tunnel was able to increase the soil average temperature to 17.7°C and 18.2°C, respectively, against that of bare soil at 15.7°C.

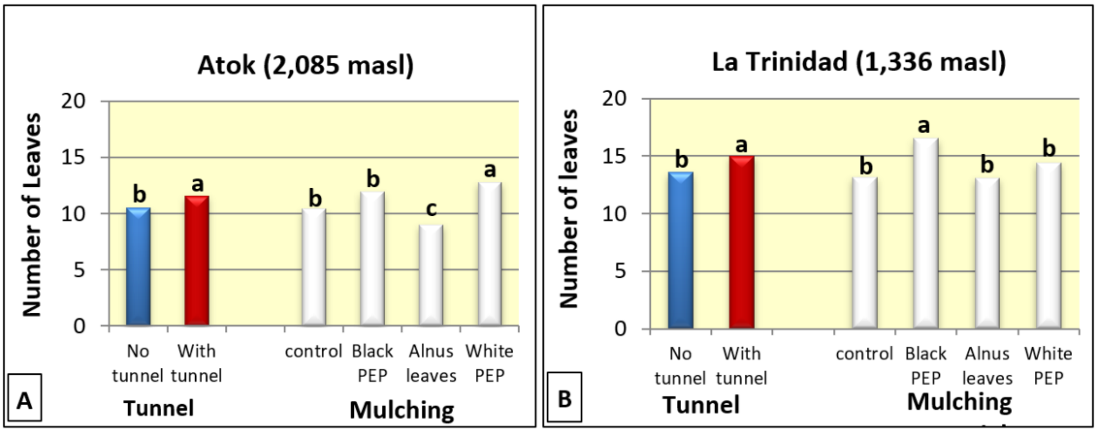

Fig. 1. Effect of low tunnel and mulching material on the number of leaves of 'Lollo Rossa' grown under chilling conditions in (A) Paoay, Atok and (B) Balili, La Trinidad, Benguet. Significant factors: main effects of low tunnel and mulch for both locations. Means with a common letter within factors in each location are not significantly different at 5% level using LSD. Legend: masl= meters above sea level.

Shoot fresh weight. Shoot fresh weight of 'Lollo Rossa' grown in both locations were significantly influenced by the interaction between low tunnel and mulch (Fig. 2). A four-fold increase in shoot fresh weight was obtained in 'Lollo Rossa' grown in Atok under white PEP, while about three-fold increase under black PEP mulch. All plants grown under bare and mulched soil further increased in shoot fresh weight, but the combination of white PEP and low tunnel gained the highest shoot weight. In La Trinidad, the use of black or white PEP mulch significantly increased shoot fresh weight of 'Lollo Rossa' by about two-fold relative to those grown in bare soil. Except for the use of white PEP and alnus mulch, plants under bare soil and black PEP mulch had significantly higher shoot fresh weight when combined with low tunnel. Overall, the use of black PEP mulch combined with low tunnel obtained the heaviest shoot fresh weight.

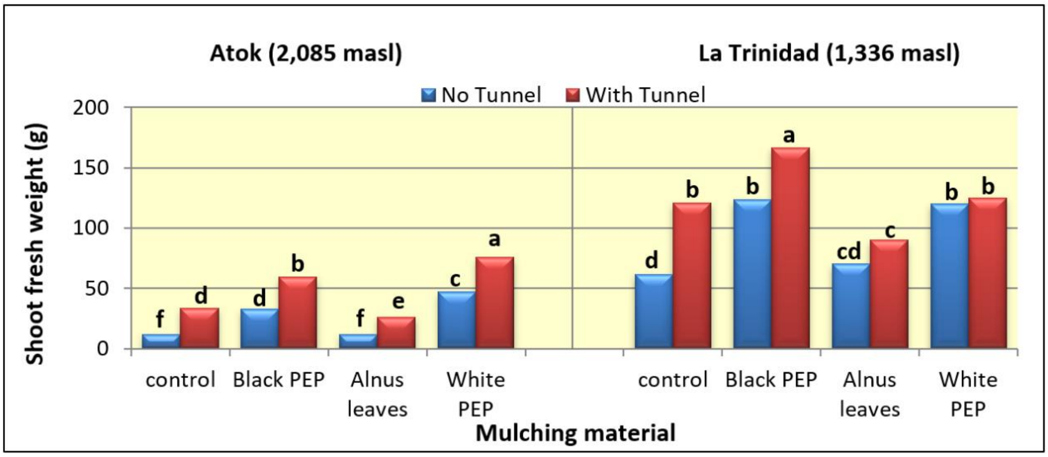

Fig. 2. Effect of low tunnel and mulching material on the shoot fresh weight of 'Lollo Rossa' grown under chilling conditions in Paoay, Atok and Balili, La Trinidad, Benguet. Significant factors: interaction effect of low tunnel and mulch for 'Lollo Rossa' grown in Atok and La Trinidad. Means with a common letter are not significantly different at 5% level using LSD. Legend: masl= meters above sea level

Aside from the capacity of mulches to increase soil temperature (Hochmuth et al. 2009; Jenni et al. 2003), low tunnels also work by trapping radiant heat during the day and delaying its loss at night (Decoteau 2005); thus, maintaining a relatively higher temperature around the plant. The control of microclimate by row cover and plastic mulches benefits the plants by enhancing crop growth and development in terms of biomass and yield, and may reduce cold damage (Decoteau 2005). This study observed that the use of mulching material increased average soil temperature ranging from 0.2 to 3.3°C higher than that in bare soil (data not shown). In addition, low tunnel alone increased morning temperature (taken during 6 AM) by 7.9% while the combination of mulching material and low tunnel increased temperature ranging from 4.7 to 13.1%. The increase in morning temperature using mulch is probably caused by the increased mid-day and evening temperatures of the previous day. The use of low tunnel alone can increase the air temperature around the plants (Maughan et al. 2014). Early growth and maturity of lettuce, as well as enhanced yield over the control plots were observed with the combined use of low tunnel and mulch (Jenni et al. 2003).

Shoot dry weight. The main effects of low tunnel and mulch was significant in terms of shoot dry weight of 'Lollo Rossa' grown in Atok and La Trinidad (Fig. 3). In both locations, 'Lollo Rossa' grown under low tunnel had significantly higher shoot dry weight compared to those grown without while the use of plastic mulch increased shoot dry weight of lettuce by about two-fold. However, alnus mulch was ineffective.

The use of protected cultivation such as low tunnel and mulch improved the shoot dry weight, and yield of 'Lollo Rossa' grown in Atok and La Trinidad. Protected cultivation is known to improve the dry weight of lettuce plants compared to open field (Santos-Filho et al. 2009). PEP mulches are commonly used in vegetable and fruit production (Decoteau 2005). The advantages of PEP mulches include earlier yields, better fruit quality, and higher yields. These responses may be due to enhanced soil temperature, improved water and nutrient use efficiency.

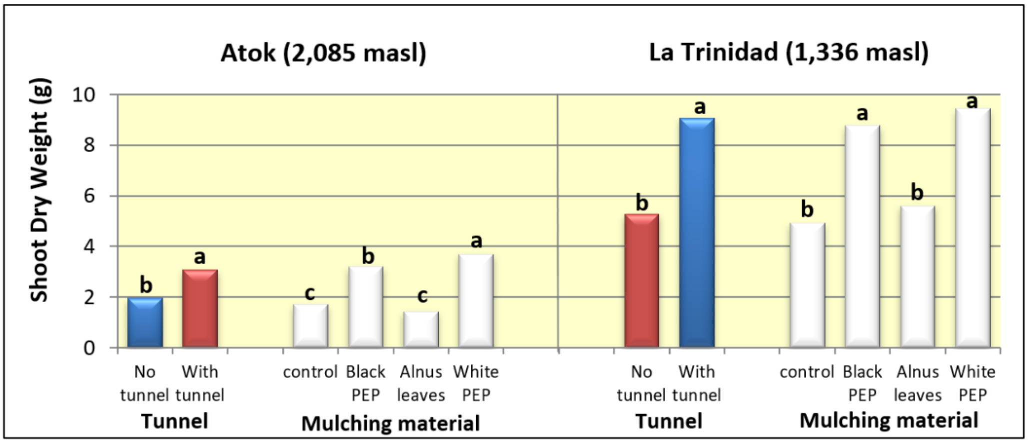

Fig. 3. Effect of low tunnel and mulching material on shoot dry weight of 'Lollo Rossa' grown under chilling conditions in Paoay, Atok and Balili, La Trinidad, Benguet. Significant factors: main effects of low tunnel and mulch for 'Lollo Rossa' grown in both locations. Means with a common letter are not significantly different at 5% level using LSD. Legend: masl= meters above sea level.

Yield. Interaction effects of low tunnel and mulch was significant on the yield of 'Lollo Rossa' grown in both locations (Fig. 4). A significant three- and four-fold increase in yield was obtained in 'Lollo Rossa' grown in Atok under black and white PEP mulch, respectively. Yield was further increased significantly in plants grown under bare and mulched soil, wherein the combination of white PEP and low tunnel gained the highest. On the other hand, the use of black or white PEP mulch increased shoot fresh weight of 'Lollo Rossa' grown in La Trinidad by about two-fold compared to those grown in bare soil. Plants under bare soil and black PEP mulch increased yield when combined with low tunnel, except for the use of white PEP and alnus mulch. Overall, the use of black PEP mulch combined with low tunnel obtained the heaviest shoot fresh weight.

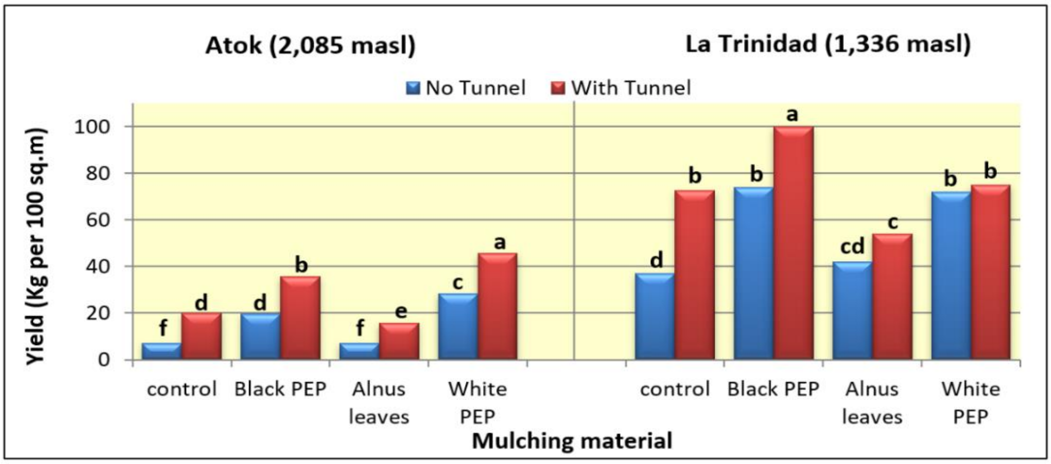

Fig. 4. Effect of low tunnel and mulching material on the yield of 'Lollo Rossa' grown under chilling conditions in Paoay, Atok and Balili, La Trinidad, Benguet. Significant factors: interaction effect of low tunnel and mulch for 'Lollo Rossa' grown in both locations. Means with a common letter are not significantly different at 5% level using LSD. Legend: masl= meters above sea level.

Yield of lettuce in Philippine setting is around 12-15 tons ha^{-1} (Maghirang et al. 2010). However, the yield of 'Lollo Rossa' grown in Atok was 0.7 to 4.55 t/ha while those grown in La Trinidad ranged from 3.7 to 10.0 t/ha. The low yield obtained in Atok was due to the effect of chilling temperatures during the experiment where *andap* was experienced in the locality. Lower growth in 'Lollo Rossa' may suggest that it is sensitive to chilling conditions. Low temperatures were also experienced in La Trinidad, but the low yield in 'Lollo Rossa' was most likely varietal in nature. The varietal differences were also observed by Koudela and Petříková (2008) using selected cultivars of leaf lettuce. Since yield and its component characters are polygenic in nature (Meena et al. 2010) they are highly influenced by environmental factors. Temperature below optimum causes reduction in the rates of metabolism, growth and development (DAFF 2010). In addition, there is slow uptake of nutrients at low temperature, which influences the root and overall plant growth and development (Moorby and Graves 1980).

Chilling injury index. Interaction effect of low tunnel and mulch was noted on the chilling injury index of 'Lollo Rossa' grown in Atok (Fig. 5). The use of white PEP mulch significantly lowered the CI index of 'Lolo Rossa' under chilling stress in Atok. Slight to moderate CI index were observed on plants grown under white or black PEP mulch, while moderate to severe CI index from plants grown on bare soil or under alnus mulch. Except for plants grown in alnus mulch, those grown under bare soil and plastic mulch significantly lowered CI index values when combined with low tunnel. The highest benefit of low tunnel utilization was observed when combined with white or black PEP, with two-fold decrease in CI index values. In La Trinidad, no signs of chilling stress were observed on 'Lollo Rossa'.

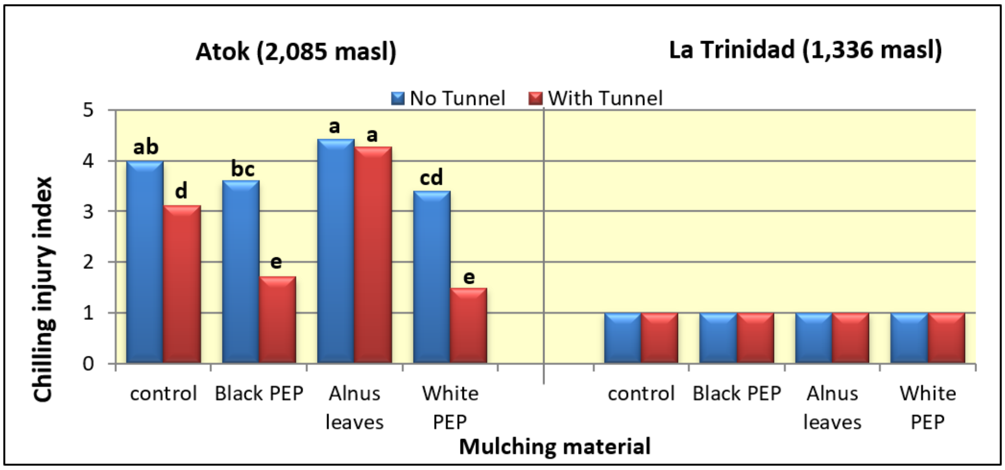

Fig. 5. Effect of low tunnel and mulching material on chilling injury index of 'Lollo Rossa' grown under chilling conditions in Paoay, Atok and Balili, La Trinidad, Benguet. Significant factors: interaction effect of low tunnel and mulch for 'Lollo Rossa' grown in Atok. Means with a common letter are not significantly different at 5% level using LSD. Legend: masl= meters above sea level.

The CI scores in this study were based on the severity of necrosis or stunting of growth. The combination of plastic mulch and low tunnel decreased CI index in 'Lollo Rossa'. A similar study showed the use of plastic mulches alone did not protect muskmelon plants from chilling injuries with seven sequential nights with temperatures between 1.6 and 1.8 °C. The combination of plastic mulches and tunnels protected the plants from chilling injuries (Jenni et al. 2003). Protected cultivation, through the use of mulch and low tunnel affected microclimate by increasing the temperature around the plants that may reduce cold damage (Faivor 2014; Maughan et al. 2014; Decoteau 2007).

Total phenolic content (TPC). Interaction effect of low tunnel and mulch was significant on the TPC of 'Lollo Rossa' grown in Atok (Fig. 6). The use of black or white PEP and alnus mulch significantly increased TPC in 'Lollo Rossa' grown in Atok relative to those grown in bare soil. Except for plants grown under alnus mulch which decreased significantly TPC when combined with low tunnel, the TPC of plants grown under plastic mulch or bare soil was not affected by low tunnel or mulching material. In La Trinidad, the main effects of low tunnel and mulching material separately affected TPC of 'Lollo Rossa'. The TPC in 'Lollo Rossa' was significantly higher when grown under low tunnel compared to those without. The use of plastic and alnus mulch increased significantly TPC in 'Lollo Rossa', where the use of white PEP obtained the highest TPC.

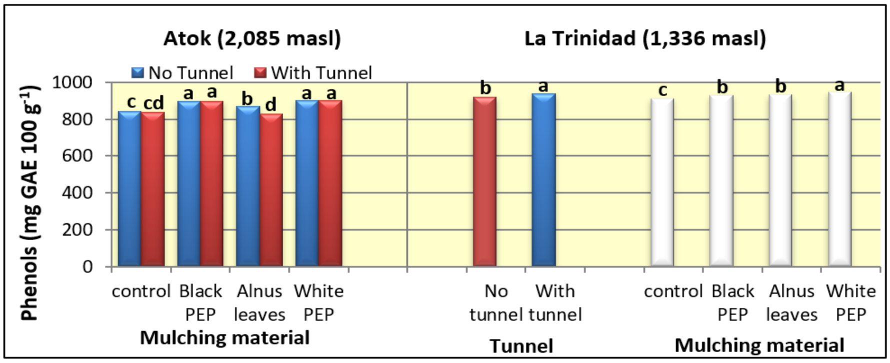

Fig. 6. Effect of low tunnel and mulching material on total phenolic content [mg gallic acid equivalent (GAE)/ 100 g] of 'Lollo Rossa' grown under chilling conditions in Paoay, Atok and Balili, La Trinidad, Benguet. Significant factors: interaction effect of low tunnel and mulch for 'Lollo Rossa' grown in Atok; significant main effects of low tunnel and mulch for 'Lollo Rossa' grown in La Trinidad. Means with a common letter are not significantly different at 5% level using LSD. Legend: masl= meters above sea level.

In this study, protected cultivation such as the use of mulch and low tunnel increased significantly the total phenolic compounds in 'Lollo Rossa' grown in Atok compared to that in bare soil, except for the use of low tunnel alone which was comparable to those grown in bare soil. Plants grown under low tunnel or the use of white PEP increased TPC relative to the control. Contrastingly, Oh et al. (2011) observed that the two lettuce cultivars, 'Baronet' and 'Red Sail', accumulated leaf phenolic concentration better when grown in open field than in high tunnel. The difference in response of lettuce may be due to the temperature at the time of the experiment. There was higher temperature of about 20°C during the conduct of the experiment of Oh et al. (2011) compared to the present study which was 15 °C. Furthermore, 'Baronet' and 'Red Sails' only obtained the highest value of TPC at about 35 and 70 mg GAE/ 100 g, while 955 mg GAE/ 100 g for 'Lollo Rossa' was noted in the present study. This implies that cold stress may have still prevailed even with the use of mulch or low tunnel.

Total flavonoid content (TFC). The interaction effect of low tunnel and mulch was significant for 'Lollo Rossa' grown in Atok and La Trinidad (Fig. 7). The use of plastic or alnus mulch significantly increased TFC in 'Lollo Rossa' grown in Atok relative to the control. The TFC further increased when combined with low tunnel. In La Trinidad, the use of plastic or alnus mulch slightly increased the TFC in 'Lollo Rossa' compared to the control. Those grown in bare soil and black PEP mulch significantly increased in TFC when combined with low tunnel, while the TFC of those grown under white PEP and alnus mulch was not affected when combined with low tunnel. The most bioactive secondary metabolites in plants are flavonoids. The biosynthesis of flavonoids is often greatly stimulated in stress-related conditions (Agati et al. 2012). Aside from environmental factors, varietal or genetic factor can influence the biosynthesis and accumulation of secondary metabolites (Yang et al. 2018). García-Macías et al. (2007) added that the intervarietal genetic variation is in general more critical than environmental influences. In this study, the TFC of 'Lollo Rossa', taken 40 days after transplanting, ranged from 246.5 to 262.7 mg quercetin equivalent (QE)/ 100 g. On the other hand, flavonoid content was found to be 138.4 mg QE/ 100 g and 20.7 mg QE/ 100 g in red tissues of 'Lollo Rosso' (Ferreres et al. 1997; DuPont et al. 2000). Furthermore, 'Lollo Rosso' lettuce cultivated under UV block, UV low and UV window plastic film accumulated flavonoid averaging at 38.2, 141.2 and 182.5 QE/ 100 g, respectively (García-Macías et al. 2007).

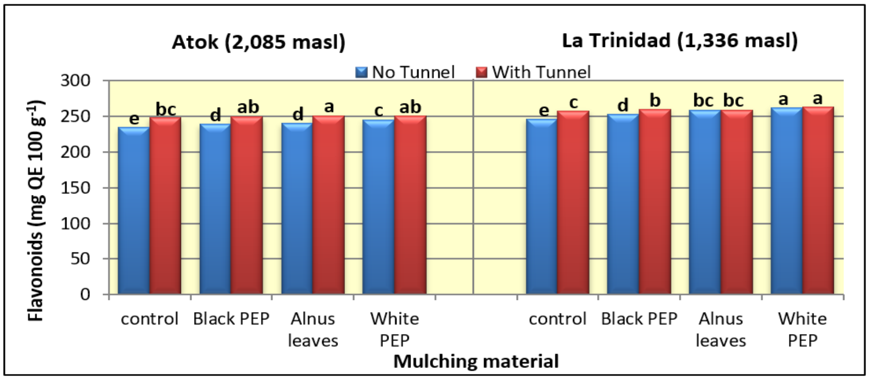

Fig. 7. Interaction effect of low tunnel and mulching material on total flavonoid content [mg quercetin equivalent (QE) 100 g^{-1}] of 'Lollo Rossa' grown under chilling conditions in Paoay, Atok and Balili, La Trinidad, Benguet. Means with a common letter are not significantly different at 5% level using LSD. Legend: masl= meters above sea level.

Antioxidant activity. Low tunnel and mulch separately affected the antioxidant activity of 'Lollo Rossa' grown in Atok (Fig. 8). The use of white PEP or alnus mulch had significantly higher antioxidant activity in 'Lollo Rossa' grown in Atok relative to those grown in bare soil. Plants grown in mulching materials significantly increased in antioxidant activity when combined with low tunnel. Antioxidant activity in 'Lollo Rossa' grown in La Trinidad was influenced separately by the main effects of low tunnel and mulching material. Antioxidant activity was significantly higher in lettuce grown under low tunnel compared to those grown without, while plastic and alnus mulch significantly increased antioxidant activity in lettuce. The average antioxidant activity in 'Lollo Rossa' grown in Atok and La Trinidad was 80 and 87%, respectively.

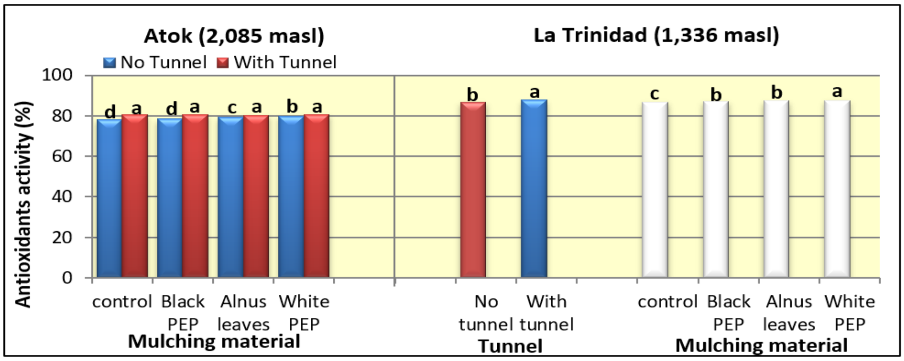

Fig. 8. Effect of low tunnel and mulching material on antioxidant activity of 'Lollo Rossa' grown under chilling conditions in Paoay, Atok and Balili, La Trinidad, Benguet. Significant factors: interaction effects of low tunnel and mulch for 'Lollo Rossa' grown in Atok; main effects of low tunnel and mulch for 'Lollo Rossa' grown in La Trinidad. Means with a common letter are not significantly different at 5% level using LSD. Legend: masl= meters above sea level.

The use of low tunnel alone or combined with mulch significantly increased the antioxidant activity of 'Lollo Rossa' grown in Atok as compared to the control. In La Trinidad, there was an increase in antioxidant activity from 'Lollo Rossa' grown in low tunnel. In contrast with antioxidant activities recorded from Atok and La Trinidad, two lettuce cultivars, 'Baronet' and 'Red Sail', accumulated phenols and antioxidants better when grown in open field than in high tunnel. In addition, antioxidant capacity followed the trend in the accumulation of total phenols (Oh et al. 2011). In this study, there was an increase in total phenolic content with increasing antioxidant activity. This trend was followed by 'Lollo Rossa' grown in both locations under low tunnel, white PEP or alnus mulch alone. Previous studies showed a larger overall effect for some combinations of antioxidants compared to the effect expected from a simple addition of the effects of the individual antioxidants (Uri 1961).

CONCLUSION

The use of low tunnel or mulching material was beneficial in 'Lollo Rossa' on TPC, TFC, and antioxidant activity grown in Atok and La Trinidad, Benguet, while the number of leaves, shoot fresh and dry weight, yield, chilling injury was in Atok conditions only. In general, white PEP mulch was advantageous when used in Atok, while black PEP mulch was more beneficial when used in La Trinidad. Overall, the use of alnus mulch against chilling stress was ineffective.

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THE FINANCIAL VIABILITY OF MULTI-ROW ONION SEEDER: THE CASE OF PANGASINAN, PHILIPPINES

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ABSTRACT

A multi-row onion seeder (MROS) was designed and developed to address the inefficiencies of the current practice of direct seeding method of bulb onion. Based on field evaluation, the MROS was found to be technically feasible passing the minimum standard prescribed under the Philippine Agricultural Engineering Standard (PAES 123:2001). A technically feasible machine needs financial evaluation as it provides useful input decision to farmers and other stakeholders in adopting the technology. This paper discusses the financial viability of MROS by accounting for the direct costs and benefits associated with the use of the seeder in comparison with the traditional manual broadcasting method (MBM). These were considered from two perspectives, that is, a private investor operating the MROS as service provider analysed using investment analysis, and from the point of view of onion farmer adopting the MROS over the traditional MBM, analyzed using partial budget analysis. The study was conducted in 2017 in Pangasinan, a major onion producing province in the Philippines that predominantly practice direct seeding of onion. The adoption of MROS and replacement of the traditional MBM would provide farmer benefits coming from reduction in labor cost, reduced seed requirement and increase in average yield estimated at PhP47,973.95/ha. Investment analysis revealed that it is financially viable given an internal rate of return of 48% and BCR of 1.77. The minimum efficient level of service area that would make its operation viable is 40 hectares per season. This condition must be satisfied in areas where it will operate to attain the potential benefits of the MROS. The following information can be used for the wider adoption and promotion of the MROS in areas where MBM is still practiced.

Key words: financial viability, labor shortage, manual broadcasting method, multi-row onion seeder

INTRODUCTION

Onion (*Allium cepa L.*) is an essential condiment and is regarded as one of the high value crops strongly supported by the Department of Agriculture (DA) of the Philippines. In 2018, the country registered a total production of 172, 665 MT that was harvested in 17,904.81 ha with a recorded average yield of 9.64 MT/ha. Central Luzon is the highest onion producing region accounting for 61.53 % of the country's total production, followed by Ilocos Region and Cagayan Valley at 22.19 % and 5.12 %, respectively (PSA 2020). Onion is a capital-intensive crop to grow requiring an average capitalization of P180,000/ha/season. Of the total cost of production 42 %

accounts for the labor cost and more than half of this cost is incurred in planting (Antolin et al. 2017). Planting of onion is traditionally done manually making it costly considering the scarcity of labor in the area particularly during peak season One way to reduce labor cost in planting is through adoption of labor-saving technology and this can be done using the appropriate type of machinery (Goreppati et al. 2017) mechanizing the operation to save labor and time and even reduce the drudgery.

In 2017, the Philippine Center for Postharvest Development and Mechanization (PHilMech) of the Department of Agriculture, designed and developed a multi-row onion seeder (MROS) to address the inefficiencies of the traditional manual broadcasting method (MBM) of direct seeding. Among the major limitations associated with MBM is the scarcity of manual labor during peak planting season and high labor cost. This was corroborated by the study of Briones (2017) who had estimated that since 2011, there was a declining work force in agricultural sector with an average of 250,000 workers leaving the agriculture for other high paying jobs in other sectors.

The MROS developed by PHilMech was subjected to a series of field testing and was evaluated based on the following technical parameters: a) actual delivery/seeding rate; b) actual field capacity (AFC); c) theoretical field capacity (TFC); d) field efficiency, and e) fuel consumption, as prescribed under Philippine Agricultural Engineering Standards (PAES 123:2001) passing all the technical parameters. As a proof of innovativeness and novelty in design, the MROS application for patent was granted by the Intellectual Property of the Philippines (IPOPhil) in 2021.

While the MROS was found to be technically acceptable, another equally important aspect of the technology that must be satisfied is its financial viability. To attain this, MROS was pilot tested in Pangasinan, a major producer of bulb onion in Region I. In 2018, Pangasinan recorded a harvest of 2,488.66 MT (PSA 2020). One unique characteristic of this production area is almost all of the farmers practice the traditional MBM. One of the limitations identified in this method of planting is the relatively higher seed and labor requirement. During planting season local farmers were forced to source-out laborers from neighboring areas to address the manual labor deficit causing delays in planting operation as well as increasing expenses incurred for planting. Given this condition, Pangasinan became the appropriate study area to pilot test the MROS.

This paper discussed the financial viability of MROS in areas where MBM is predominantly practiced. Specifically, it sought to evaluate its viability from two different perspectives, that is: 1) from the point of view of onion farmer who will be adopting the MROS over the traditional MBM, and 2) from the point of view of a private investor who will invest in MROS and operate the seeder as a service provider.

METHODOLOGY

Conceptual framework. The study compared the technical and financial performance of MROS with the MBM (Fig. 1). The financial viability of MROS was assessed using the technical and financial data gathered during the pilot testing of the machine. The study performed costs and returns analysis, partial budget analysis and investment analysis. The financial viability of using MROS was evaluated from two perspectives, that is: 1) from the point of view of a farmer adopting the MROS, analysed using partial budget analysis; and 2) from the point of view of a private investor investing in MROS and operating as service provider, analysed using investment analysis.

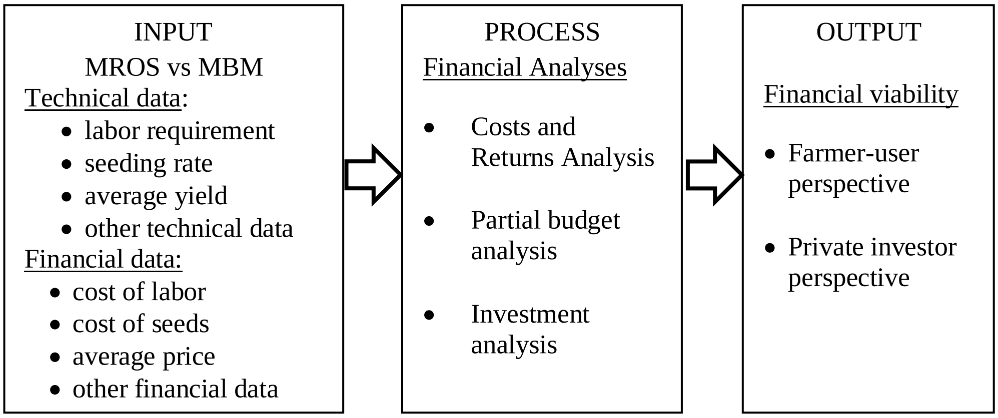

Fig. 1. Conceptual framework of the study.

Time and place of the study. The study was conducted in Pangasinan in 2017 in cooperation with onion farmers as project co-operators. The co-operators were selected based on three criteria: a regular onion farmer that practices direct seeding, willing to use the MROS and availability of required production area where both MBM and MROS can be applied at the same time for direct seeding. With only the two types of direct seeding method, that is MBM and MROS, as the only source of variation, the project cooperators applied the traditional production practice such as cropping calendar, onion varieties planted, land preparation techniques, rate of fertilizer and chemical applications, water management, and harvest practices.

Data collected. The study used primary and secondary data. Primary data were gathered from the field by actual measurement and observation of technical parameters such as labor used, seeding rate, planting density and average yield, among others. Farmers' survey with the aid of structure questionnaire was conducted to obtain information on socio-economic data and production-related practices. For financial data such as average cost incurred for seeds, fertilizers, chemicals, packaging material/red sacks, labor for land preparation, furrowing, boxing, planting, weeding, thinning, fertilizer and chemical application, harvesting, cutting and sorting as well as irrigation, food and interest on loan, average farm-gate price. The following information were obtained from actual observations, key informants interview and secondary data from the Provincial Agriculturist Office.

Manual broadcasting method. MBM is the predominant method of planting practiced by farmers in the study area (Antolin et al. 2017). This method is a quick means of sowing onion seeds. In this method, seeds are broadcasted manually in well-pulverized and equally spaced raised beds. After sowing, onion seeds are covered with thin layer of fine soil using steel hoe. Since this operation is done manually, the spacing and the density of plants are dependent on the skill of the person doing the broadcasting. This practice of direct seeding often results to uneven spacing, overcrowding and non-uniformity of growth and development of plants, leading to low yield and consequently low income (Khan et al. 2015; Khan et al. 2016). Seed requirement using MBM ranges from 6.75 to 9 kg/ha (Idago et al. 2019). Also, this practice is labor-intensive requiring 25 to 35 persons to accomplish seed sowing, covering the seeds and creation of levee. Since the density of plants is uneven, additional labor for thinning is required 35 to 45 days after sowing to attain optimum plant spacing, increasing further the manual labor required.

MROS. The multi-row onion seeder is a tractor-hitched drill type seeder that is capable of opening furrow, delivering the seed, covering the seed and firming the bed in just one passing (Idago et al. 2019). It is designed with 10 units' seed delivery system hence it is sometimes called 10-row onion mechanical seeder. Each of the ten seed bin has a capacity of 500 grams thus in a single run it can directly sow 5kg of onion seeds in the field. The seeder has three major components, namely: soil-engaging component, seed metering system and distribution system. The seeding rate ranges from 3.60 to 4.80 kg/hectare and requires 1 to 2 persons to operate (Dela Cruz et al. 2017). This method of planting does not require thinning after planting since the onion seeds are delivered equally-spaced and seeding rate is already pre-adjusted based on the requirement.

Table 1 shows the technical performance of MROS based on the study conducted by Idago et al. (2019). Results of the study indicated that the MROS has an effective field capacity 0.41ha/hr, field efficiency of 77.23%, fuel consumption of 2.45 L/ha, and seeding rate of 4.80 kg/ha. In comparison with manual broadcasting, the MROS had higher field capacity, lower seeding rate and lower labor requirements by 2.44 person-days/ha (Table 2).

Table 1. Summary of the field performance of MROS, 2017.

Parameters	Mean values across farms
1. Area (m^2)	1,158
2. Time of operation (min)	17.58
3. Speed (km/h)	3.35
4. Effective field capacity, ha/hr	0.41
5. Theoretical field capacity, ha/hr	0.53
6. Field efficiency, %	77.23
7. Seeding rate, kg/ha	4.80
8. Fuel consumption, L/ha	2.45

Source: Idago et al. (2019)

Table 2. Comparison of MROS and MBM in Pangasinan, 2017.

Method of Direct Seeding	Actual field capacity (ha/hr)	Seeding rate (kg/ha)	Labor requirements (person-days/ha)
MROS	0.37	4.94	0.90
MBM	0.35	7.68	3.34
Difference	0.06*	-2.74*	-2.44

Note: * Significant at 5% level.
Source: Idago et al (2019)

Assumptions used in assessing the financial viability of MROS. The data used in the assessment of MROS' financial viability were based on the results of the pilot testing from farmer co-operators and the technical performance of the MROS (Table 1) from the study of Idago et al. (2019). In one of the analyses using farmer-user perspective, it is assumed that MROS will replace the traditional MBM and this will have corresponding implications on the amount of labor used, seeding rate (Table 2) as well as average yield which would determine if the adopter will be better-off using the proposed technology. In addition, the analysis using private investor's perspective assumed that the MROS will be operated as a service provider using service rate that is lower than the existing rate used for MBM. This will create incentive and will therefore facilitate its acceptance and adoption. The capacity of the seeder is 0.37 ha/hr (Table 2) and will be operated for 20 days per season.

Using MROS the cost of seeding and land preparation is reduced. The MBM needs 6.75 to 9 kg /ha and requires about 25 to 35 persons to plant and cover the seeds per hectare. MROS required 3.60 to 4.5 kg/ha and 1 to 2 persons to operate the machine. The reduced labor will be coming from operations eliminated such as: labor from MBM, boxing and thinning. On the other hand there will be additional costs incurred from service fee for the use of MROS, furrowing, harvesting fee, tying and hauling, and cost of packaging/red bag. The material inputs (i.e. fertilizers, chemicals) are all the same except for the seed requirement.

To analyze the changes in costs and benefits associated with the use of MROS partial budget analysis was used. This method compares the marginal cost of an activity within a certain enterprise with the marginal increase in the benefit that the new activity will provide (Gittinger 1982). It measures the changes in income resulting from the proposed intervention. The partial analysis was computed using the formula below:

$$I = (Ra + Cr) - (Rr + Ca)$$

where:

I = incremental income
Ra = added revenue
Cr = reduced cost
Rr= reduced revenue
Ca= added cost

Financial viability. The financial viability of MROS was assessed from the point of view of private investor operating the seeder as service provider. It is assumed that the seeder will be operating for about 20 days/cropping. Based on its capacity its effective area coverage per year is 40 has. The cost of MROS is PhP140,000 and the minimum working capital is PhP7,000 to cover for the initial cost of fuel, operator and other incidental expenses. Other costs that were included in the financial analysis were: depreciation cost, repair and maintenance, and miscellaneous expenses which included the cost of barangay permit and other expenses. Sensitivity analyses were also performed to determine the sensitivity of this type of investment to other variables specifically by applying different levels of area coverage and service rates.

The financial viability indicators used were benefit-cost ratio (BCR and internal rate of return (IRR). The benefit cost ratio (BCR) is obtained when the current worth of the benefit is divided by the current worth of the cost (Gittinger 1982). When the BCR is less than 1, the costs of the project outweigh the benefits of a proposed project. BCR was computed using the equation below:

$$\frac{\sum_{t=n}^{t=1} \frac{B_t}{(1+i)^t}}{\sum_{t=n}^{t=1} \frac{C_t}{(1+i)^t}}$$

The internal rate of return is "rate of return on capital outstanding per period while it is invested in the project" (Merret and Skyes 1973). It is the rate of return in which the project is able to generate, computed as follows:

$$\sum_{t=1}^{t=n} \frac{B_t - C_t}{(1+i)^t} = 0$$

Where: $B_t =$ benefit each year
C_t = cost in each year
t = 1, 2… n
n = number of years
I = interest discount rate

RESULTS AND DISCUSSION

Cost and returns analysis. The use of MROS can reduce the cost of production and increase income (Table 3). The decrease in the expenses can be attributed to the reduction in the quantity of seed requirements and labor. The seed requirement of using MROS is 2.74 kg/ha less than MBM. In a similar study, the use of mechanized planting reduced the quantity of seed requirements in comparison with MBM by 1.6 to 2 kg/ha (Austria et al. 2020).

For labor requirement, MBM needed 25 to 35 persons/ha in sowing seeds, furrowing and boxing compared to MROS wherein 1 to 2 persons were needed. Onion is a high labor intensive crop and around 50 % of total production cost can be attributed to labor cost in planting (Mahajan et al. 2017). The high labor requirement in MBM is one of the problems of the farmer-growers. The farmer-growers needed to outsource laborers to plant onion in neighboring municipalities/provinces. In Pangasinan, hired laborers from Nueva Ecija were necessary because of insufficient labor within the area. The lack of labor resulted in delayed planting and high labor cost due to transportation and meal expenses. In addition, the use of MROS eliminated the cost of labor in thinning. MBM dropped many seeds in a single place which required thinning and increased labor cost (Gorrepati et al. 2017).

The use of MROS showed equal distance of planting and consistent depth of planting. When using MBM, there is tendency for the laborer to uproot onion plant because of uneven distance. Hence, the potential yield of MROS is higher by 1.16 MT/ha. The marketable bulbs using MROS and MBM are 92.5 % and 90.7 % respectively. The total cost of producing onion in a hectare using MROS is PhP149, 717 while in MBM is PhP180, 234, difference of PhP30, 517.

Table 3. Costs and returns analysis on the use of MROS and MBM, Pangasinan, 2017.

Item	Planting Method		Difference	Percent
	MROS	MBM	A-B	Change
Materials (PhP)				
Seeds	27,599.10	46,560.40	(18,961.30)	-40.70
Fertilizer	25,780.00	25,780.00		
Pesticides	6,340.00	6,340.00		
Herbicides	2,720.00	2,720.00		
Packaging materials/red bag	10,260.00	9,792.00	468.00	4.8
Cost of labor (PhP)				
Land preparation	7,200.00	7,200.00		
Levelling	1,500.00	1,500.00		
Furrowing & boxing	1,200.00	2,400.00	(1,200.00)	-50
Planting/seeding	3,500.00	5,200.00	(1,700.00)	-32.7
Weeding	6,000.00	6,000.00		
Thinning		12,000.00	(12,000.00)	-100
Fertilizers & chemicals application	9,000.00	9,000.00		
Harvesting, cutting & sorting	25,650.00	24,480.00	1,170.00	4.8
Tying & hauling	10,260.00	9,792.00	468.00	4.8
Other expenses (PhP)				
Interest	3,750.00	3,750.00		
Irrigation	6,720.00	6,720.00		
Food		1000.00	(1,000.00)	-100
Depreciation cost	2,238.00		2,238.00	100

Item	Planting Method MROS	MBM	Difference A-B	Percent Change
Total cost/ha (TC) PhP	149,717.10	180,234.00	30,517.30	-16.9
Yield per ha (kg)	25,662.58	24,460.60	1,161.98	4.8
Price: Good @PhP23.00/kg	540,520.44	518,880.68	21,639.76	4.2
Bottleneck & pickles @PhP15/kg	31,825.47	28,508.58	3,316.89	11.6
Excess planting materials for thinning		7,500.00	(7,500.00)	-100
Gross income/ha (PhP)	572,345.91	554,889.26	17,456.65	3.1
Net Income/ha (PhP)	422,628.00	374,654.86	47,973.95	12.8
Cost/kg (PhP) (TC/PY)	5.84	7.21	1.37	-19
Net income (PhP)	16.49	15.32	1.18	7.7

Financial attractiveness of MROS to farmers. Using partial budget analysis, the effects of adopting MROS was measured particularly by the changes in the cost, its effect on yield and income. The use of MROS, provide an additional income of PhP47, 973.95 (Table 4). The increase in income emanated from reduction of expenses of around PhP30,517.30, increase in yield of around 1,161.98 kilograms valued at PhP24, 956.65 and sale of excess onion planting materials from thinning sold at PhP 7,500 Other benefits derived by the farmers in using MROS are: less time required to locate and schedule laborers to broadcast and thin out.

Table 4. Partial budget analysis of MROS and MBM of planting onion, Pangasinan, 2017.

Incremental Benefits (IB) PhP		Incremental Cost (IC) PhP	
Added benefits/ha	24,956. 65	Added cost/ha:	9,044.00
Good 28.83bags x 30kg/bag x PhP23.0/kg	21,639.76	Service fee for MROS/ha	3,500.00
Class B 6.79bags x 30kg/bag x PhP15.0/kg	3,316.89	Furrowing, 4WT	1,200.00
		Harvesting Fee, 35.62 bags x PhP32.84.00	1,170.00
		Tying & hauling, 1.78/bag x 36 bags	468.00
		Red bag, 38.73 bags x PhP12.00/bag	468.00
		Depreciation cost	2,238.00
Reduced cost/ha	39,561.30	Reduced benefits/ha	7,500.00
Boxing, 80 boxes x PhP30.00/box	2,400.00	5 sacks of excess planting material	7,500.00
Broadcasting, 80 boxes x PhP65.00/box	5,200.00	(5 sacks x P1,500.00/sack)	
Thinning	12,000.00		
Seeds, 10.95 cans x PhP1,850.00/can	18,961.30		
Other indirect expenses	1,000.00		
Subtotal	64,517.95	Subtotal	16,544.00
Net change in income (IB-IC=Δ in income)		47,973.95	

Financial analysis of MROS for custom servicing. Investing in MROS is financially viable with a BCR of 1.77 and an IRR of 48 %. A BCR of 1.77 indicates that there is a net return of 0.77 for every unit of peso invested. The IRR of 48% is higher than the existing hurdle rate which was pegged at 10% (Table 5). Sensitivity analysis showed the project must operate beyond 67% of the projected service area (40has) or equivalent to service area greater than 27 hectares per year to remain viable (Fig.2). The effect of rate of service fee on the financial viability is presented in Fig. 3. The result suggests that the minimum service fee that should be charged per hectare is PhP2, 500. Operating below this level would result to financial loss. Interestingly, this rate is reasonable since the rate of being charged using the traditional MBM to plant a hectare is PhP5, 200 which means that promotion of MROS will be easier because of the obvious incentive over the traditional method (Fig 3).

Table 5. Investment analysis of MROS, 2017.

Particular	PhP/year	PhP/year
Investment cost		147,000.00
MROS	140,000.00	
Operating capital	7,000.00	
Fixed costs		35,000.00
Depreciation	14,000.00	
Repair & maintenance	21,000.00	
Variable costs		59,200.00
Salaries and wages	44,000.00	
Miscellaneous costs	15,200.00	
Total operating costs		94,200.00
Area coverage per year		
Service area	40	
Service charge/ha (PhP/ha)	3,500.00	
Gross income		140,000.00
Net income		45,800.00
Benefit cost ratio		1.77
Internal rate of return		48%
Breakeven hectare/year		26.91
Breakeven service fee (PhP/ha)		2,355.00

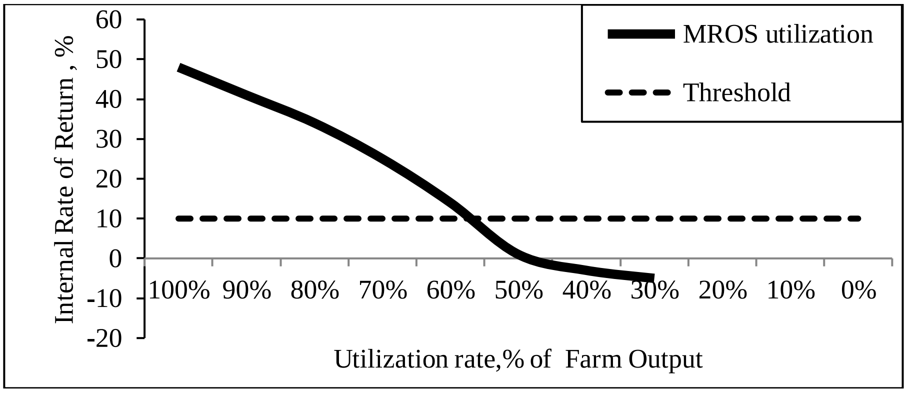

Fig. 2. Effect of utilization rate on IRR.

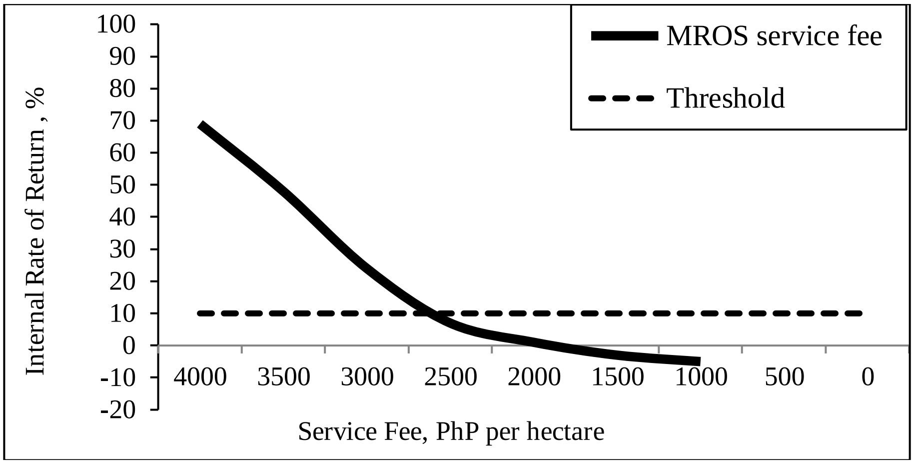

Fig. 3. Effect of service fee on IRR.

CONCLUSION AND RECOMMENDATIONS

This paper discussed the financial viability of MROS, a technology developed by PHilMech to address the inefficiencies of the current direct seeding method using the traditional MBM. The MROS was pilot tested in Pangasinan in 2017 where farmer cooperators operated the seeder for a year applying their production and cultural management techniques. The financial viability was assessed from two perspectives, that is: 1) from the point of view of farmer-user, and 2) from the point of view of a private investor operating as service provider. Using the data generated from the pilot testing and available data on technical performance, the financial viability was assessed using costs and returns analysis, partial budget analysis, and investment analysis. Farmers adopting the MROS over the traditional MBM would be better-off because of the advantages offered from reduced labor costs, reduced seed requirement and increase in average yield. From investor's point of view, investing in MROS is financially viable as indicated by an IRR of 48% and a BCR of 1.77. Based on the results of the sensitivity analyses, the minimum service coverage should not be less than 27 has and service rate of not lower than PhP2,500/ha. These conditions must be satisfied for the MROS operated as a service provider to remain viable. The information generated from this study can be valuable inputs for identifying specific onion producing areas where the potential benefits of this technology can be effectively maximized.

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EFFECTS OF LARVAL DIETS ON GROWTH AND DEVELOPMENT OF *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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ABSTRACT

The fall armyworm, *Spodoptera frugiperda* (*J.E.* Smith) (FAW) is an invasive pest of corn worldwide. Due to alarming spread and continuous damage in newer geographical regions in Africa and in Asia including the Philippines, it becomes imperative to understand growth and development of this invasive species in selected crops. In this study we investigated the effect of artificial diet and corn leaf on growth and development of *S. frugiperda* larvae and generated information on development time, larval survival and pupation, weight of pupa, reproduction (fecundity) and adult longevity. The artificial diet ingredients consisted of soybean flour, wheat germ, mineral salt, sucrose, vitamin mix, agar, methyl paraben, sorbic acid, aureomycin, and calcium propionate; while young leaves of corn (10-15 days) were used as natural food Larvae fed with the artificial diet showed a significant shorter larval duration and produced heavier pupae than those fed with leaves of corn seedlings. Fecundity of the female adults averaging 1,471.6 ± 365.48 eggs/female from larvae reared on artificial diet was not significantly different to the natural food (X=1,534.36 ± 742.50 eggs per female). Moreover, larval survival (87.33 %), pupation rate (86.67 %), adult emergence (100 %) was also high in the artificial diet. These results suggest that the pupal weights, fecundity, and survivability of *S. frugiperda* is significantly affected by larval diets. In addition, the artificial diet as a substitute for the natural food of *S. frugiperda* is suitable for rearing this pest successfully under laboratory conditions. Information from this study was discussed in terms of biological attributes of fall armyworm and why larval growth and development are important in colony maintenance under laboratory conditions.

Key words: fall armyworm, biological control, invasive pest, artificial diet, rearing technique

INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a polyphagous migratory pest native to the North and South America (Montezano et al. 2018; Igyuve et al. 2018). It is recently considered as the most important lepidopterous pest of corn (*Zea mays* L.) in the Philippines (Navasero et al. 2019; IPPC 2019) and since its detection in 2019, FAW has been monitored across the country damaging corn, sugarcane and rice. Research showed that control of *S. frugiperda* in corn mainly used chemical insecticides, but continuous use of these products may lead to development of insect resistance, negative impacts to beneficial insects, accumulation of residues in food, water and soil and air pollution. To reduce such effects, but at the same time, mitigate *S. frugiperda*, strategies as biological control, using entomopathogenic fungi and viruses have been explored (Navasero et al. 2020, 2021; Montecalvo and Navasero 2021).

Spodoptera frugiperda is adaptable to climate conditions in the Philippines and highly suitable for FAW population establishment all year round. It has high reproductive potential, short life cycle, and easy to handle. The biology of *S. frugiperda* had been reviewed by Sparks (1979) in the United States, as follows: it has no diapause mechanism and overwinters in south Florida and Texas where hosts are continually available and temperatures below 50 ^{0}F are rare; eggs are laid in clusters and densely covered with scales containing from a few to hundreds of eggs which hatched in 2-4 days; as larvae hatched from the eggs, they eat the chorions, and then continue to feed on leaves until they completed six instars and pupated, mostly in the soil. During warm weather, the life cycle requires about four weeks but longer during colder seasons. In Sub-Saharan Africa, on the other hand, the development cycle of FAW takes 25 days on average the durations of the different stages are distributed as follow: egg- 5days, larva-14d, pupa-7d and adult-16d (Tendeng et al. 2019). In the Philippines, Navasero and Navasero (2020) studied the life history and morphometry of FAW in the laboratory in native variety of corn (Lagkitan) and some natural enemies of *S.*

frugiperda population from the field, collected from Gonzaga, Cagayan. Briefly, eggs are laid in mass which hatched in 2-3 days; six larval instars in 14 days, on average; prepupa- one day; pupa-nine days; fecundity ranging 800 to 1,639 eggs per female; adult longevity of 10.18 days for the male and 9.82 days for the female. Efforts were made to mass rear this insect for efficacy testing of entomopathogenic fungi, bacteria, nematodes and viruses to establish their virulence levels against the different life stages of the pest.

We reviewed techniques for rearing *S. frugiperda* using artificial diets and natural food as well (Ashok et al. 2021; Lekha et al. 2019; Pinto et al. 2019; Modalon et al. 2017; Da Silva and Parra 2013; Tefera et al. 2011; Vilarinho et al. 2011; Busato et al. 2006; Chapman et al. 2000; Lynch et al. 1989; Mihn 1983; Perkins 1979; Burton and Perkins 1972; Ravelo and Raur 1967; Burton 1967; Burton and Cox 1966; and Hale 1965). However, none of them used a standardized artificial diet similar to the diet used in this study, that is easy to cook or prepare (about four minutes) and can be dispensed easily into rearing units and fed to developing FAW neonates and growing larvae. Most importantly, the diet used was very palatable to FAW neonates as they readily acclimatized and fed.

This study evaluated a standardized artificial diet as compared to its natural food (corn leaf) for laboratory rearing of *S. frugiperda* to ensure a continuous supply of homogenous and stage specific insects for efficacy testing and for colony maintenance in the laboratory. Information from this study would allow us to have a better understanding on FAW survival, reproductive behavior and performance of its offspring.

MATERIALS AND METHODS

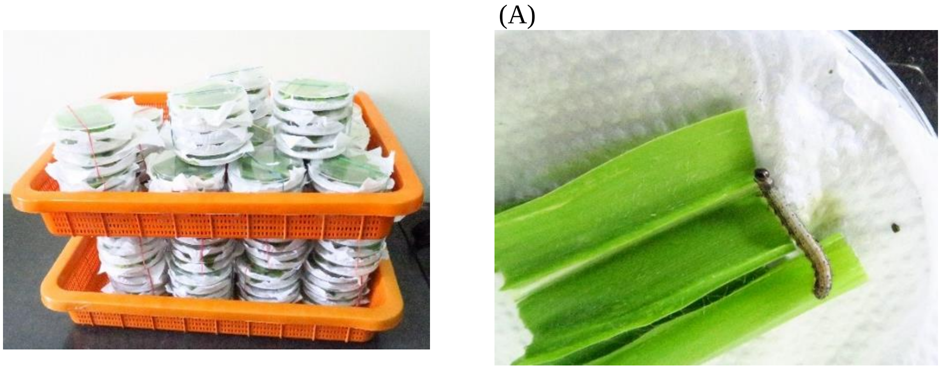
Standard artificial diet composition and preparation. The ingredients of the commercially available standardized artificial diet (Insect Media^{R}) were soybean flour, wheat germ, mineral salt, vitamin mix, agar, methyl paraben, sorbic acid, aureomycin and calcium propionate. To prepare a liter of diet, 162g of the artificial diet was diluted with 930ml boiled distilled water and blended thoroughly for about three minutes at a high speed. While hot, the mixture was poured into sterilized plastic container (21 cm in length x 14.5 wide x 9.5 in height) and allowed to cool. The diet was refrigerated until use after solidifying at room temperature. The diet was removed from the fridge and conditioned at room temperature for 2-3h before use. The solidified diet was cut into pieces of about 2g and transferred to sterilized plastic cups (38.4mm, bottom x 47.8mm, top x 43.2mm high) for larval feeding.

Preparation of natural diet, corn. A local variety of corn, Lagkitan, was planted at high density in plots on a weekly interval as source of larval food. Excised leaves were cut into small pieces, 5cm long, disinfected in 0.5% sodium hypochlorite for 10 min, and washed two times with sterile distilled water. Finally, these were blot- dried with sterile tissue paper, prior to use.

Stock culture of Insect. Egg clusters of fall armyworm were obtained from an established colony maintained on leaves of Lagkitan as food at the National Crop Protection Center, College of Agriculture and Food Science, University of the Philippines Los Baños. Neonates were reared for one generation for acclimatization on the standardized diet and the following generation was evaluated. Neonate larvae were used in the feeding test. Neonates fed with young leaves of corn were hatched from egg clusters directly obtained from the stock culture maintained on leaves of Lagkitan.

Feeding test. Neonates hatched on the same day were individually placed in rearing cups containing 2g diet until pupation (Fig. 1). The opening of each cup was covered with sterilized tissue paper and perforated lid. Pupation occurred inside the pupal cells formed with artificial diet and frass. On the third day of pupation, the pupae were removed from the pupal cells, sex determined, weighed, and kept in the same rearing cup, cleaned off unspent diet and frass, until adult emergence. A male and a female adult emerging on the same day were paired in a cylindrical plastic cage (8.93 cm inside diameter, 11 cm high) and fed with 10% sucrose. In another set-up, larval feeding on the natural diet (corn) was prepared by placing one neonate into a plastic plate (9cm diameter x 1.5cm thickness) containing pieces of leaves taken from seedlings at 10-15 DAS (days after sowing). Daily thereafter, o fresh pieces of leaves were supplied to replace the previous ones, the plate cleaned off spent food and frass, until pupation. Fresh leaves were supplied daily since young leaves of corn dried up easily and not palatable to larvae anymore. Moreover, during the fourth larval stage, larvae consumed mostly all the pieces of leaves offered, hence the need for additional food. Pupation occurred inside the pupal cell formed with uneaten leaves or tissue lining of the plate. On the third day of pupation, pupae were removed from the pupal cells, sexed, weighed, and kept in plastic plate until adult emergence. As in the artificial diet, a male and a female adult emerging on the same day from this food source, were paired in cylindrical plastic cage, fed with 10% sucrose for recording of eggs laid (fecundity) and longevity. The number of neonates tested for the standardized diet and natural diet was 300 and 200, respectively.

(A)



(B)

(C)

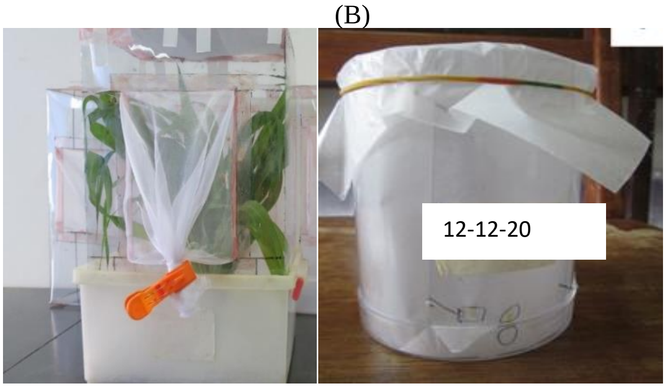


Fig. 1. Set-up for rearing *Spodoptera frugiperda* on artificial (standard) diet (A), natural diet (B), and oviposition cages (C) for multiple pairs (left) and individual pair (right).

Rearing in individual containers for *S. frugiperda* is commonly used (Pinto et al. 2020; Vilarinho et al., 2011; Chapman et al. 2000; Perkins 1979; Burton and Perkins 1972; Burton and Cox 1966, among others) due to cannibalism of older larvae, although Da Silva and Parra (2013) proved that cannibalism is not obligatory.

Biological attributes of *S. frugiperda* observed on the standardized artificial and natural diets were as follows: 1) larval periods, reckoned from the day of hatching until pupation, 2) pupal periods, duration of quiescent period until adult emergence, 3) pupal weights taken at 3d old, 4) percent pupation expressed as the proportion of larvae that developed into pupae, 5) percent larval survival, the proportion of larvae that successfully pupated, 6) percent adult emergence, the proportion of adults that successfully emerged from pupae, 7) fecundity (eggs/female), 8) longevity of male and female adults, reckoned from the day of emergence from pupa until death, 9) sex ratio or male to female ratio.

The growth index (GI) was calculated using the method of Setamou et al. (1999) where:

Larval growth index= % pupation/larval period, in days; Pupal growth index= % adult emergence/pupal periods, in days; Total development index= % Survival of larva/total development of larva and pupa, in days.

The experiments were performed under laboratory conditions at 27-28^{0}C, 65% RH and 12D:12L.

Statistical analysis. The effects of artificial/standardized and natural diets on growth and development (e. g. larval period, pupal period, adult emergence, pupal weight) of *S. frugiperda* were compared and analyzed using t-test for independent samples in SAS (SAS 2018). The test was carried out using 30 samples/replication per group.

RESULTS AND DISCUSSION

Comparative effect of artificial diet and corn leaf on growth and development of FAW larva. Larvae of *S. frugiperda* reared on the standard artificial diet appeared as healthy and active as the larvae reared on leaves of their natural host, corn. The two food sources are sufficient in terms of larval development time from larva to pupa for both males and females. Larval durations were significantly shorter in the standard artificial diet than the natural diet (corn) for both male (t=-5.97; df= 210; P<0.0001) and female (t=2.48; df= 187; P=0.0140) larvae (Table 1). However, pupal duration was significantly longer in males(9.36 d in corn, 8.62d in artificial diet, t= 8.53; df=213; P<0.0001) than in females(8.26 d in corn, 7.57 in artificial diet, t=8.48; df= 205; P<0.0001). In terms of total development (Larval duration + Pupal Period), generally, the two diets were equally efficient for both males (t=2.19; df= 212; P=0.2940) and females (t=7.99; df=202; P=7.99).

Table 1. Developmental periods of larvae and pupae of *Spodoptera frugiperda* reared on standardized artificial diet and natural food, corn.

Diet	Larval Development (Days ± SD)		Pupal Development (Days ± SD)		Total Development (Days ± SD)	
	Male	Female	Male	Female	Male	Female
Standard	13.02 ± 0.69b	13.32 ± 0.70b	8.62 ± 0.73a	7.57 ± 0.70a	21.89±0.60a	19.55±1.10a
Natural	12.52± 0.55a	12.24± 0.66a	9.36 ± 0.55b	8.26 ± 0.47b	21.65±1.02a	20.50±0.61a

Means for each parameter followed by a common letter are not significantly different for comparison between treatments within each column (t-test, P<0.05).

The survival of larvae on natural diet (98.91 %) was greater than the standard diet (87.33%) and no differences were observed on sex ratio on either food source (Table 2). Percent pupation was higher when larvae were reared in the natural diet than in the standard diet. For overall assessment of the effects of larval food, we also focused on the larval growth index, pupal growth index and total development index. The growth index emphasizes the importance of both survival and developmental time in measuring food quality and suitability (Setamou et al. 1999). Higher survival rates and shorter development times yields higher values, thus indicating better food quality. No statistical analysis was done on these indices because only one value was obtained for each food source.

Table 2. Percent larval survival, pupation, adult emergence, growth indices and sex ratio of *Spodoptera frugiperda* on standard and natural diets.

Parameters	Diet	
	Standard	Natural
Survival, larva (%)	87.33	98.91
Pupation (%)	86.67	91.00
Adult emergence (%)	100.00	93.91
Larval growth index	6.46	7.34
Pupal growth index	13.89	10.58
Total development index	4.03	4.43
Sex ratio (Male:Female)	1.04: 1	1.07: 1

Reports show that several diets for rearing *S. frugiperda* have been developed and used successfully: wheat germ-casein diet (Burton 1967), modified pinto diet (Burton 1969) and the wheat and-soy-blend diet (Burton and Perkins 1972). All performed equally well on *S. frugiperda* (Perkins et al. 1973). Recently, Pinto and co-workers (2019) studied a corn-based diet for *S. frugiperda* and recorded 94.7 ± 2.49 % larval survival and 89.3 ± 7.59% pupation. Lynch et al. (1989) reported that peanut and soybean meal were unsuitable as diet ingredients due to low survival rate of larvae.

The development of *S. frugiperda* in the standard artificial diet we used was shorter than that observed by Pinto et al. (2019) on their corn-based artificial diet. The latter compared their results to be similar to Giongo et al. (2015) who obtained a larval period of 17.25 days and a pupal weight of 279.51mg using the diet developed by Greene et al. (1976). He Li et al. (2021) on the other hand obtained a shorter larval period of 14.1 days for FAW using Green's artificial diet but similar to the values we obtained. On different artificial diets, Lekha et al. (2020) recorded larval periods between 14.0 days and 18.5 days on cowpea, chickpea, black gram, green gram and soybean- based diets.

In the present study, percent larval survival and pupation in the standard artificial diet were lower than the natural diet, however, the pupae produced all emerged into adults (100%). The larval and pupal growth indices were similar, in the standard and natural diets, but the pupation index was higher in the standard diet. Sex ratio in both diets conform to the conventional 1:1. Inbreeding depression was not observed, a situation generally observed after the fourth/fifth generation in the laboratory- reared colonies of the insect culture where larval survival and pupation decreased tremendously (Gupta et al. 1998).

Comparative effect of artificial diet and corn leaf on weight of FAW pupa. Female pupal weight of *S. frugiperda* was significantly higher in standard artificial diet, 248 ± 43.84mg and 207.6 ± 20.60 mg in corn (t=- 8.94; df=190; P<0.0001). Likewise, male pupae were significantly heavier in the standard artificial diet (245.8 ± 48.94 mg) than in the natural diet (251.7 ± 18.28 mg) (t=-6.39; df=177; P<0.0001) (Fig. 2A). When evaluating different artificial diets as food for *S. frugiperda* under laboratory conditions (temperature 25± 1^{0}C, 70± 10% RH and 12D:12L). Truzi et al. (2021) found a reduction in pupal weight in diet with double protein, whereas intermediate levels generated heavier pupae. Giongo et al. (2015) using an artificial diet developed by Greene et al. (1976) obtained pupae weighing 279.51mg, similar but higher than those obtained in the present study.

Comparative effect of artificial diet and corn on fecundity and adult longevity of FAW. Lifetime fecundity of females reared on artificial/standard diet during their larval stages was lower, (X= 1,471.6 ± 734 eggs/female) than in natural diet (corn) (X=1,534.36 ± 742.50 eggs/female) but the difference was not statistically significant (t=0.25; df=27;P=0.8027) (Fig. 2B). Likewise, longevity of adults was shorter on standard diet (11.67 ± 1.92 days) than in corn (12.25 ± 0.45 days) (Fig. 2C) . Likewise, the difference was not statistically significant (t=1.56; df=36; P=0.1273). Thus, it shows that the artificial diet is comparable to corn, its natural food, in providing the nutritional requirements of larvae needed by adult females for oviposition and survival.

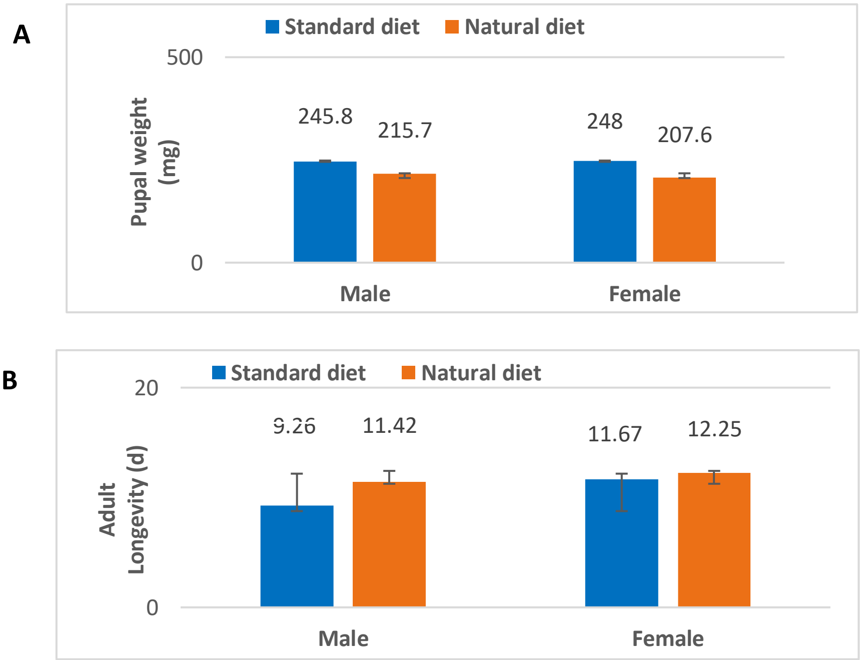


Fig. 2. (A) Pupal weight in mg (means±SD) of insects reared on artificial diet and natural food; (B) Adult longevity in days (means±SD) from adult emergence to death, of *Spodoptera frugiperda* moths obtained from larvae reared on artificial diet and natural food.

The general trend in insects is a positive relationship between pupal weight and adult fecundity, that is, heavy pupae produced bigger adults that lay more eggs (Miller et al. 1982; Bernardi et al. 2014). This relationship was not observed in the present study, since the diets offered during the larval stages did not significantly affect female fecundity and adult survival, possibly because the diets used resulted in similar pupal weight. However, the fecundities recorded in this study are within the range reported by other workers (between 1061.0 and 1850.0 eggs/per female) (Bernardi et al. 2014; Pinto et al. 2019) but higher than those reported by Truzi et al. (2021), varying from 592.9 and 667.5 eggs/female and 544.07 eggs by He Li et al. (2021). Furthermore, in this study, the fecundity of *S.*

frugiperda on the natural diet (1,534.36± 742.50 eggs/female) was higher than that reported by Murua et al. (2008), as follows: 955.05 eggs /female for population collected in maize, 555.89 in alfalfa, 519.83 in soybean, 978.10 in wheat, and 758.89 in weeds. Silva Lopez and co-workers (2008) reported a mean fecundity of 1,125 eggs/female in cassava leaves; 699 eggs/female on 2-leaf stage maize, 484 on 4 or 5 leaf stage corn, 525 on 2-leaf stage sorghum, 587 on 2-leaf stage wheat by He Li et al. (2021) for *S. frugiperda.*

Female longevities in this study are within the range reported by Truzi et al. (2021), ranging from 6.5 days to 11.5, but shorter than 17.0 days by Bernardi et al. (2014) and Pinto et al. (2019). This is possibly due to difference in rearing conditions, where room temperature was about 27-28ºC and 65% RH in our laboratory.

A diet is considered suitable for insect rearing if after undergoing evaluation biological parameters for survival, reproduction and behavior are satisfactory (Cohen 2001). Our findings suggest that the standard artificial diet evaluated supplied all the essential elements in balanced proportions needed for normal growth, development, and reproduction, allowing continuous rearing of *S. frugiperda* under laboratory conditions.

CONCLUSION

Spodoptera frugiperda reared on standard artificial diet produced healthy larvae and pupae which emerged into healthy adults that laid eggs comparable to those reared on natural food, corn. The diet was able to support growth and development of *S. frugiperda* ensuring continuous supply of homogenous stage specific insects for efficacy testing and for colony maintenance in laboratory.

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