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# FIRST RECORD ON THE OCCURRENCE OF Q BIOTYPE *BEMISIA TABACI* ON POTATOES IN SYRIA

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## ABSTRACT

In Syria, potatoes are usually infested with whiteflies, such as the sweet potato whitefly (*Bemisia tabaci*). We report the occurrence of a Q biotype of *B. tabaci*, which were collected in potato fields in Syria. The identification of the biotype was confirmed by mitochondrial cytochrome oxidase I (mtCOI) sequence analysis. The occurrence of whiteflies was observed in all of the 11 potato fields investigated from early September 2006, when the investigations in Syria began, to early November 2006, just before harvest. The cultivation system of the potato investigated in this study is generally called "autumn culture potato" because these are planted in August and harvested in November.

**Key words:** whitefly, identification, mtCOI, insect pest, Aleppo

## INTRODUCTION

In Syria, many kinds of crops, such as potato, tomato, cucumber, egg plant, green pepper, red pepper, tobacco, cotton, bean, sugar beet and sunflower, are cultured in a limited number of areas where irrigation systems are set up. Wheat and fruit trees are the main crops in non-irrigated areas. Among them, the potato (*Solanum tuberosum*) is one of the most prosperous crops in irrigated fields in Syria.

The General Organization for Seed Multiplication (GOSM), a national organization in Syria, exclusively imports, produces and distributes many kinds of crop seeds including seed potatoes (GOSM, 2006). Although a large quantity of seed potatoes are annually imported from Europe, the national project to switch from importation of seed potatoes to domestic production is now proceeding under a partial cooperation with the Japan International Cooperation Agency (JICA). However, during attempts of domestic production of potatoes, many kinds of viral, fungal and bacterial diseases and damage by insect pests result in serious yield losses (GOSM, 2005).

In Syria, the main diseases of potato are mosaic and other virus diseases, late blight, early blight, skin spot, *Rhizoctonia* canker, black scurf, silver scurf, common scab and bacterial tuber soft rot. The main insect pests are aphids, wireworms and the potato tuber moth (Netherlands Potato Consultative Institute, 1996; GOSM, 2005). Moreover, we observed frequently whiteflies on potatoes. This study was therefore conducted to assess the occurrence of whiteflies in potato fields in Syria.

In this study, samples were collected from whitefly-infested potato plants in Aleppo and Hama, Syria. Subsequently, molecular analysis was employed for identification of whitefly populations by comparison with a suite of reference sequences for well-studied biotypes and haplotypes (Brown, 2000). To our knowledge, this is the first report on the identification of Q biotype of *Bemisia tabaci* in Syria. Investigations to define their status as a pest were also carried out in potatoes cultured during autumn.

On the other hand, the greenhouse whitefly (*Trialeurodes vaporariorum*i) is recognized as a potato pest in Japan (The Japanese Society of Applied Entomology and Zoology, 2006). In our investigation until now, we could not find this species on potatoes in Syria. We, however, need to continue the survey to avoid unexpected damage by this pest.

The Q biotype of *B. tabaci* possesses the ability to transmit several viruses, and moreover, the resistance against chemical pesticides is stronger than other biotypes (Nauen et al., 2002;Horowitz et al., 2005). The management of this biotype is rather difficult, therefore, this biotype is recognized as a vicious and invasive insect pest. In our investigation, the Q biotype from the samples on potatoes in Syria was identified, so this information would basically be useful for managing this biotype.

## MATERIALS AND METHODS

Four autumn cultured potato fields (AG1, AG2, and AG3 in Aleppo and HG1 in Hama) under contract with GOSM and 7 fields (AF1, AF2, and AF3 in Aleppo and HF1, HF2, HF3, and HF4 in Hama) without contract were selected for collection of samples and assessment of the occurrence of whiteflies in 2006 (Table 1 and Fig. 1).

Almost all potatoes investigated were of the “Afamia” variety, which is extremely popular in Syria. Whiteflies were also collected in a 2.5m×8.5m net house (NH) located on the GOSM Tissue Culture Laboratory site in Aleppo. Aleppo is in the northern part and Hama in the midland of Syria (Fig. 1).


**Fig. 1.** Map showing the geographic locations in Syria from where whitefly populations were collected and assessed.

Six samples of whiteflies, two individuals per sample, were identified using mitochondrial cytochrome oxidase I (mtCOI) sequence analysis (Brown, 2000; Ueda and Brown, 2006). The numbers of adult whiteflies were counted on 200 random compound leaves in each of the selected fields once or twice per month, and the average number of whiteflies per leaf was estimated.

**Table 1.** Potato fields and potato varieties investigated in Syria in 2006.

| Area and field abbreviation | Field area (ha) | Potato variety |
|---|---|---|
| Aleppo | | |
| AG1 | 1.0 | Afamia |
| AG2 | 1.5 | Afamia |
| AG3 | 3.0 | Afamia |
| AF1 | 2.0 | Afamia |
| AF2 | 2.5 | Afamia, Merabl, Agria |
| AF3 | 30.0 | Afamia, Spunta, Marfona |
| Hama | | |
| HG1 | 2.0 | Afamia |
| HF1 | 1.5 | Afamia |
| HF2 | 2.0 | Afamia, Spunta |
| HF3 | 1.5 | Afamia |
| HF4 | 1.0 | Afamia |

## RESULTS AND DISCUSSION

Six samples of whiteflies were subjected to the mtCOI sequence analysis to confirm their biotype. The Q biotype of *B. tabaci* from the samples, which were collected from potatoes as sample No. 1 (Aleppo WF3-4), 3 (Aleppo WF7-4) and 6 (Hama WF2-3 and WF2-5), was the first confirmation of its presence in Syria (Table 2 and Fig. 2).

The sequences have been submitted to the DDBJ/GenBank databases under the following accession numbers: Aleppo WF3-4=AB297895, Aleppo WF7-4=AB297896, Hama WF2-3=AB297897, Hama WF2-5=AB297898.

**Table 2.** Identification of the whitefly populations collected on potatoes in Syria in 2006.

| Sample No. | Field abbreviation collected [1)] (*Bemisia tabaci* abbreviation) | Date collected | Biotype of *Bemisia tabaci* |
|---|---|---|---|
| 1 | AG1,AG2,AG3 (Aleppo WF3-4) | Sept. 20 | Q |
| 2 | NH | Sept. 24-28 | Not identified |
| 3 | NH (Aleppo WF7-4) | Oct. 1-4 | Q |
| 4 | HG1 | Sept. 18 | Not identified |
| 5 | HG1 | Sept. 27 | Not identified |
| 6 | HF1, HF2, HF3, HF4 (Hama WF2-3, WF2-5) | Sept. 18 | Q |

1) Field abbreviations are shown in Table 1. NH shows a net house in GOSM Tissue Culture Laboratory site in Aleppo.

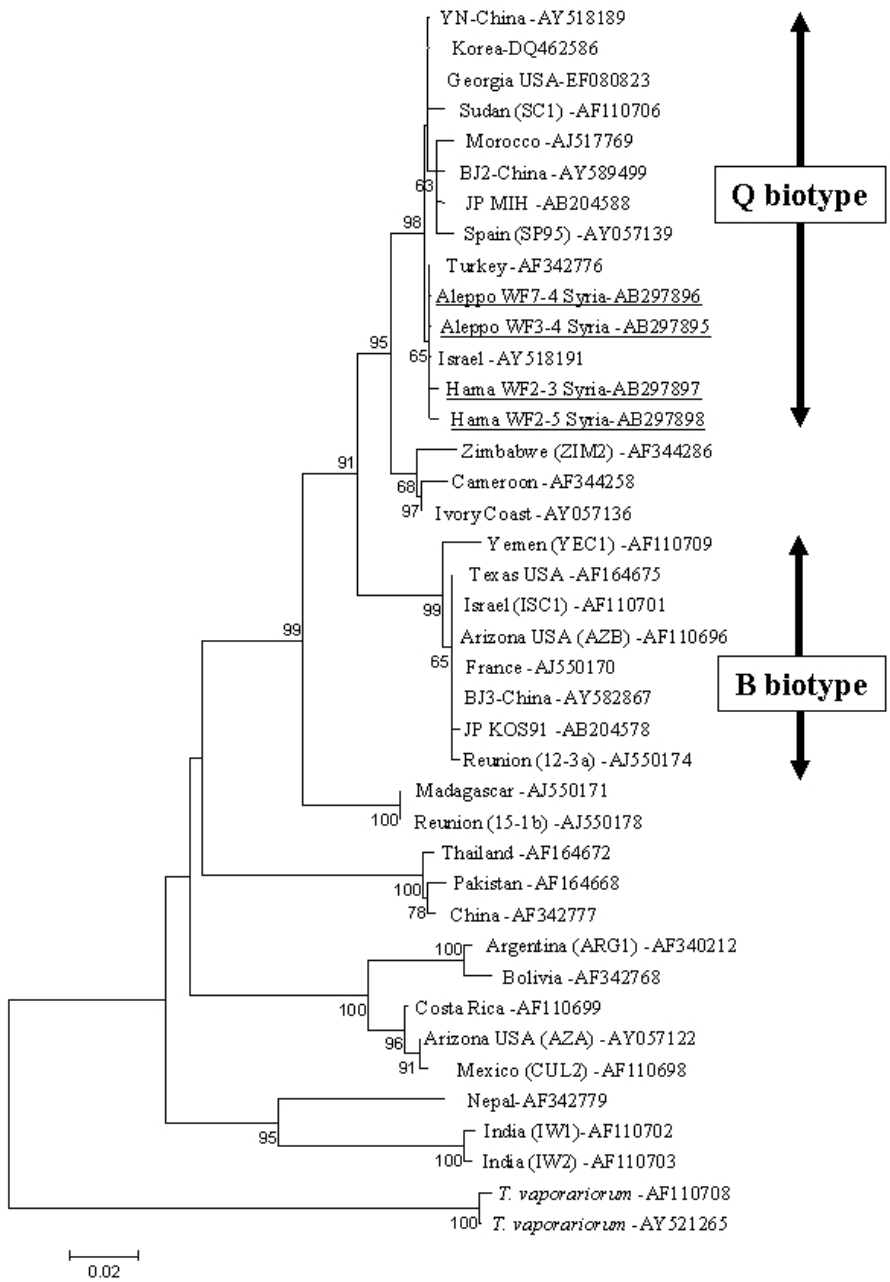


**Fig. 2.** Neighbor-joining phylogenetic tree reconstructed using the whitefly mitochondrial cytochrome oxidase I (mtCOI) sequence as a molecular marker. The numbers placed at each node indicate bootstrap support for values > 50.

The horizontal branch length in Fig 2 is drawn to scale, and the bar indicates 0.02 nt replacements per site. Two *Trialeurodes vaporariorum* mtCOI sequences were included as out-group sequences. The populations from Syria are underlined in the tree; all other populations are sources of reference sequences used in the analysis.

Whitefly populations were assessed in potato fields from September to November. Not all whiteflies were identified to the species level, but the whitefly populations consisted mostly of *B tabaci* with some other not identified species. Whiteflies were observed in almost all fields throughout the investigation period from early September to early November (Table 3). All potato varieties, such as "Afamia", "Spunta" and others, were infested with whiteflies (data not shown). However, farmers scarcely wrestle with the management of whiteflies, because at present they are not aware of the risk of whiteflies as virus vectors.

*B. tabaci*, which is known as a species complex (Perring, 2001) with many biotypes, has native varieties distributed throughout the world, the A biotype, B biotype (the silver leaf whitefly; *Bemisia argentifolii*), Q biotype, and others. Although *B. tabaci* is known to occur on potatoes, tomatoes, eggplants, pepper and melons in Syria (Personal communication), confirmation of the occurrence of the Q biotype is relevant here.

The Q biotype was first reported to be unevenly distributed in the Iberian Peninsula, where *B. tabaci* had damaged the various crops such as tomato, pepper, cotton, potato, bean and tobacco as a native pest (Guirao et al., 1997). This biotype was recently reported from China (Zhang et al, 2005; Chu et al., 2006), the United States of America (Brown et al., 2005), and Japan (Ueda and Brawn, 2006), and has become a serious threat for crops and ornamental plants like tomato, melon, pumpkin, cotton and poinsettia. Moreover, the occurrence of the Q biotype was known to occur in neighboring countries of Syria (Horowitz et al., 2003). In this study, we recorded for the first time the invasion and establishment of the Q biotype in Syria.

In Japan, unprecedented upsurges of the B biotype have occurred, and moreover the existence of the Q biotype was confirmed (Ueda and Brown, 2006), although the B biotype, which is associated with imported plants, was introduced and established approximately in 1989. The B and Q biotypes have the ability to transmit the *Tomato yellow leaf curl virus* and other begomoviruses. The congeniality between *B. tabaci* populations and host plants is more important in transmitting begomoviruses (Brown et al., 1995).

Moreover, the Q biotype is difficult to manage, because the resistance to chemical pesticides of this biotype is stronger than of the B biotype (Nauen et al., 2002; Horowitz et al., 2005). Several species of predators and parasitoids were reported as natural enemies of *B. tabaci* in Turkey (Bayhan et al., 2006). For the protection against the Q biotype, the parasitoid, *Eretmocerus mundus*, is used commercially as a biological pesticide in Spain (Urbaneja and Stansly, 2004; Stansly et al., 2005). Therefore, the search for parasitoid insects against the Q biotype in Syria can be an important research target.

Before this report, whiteflies have not been adequately recognized as pests in potatoes (Netherlands Potato Consultative Institute, 1996;GOSM, 2005), although many crops have been infested with several kinds of whiteflies in Syria. Hereafter, we should watch carefully the population dynamics of whiteflies, especially the Q biotype of *B. tabaci* populations in order to protect the domestic potato production in Syria.

**Table 3.** Occurrence of whiteflies in potato fields in Aleppo and Hama, Syria (2006).

| Area | Date | Field abbreviation [1)] | Whitefly No. (No./leaf) [2)] |
|---|---|---|---|
| Aleppo | Sep. 3 | AG1 | 0.01 |
| | | AG2 | 0.02 |
| | | AG3 | 0.04 |
| | | AF2 | 0.11 |
| | | AF3 | 0.04 |
| | Sep. 20 | AG1 | 0.07 |
| | | AG2 | 0.25 |
| | | AG3 | 0.16 |
| | Oct. 4 | AG3 | 0.14 |
| | | AF1 | 0.02 |
| | | AF2 | 0.03 |
| | | AF3 | 0.04 |
| | Oct. 18 | AG1 | 0.01 |
| | | AG2 | 0.01 |
| | | AG3 | 0.04 |
| | Nov. 1 | AG3 | 0.08 |
| | | AF1 | 0.04 |
| | | AF2 | 0.08 |
| | | AF3 | 0.14 |
| | Nov. 8 | AG1 | 0 |
| | | AG2 | 0.02 |
| | | AG3 | 0.10 |
| Hama | Sep. 18 | HG1 | 0.11 |
| | | HF1 | 0.06 |
| | | HF2 | 0.23 |
| | | HF3 | 0.09 |
| | | HF4 | 0.06 |
| | Sep. 27 | HG1 | 0.03 |
| | | HF1 | 0.09 |
| | | HF2 | 0.04 |
| | | HF3 | 0.10 |
| | | HF4 | 0.02 |
| | Oct. 12 | HG1 | 0.01 |
| | | HF1 | 0.02 |
| | | HF2 | 0.01 |
| | | HF3 | 0.01 |
| | | HF4 | 0.02 |
| | Nov. 2 | HG1 | 0.01 |
| | | HF1 | 0.01 |
| | | HF2 | 0.02 |
| | | HF3 | 0.01 |
| | | HF4 | 0 |
| | Nov. 9 | HG1 | 0.02 |
| | | HF1 | 0.01 |
| | | HF2 | 0.02 |
| | | HF3 | 0 |
| | | HF4 | 0.01 |

1) Field abbreviations are shown in Table 1.

2) Adults whiteflies on 200 compound leaves were investigated each time.

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# INSECTICIDAL ACTIVITY OF EXTRACT MIXTURES OF FOUR PLANT SPECIES AGAINST *CROCIDOLOMIA PAVONANA* (F.) (LEPIDOPTERA: PYRALIDAE) LARVAE

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## ABSTRACT

Vegetable farmers in Indonesia usually use synthetic insecticides to control insect pests. Improper and excessive use of synthetic insecticides cause undesirable effects. Therefore, a search for novel and safer insecticides to conserve our agricultural ecosystem should be made. Previously, botanical insecticide research focused on screening and bioassay of plant extracts and making botanical insecticide formulations containing a single plant extract. Unfortunately, for mass production, the source of plants is sometimes very limited. So, one strategy to overcome the limitation of plant resources is the use of extract mixtures. This study sought to develop an effective extract mixture of four plant extracts and to search for alternatives in cabbage insect pest management by using botanical insecticides. Four plant species, *Aglaia odorata*, *Swietenia mahogani*, *Piper retrofractum*, and *Annona squamosa* were evaluated. Two extracts were combined for each formulation at the ratios of 3:7, 1:1 and 7:3 (w/w). Each combination was bioassayed against *Crocidolomia pavonana* (F.) (Lepidoptera: Pyralidae) larvae using a leaf dipping method. The extract mixtures that resulted in high larval mortality on *C. pavonana* at 0.05% were *S. mahogani* and *A. squamosa* (3:7), *A. odorata* and *A. squamosa* (3:7 and 1:1), and *P. retrofractum* and *A. squamosa* (3:7, 1:1, and 7:3).

**Key words**: Botanical insecticide, mortality, pest management, plant extracts

## INTRODUCTION

Among the insect pest management strategies, the chemical strategy of using insecticides is commonly adopted by Indonesian farmers. Farmers, particularly vegetable farmers, sometimes rely heavily on insecticides. Moreover, most of the farmers spray insecticides improperly causing environmental pollution, disturbing the ecosystem balance as well as affecting human health. In order to conserve natural enemies and to sustain agricultural production, three improvements in relation to insecticide use should be implemented: (1) improvement in law enforcement, (2) improvement in insecticide application techniques and delivery systems, and (3) improvement in insecticide quality. The improvement of insecticide quality means that we should search for novel and safer compounds that are compatible with the IPM program. Neumann (1997) mentioned that high yielding varieties and new agrochemicals including insecticides with further improved characteristics will be important pillars.

Plant chemicals may exert physiological and/or behavioral effects on insects. In relation to insect pest management, researchers have explored the utilization of plant chemicals as botanical

insecticides in the insect pest control program. Botanical insecticides may be used as one of the alternative strategies in managing insect pests.

Previously, our research focused on screening and bioassay of plants for insecticidal activity. This progress to formulating botanical insecticides containing a single plant extract as the active ingredient. Several plants have been studied extensively including the isolation of the active compound. Unfortunately, for mass production of botanical insecticide formulations, plant resources are sometimes very limited. So, one strategy to overcome the limitation of plant resources is the use of extract mixtures, which could also take advantage of the possible synergism that may result in the mixture. This study sought to find out the effective extract mixtures of four plant extracts and to search for alternatives in cabbage insect pest management by using botanical insecticides.

## MATERIALS AND METHODS

### Plants

Plant sources were seeds of *Swietenia mahogani* (Meliaceae), twigs of *Aglaia odorata* (Meliaceae), seeds of *Annona squamosa* (Annonaceae), and fruits of *Piper retrofractum* (Piperaceae). All plant materials were air-dried for one week before extraction.

### Extraction

Plant materials were cut and then ground using a mill to powder. The powder of each plant species was soaked in methanol (1:10; w/v) for 48 hours. Filtrates were evaporated using a rotary evaporator under reduced pressure to yield crude extracts. Crude extracts were kept under low temperature (-4 $^{o}$C) in the refrigerator until used.

### Insect rearing

A mass culture of *Crocidolomia pavonana* (F.) (Lepidoptera: Pyralidae) was maintained in an insect room kept under temperature of 26 ± 1 $^{o}$C. Larvae, fed on insecticide-free broccoli leaves, were reared in plastic boxes (30 cm x 25 cm x 5 cm). Newly-emerged adults were allowed to mate and oviposit on broccoli leaves in a plastic cage (15 cm in diam. and 35 cm in height). Second instar larvae were used for bioassay.

### Mortality Assay

There were six combinations of extracts used in this experiment; *A. odorata* and *S. mahogani* (OM), *P. retrofractum* and *S. mahogani* (RM), *S. mahogani* and *A. squamosa* (MS), *A. odorata* and *P. retrofractum* (OR), *A. odorata* and *A. squamosa* (OS), and *P. retrofractum* and *A. squamosa* (RS). The comparisons of extract weight in an extract mixture were 3:7, 1:1 and 7:3 (w/w). Each crude extract mixture was diluted with methanol, and water containing Latron 77L as emulsifier and sticker was added to obtain the desired concentrations. The final concentration of methanol and Latron 77L in the extract emulsion was 1% and 0.1%, respectively. The concentrations of extract mixture used for bioassay were 0.05%, 0.1%, 0.2%, 0.4%, 0.8%, and 1.0%. Water containing Latron 77L and methanol only was served as control.

Bioassays were conducted by a leaf dipping method. A piece of broccoli leaf (2 cm x 2 cm) was dipped in extract emulsion for 5–10 seconds and air-dried. Four pieces of broccoli leaves were placed into each petri dish. Ten second-instar larvae of *C. pavonana* were introduced into each petri dish. Each treatment was replicated 5 times. The mortality was assessed at 24, 48 and 72 hours after treatment.

## RESULTS AND DISCUSSION

All concentrations of *S. mahogani* and *A. squamosa* (MS) extract mixture (3:7) gave more than 50% larval mortality at 24 hours after treatment (HAT) and 100% larval mortality was achieved at 48 HAT (Table 1). Treatment with MS (1:1) showed lower larval mortality than MS (3:7) and MS (7:3) showed lower mortality activity than treatment with MS (1:1) (Table 1). The results suggest that *A. squamosa* gave the major contribution in causing *C. pavonana* larval mortality in the MS extract mixture.

**Table 1.** Percent larval mortality of *C. pavonana* treated with extract mixtures of *S. mahogani* and *A. squamosa* (MS) at several concentrations

| Comparison of Extracts | Concentration (%) | Larval mortality (%) ± sd[a] (Hrs after treatment) 24 | 48 | 72[b] |
|---|---|---|---|---|
| MS 3:7 | 0.05 | 66.00 ± 33.62a | 100 ± 0a | 100 ± 0a |
| | 0.10 | 70.00 ± 18.71a | 100 ± 0a | 100 ± 0a |
| | 0.20 | 70.00 ± 14.14a | 100 ± 0a | 100 ± 0a |
| | 0.40 | 68.00 ± 29.50a | 100 ± 0a | 100 ± 0a |
| | 0.80 | 68.00 ± 21.68a | 100 ± 0a | 100 ± 0a |
| | 1.00 | 82.00 ± 10.96a | 100 ± 0a | 100 ± 0a |
| MS 1:1 | 0.05 | 34.00 ± 23.02a | 82.00 ± 29.50a | 96.00 ± 5.48a |
| | 0.10 | 38.00 ± 23.87a | 96.00 ± 8.94a | 100 ± 0a |
| | 0.20 | 58.00 ± 19.24a | 98.00 ± 4.47a | 100 ± 0a |
| | 0.40 | 40.00 ± 12.25a | 100 ± 0a | 100 ± 0a |
| | 0.80 | 58.00 ± 16.43a | 100 ± 0a | 100 ± 0a |
| | 1.00 | 62.00 ± 17.89a | 100 ± 0a | 100 ± 0a |
| MS 7:3 | 0.05 | 66.00 ± 16.73a | 94.00 ± 8.94a | 94.00 ± 8.94a |
| | 0.10 | 66.00 ± 20.74a | 94.00 ± 8.94a | 94.00 ± 8.94a |
| | 0.20 | 66.00 ± 20.74a | 96.00 ± 8.94a | 96.00 ± 8.94a |
| | 0.40 | 74.00 ± 18.17a | 100 ± 0a | 100 ± 0a |
| | 0.80 | 88.00 ± 10.95a | 100 ± 0a | 100 ± 0a |
| | 1.00 | 90.00 ± 14.14a | 100 ± 0a | 100 ± 0a |

[a] Standard deviation

[b] Percentages of mortality in the same column and the same comparison of extracts followed by the same letter are not significant different based on DMRT at 0.05%

Treatments with *A. odorata* and *P. retrofractum* (OR) 3:7 and 7:3 resulted in low mortality activity at the concentration of 0.2%, while treatment with OR 1:1 showed high enough mortality activity causing 84% larval mortality at 48 HAT (Table 2). It indicated that to make an effective extract mixture of *A. odorata* and *P. retrofractum*, the proportion of *A. odorata* and *P. retrofractum* extracts should be balanced.

Treatment with extract mixture of *A. odorata* and *A. squamosa* (OS) 3:7 showed high mortality activity. This OS (3:7) at 0.1% resulted in 88% larval mortality at 24 HAT and all concentrations caused 100% larval mortality at 48 HAT (Table 3). Treatment with other OS combinations resulted in lower mortality activity. Treatment with OS (1:1) gave 100% mortality when the larvae were treated with 0.2% at 48 HAT or with 0.1% at 72 HAT (Table 3).

**Table 2.** Percent larval mortality of *C. pavonana* treated with extract mixtures of *A. odorata* and *P. retrofractum* (OR) at several concentrations.

| Comparison of Extracts | Concentration (%) | Larval mortality (%) ± sd[a] (Hrs after treatment) 24 | 48 | 72[b] |
|---|---|---|---|---|
| OR 3:7 | 0.05 | 6.00 ± 5.48c | 14.00 ± 5.48c | 20.00 ± 12.25c |
| | 0.10 | 10.00 ± 7.07c | 24.00 ± 11.40c | 28.00 ± 13.04c |
| | 0.20 | 14.00 ± 8.94bc | 24.00 ± 11.40c | 40.00 ± 35.36bc |
| | 0.40 | 30.00 ± 18.71b | 58.00 ± 21.68b | 60.00 ± 22.36b |
| | 0.80 | 80.00 ± 18.71a | 100 ± 0a | 100 ± 0a |
| | 1.00 | 94.00 ± 8.94a | 100 ± 0a | 100 ± 0a |
| OR 1:1 | 0.05 | 4.00 ± 5.48d | 12.00 ± 13.04c | 22.00 ± 20.49b |
| | 0.10 | 16.00 ± 11.40d | 44.00 ± 25.10c | 56.00 ± 25.10b |
| | 0.20 | 30.00 ± 17.32c | 84.00 ± 20.74b | 96.00 ± 8.94a |
| | 0.40 | 64.00 ± 8.94b | 98.00 ± 4.47a | 100 ± 0a |
| | 0.80 | 88.00 ± 10.95a | 100 ± 0a | 100 ± 0a |
| | 1.00 | 88.00 ± 13.04a | 100 ± 0a | 100 ± 0a |
| OR 7:3 | 0.05 | 12.00 ± 4.47d | 26.00 ± 11.40d | 38.00 ± 20.49c |
| | 0.10 | 12.00 ± 16.43cd | 30.00 ± 15.81c | 30.00 ± 15.81c |
| | 0.20 | 18.00 ± 13.04c | 46.00 ± 16.73b | 56.00 ± 25.10b |
| | 0.40 | 34.00 ± 5.48b | 86.00 ± 16.73a | 100 ± 0a |
| | 0.80 | 82.00 ± 13.04a | 100 ± 0a | 100 ± 0a |
| | 1.00 | 88.00 ± 21.68a | 100 ± 0a | 100 ± 0a |

[a] Standard deviation

[b] Percentages of mortality in the same column and the same comparison of extracts followed by the same letter are not significant different based on DMRT at 0.05%

Treatment with OS (7:3) at 0.2% caused 28% and 92% larval mortality at 24 and 48 HAT, respectively. The results suggest that a higher concentration of *A. squamosa* extract in *A. odorata* and *A. squamosa* extract mixture resulted in higher effectivity. It indicates that *A. squamosa* plays an important role in causing larval mortality against *C. pavonana*.

Treatments with extract mixture of *P. retrofractum* and *S. mahogani* (RM) 7:3 and 3:7 showed quick action in causing larval mortality of *C. pavonana* rather than RM 1:1. Treatments with RM (7:3 and 3:7) gave 84% and 72% larval mortality, respectively at 24 HAT (Table 4). A slower reaction was observed in the 1:1 formulation. All concentrations showed low mortality activity at 24 HAT, but this increased to more than 90% at 48 HAT. Treatment with RM (1:1) at 0.05% gave more than 90% larval mortality at 48 HAT and 100% mortality at 72 HAT (Table 4).

Treatment with extract mixture of *P. retrofractum* and *A. squamosa* (RS) 3:7 resulted in high larval mortality. Treatment at 0.05% caused 90% larval mortality at 24 HAT and all concentrations caused 100% larval mortality at 48 HAT (Table 5). Treatment with RS (1:1) caused lower mortality activity than treatment with RS (3:7) but larval mortality was still high (Table 5). Treatment with RS (7:3) resulted in lowest mortality activity than treatments with RS (3:7 and 1:1). Treatment with RS (7:3) at 0.05% caused 98% at 48 HAT (Table 5). Again this indicates that larval mortality will increase with increasing *A. squamosa* concentration in the extract mixture.

**Table 3.** Percent larval mortality of *C. pavonana* treated with extract mixtures of *A. odorata* and *A. squamosa* (OS) at several concentrations.

| Comparison of Extracts | Concentration (%) | Larval mortality (%) ± sd[a] (Hrs after treatment) 24 | 48 | 72[b] |
|---|---|---|---|---|
| OS 3:7 | 0.05 | 76.00 ± 23.02b | 100 ± 0a | 100 ± 0a |
| | 0.10 | 88.00 ± 13.04ab | 100 ± 0a | 100 ± 0a |
| | 0.20 | 96.00 ± 5.48a | 100 ± 0a | 100 ± 0a |
| | 0.40 | 100.00 ± 0a | 100 ± 0a | 100 ± 0a |
| | 0.80 | 98.00 ± 4.47a | 100 ± 0a | 100 ± 0a |
| | 1.00 | 98.00 ± 4.47a | 100 ± 0a | 100 ± 0a |
| OS 1:1 | 0.05 | 74.00 ± 34.35a | 94.00 ± 13.42a | 94.00 ± 13.42a |
| | 0.10 | 70.00 ± 44.72a | 86.00 ± 31.30a | 100 ± 0a |
| | 0.20 | 86.00 ± 19.49a | 100 ± 0a | 100 ± 0a |
| | 0.40 | 98.00 ± 4.47a | 100 ± 0a | 100 ± 0a |
| | 0.80 | 98.00 ± 4.47a | 100 ± 0a | 100 ± 0a |
| | 1.00 | 100 ± 0a | 100 ± 0a | 100 ± 0a |
| OS 7:3 | 0.05 | 12.00 ± 13.04b | 60.00 ± 12.25a | 82.00 ± 13.04a |
| | 0.10 | 18.00 ± 13.04b | 66.00 ± 21.91a | 90.00 ± 17.32a |
| | 0.20 | 28.00 ± 16.43b | 92.00 ± 13.04a | 98.00 ± 4.47a |
| | 0.40 | 82.00 ± 23.87a | 100 ± 0a | 100 ± 0a |
| | 0.80 | 100.00 ± 0.00a | 100 ± 0a | 100 ± 0a |
| | 1.00 | 92.00 ± 10.95a | 100 ± 0a | 100 ± 0a |

[a] Standard deviation

[b] Percentages of mortality in the same column and the same comparison of extracts followed by the same letter are not significant different based on DMRT at 0.05%

Bioassays of treatments with extract mixtures of *A. odorata* and *S. mahogani* (OM) were conducted to verify the previous study conducted by Dadang and Ohsawa (2003) which showed mortality activity of extract mixtures against *Plutella xylostella* (Lepidoptera: Yponomeutidae) larvae, another major pest of cabbage. Present results indicate that the activities were comparable. Treatment with OM (7:3) was more effective than treatment with OM (1:1) and (3:7) (Table 6). One of the important things is the occurrence of phytotoxic effect. The phytotoxic effect was observed when broccoli plants were sprayed with OM (1:1) and OM (3:7) (Dadang and Ohsawa, 2003), but not on OM (7:3) treatment.

## DISCUSSION

Several extract mixtures caused high mortality activity against *C. pavonana* at low concentration (0.05%) such as MS (3:7), OS (3:7, 1:1), and RS (3:7, 1:1, 7:3). These caused mortality activity ranging 94-100% at 48 HAT and 100% larval mortality was achieved at 72 HAT. These results indicate the potential of plant extracts as pest control agents.

Single tests of each extract has been conducted and reported. *A. odorata* twig extract was effective against second instar larvae of *C. pavonana* (Prijono, 2001). *S. mahogani* inhibited feeding activity and caused mortality activity to *C. pavonana* larvae. Moreover, *S. mahogani* extract also caused larval mortality and feeding inhibition against *P. xylostella* (Dadang and Ohsawa 2000). A

triterpenoid compound might be responsible for its feeding inhibition activity (Dadang and Ohsawa, 2000).

**Table 4.** Percent larval mortality of *C. pavonana* treated with extract mixtures of *P. retrofractum* and *S. mahogani* (RM) at several concentrations.

| Comparison of Extracts | Concentration (%) | Larval mortality (%) ± sd[a] (Hrs after treatment) 24 | 48 | 72[b] |
|---|---|---|---|---|
| RM 3:7 | 0.05 | 32.00 ± 35.64b | 50.00 ± 46.37c | 50.00 ± 46.37c |
| | 0.10 | 38.00 ± 21.68b | 62.00 ± 38.99b | 62.00 ± 38.99bc |
| | 0.20 | 84.00 ± 26.08a | 92.00 ± 17.89ab | 92.00 ± 17.89ab |
| | 0.40 | 100.00 ± 0.00a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 0.80 | 100.00 ± 0.00a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 1.00 | 100.00 ± 0.00a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| RM 1:1 | 0.05 | 40.00 ± 14.14a | 92.00 ± 10.95a | 100.00 ± 0.00a |
| | 0.10 | 40.00 ± 17.32[a] | 92.00 ± 13.04a | 100.00 ± 0.00a |
| | 0.20 | 40.00 ± 33.17a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 0.40 | 46.00 ± 5.48a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 0.80 | 56.00 ± 15.17a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 1.00 | 58.00 ± 20.49a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| RM 7:3 | 0.05 | 20.00 ± 12.25c | 42.00 ± 36.33b | 42.00 ± 36.33c |
| | 0.10 | 46.00 ± 32.09b | 58.00 ± 30.33b | 72.00 ± 23.87b |
| | 0.20 | 72.00 ± 26.83a | 94.00 ± 5.48a | 94.00 ± 5.48ab |
| | 0.40 | 78.00 ± 13.04a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 0.80 | 82.00 ± 10.95a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 1.00 | 86.00 ± 8.94a | 100.00 ± 0.00a | 100.00 ± 0.00a |

[a] Standard deviation

[b] Percentages of mortality in the same column and the same comparison of extracts followed by the same letter are not significant different based on DMRT at 0.05%

The *Annona squamosa* extract played an important role in causing mortality activity to *C. pavonana* larvae. All extract mixtures containing *A. squamosa* extract showed high mortality activity. Annonin I and squamosin are two toxic compounds isolated from the seed of *A. squamosa* that were responsible for mortality activity of *A. squamosa*. These compounds together with asimisin, isolated from seeds of *Annona glabra* (Where is this compound from? Dadang this is your question. No, I found that question in the first editing) increased the toxicity against *P. xylostella* and *C. pavonana* (Ohsawa and Dadang, 1998).

Besides having mortality activity, the *A. squamosa* extract also had feeding inhibition activity as reported by Herawati (1998). On the single assay of *A. squamosa*, the extract showed highly feeding inhibition activity to *C. pavonana* when the larvae were fed with broccoli leaves containing 0.4% and 2% of *A. squamosa* extracts. It means that *A. squamosa* has both feeding inhibition and mortality activities but the feeding inhibition activity seems to be present at the higher concentration only. The presence of a feeding inhibitor in *A. squamosa* extract could increase the effectiveness of the extract. Inhibiting feeding activity will make the larvae weak, and if the larvae still consumes the leaf containing the extract, larval death will occur earlier.

*A. odorata* is one of plant species that was exhaustively researched by many researchers. Nugroho (1999) isolated six rocagalmide compounds from *A. odorata* which were responsible for feeding inhibition, mortality and growth regulatory activity against *Spodoptera liitoralis* using the

same plant materials, plant twigs. *A. odorata* extract at 0.25% and rocaglamide at 80 ppm caused 90% mortality against *C. pavonana* larvae.( (Sudarmo, 2001).

**Table 5.** Percent larval mortality of *C. pavonana* treated with extract mixtures of *P. retrofractum* and *A. squamosa* (RS) at several concentrations.

| Comparison of Extracts | Concentration (%) | Larval mortality (%) ± sd[a] (Hrs after treatment) 24 | 48 | 72[b] |
|---|---|---|---|---|
| RS 3:7 | 0.05 | 90.00 ± 10.00b | 100 ± 0a | 100 ± 0a |
| | 0.10 | 90.00 ± 7.07b | 100 ± 0a | 100 ± 0a |
| | 0.20 | 100 ± 0a | 100 ± 0a | 100 ± 0a |
| | 0.40 | 100 ± 0a | 100 ± 0a | 100 ± 0a |
| | 0.80 | 100 ± 0a | 100 ± 0a | 100 ± 0a |
| | 1.00 | 100 ± 0a | 100 ± 0a | 100 ± 0a |
| RS 1:1 | 0.05 | 88.00 ± 13.04b | 100 ± 0a | 100 ± 0a |
| | 0.10 | 94.00 ± 5.48ab | 100 ± 0a | 100 ± 0a |
| | 0.20 | 98.00 ± 4.48a | 100 ± 0a | 100 ± 0a |
| | 0.40 | 100 ± 0a | 100 ± 0a | 100 ± 0a |
| | 0.80 | 100 ± 0a | 100 ± 0a | 100 ± 0a |
| | 1.00 | 100 ± 0a | 100 ± 0a | 100 ± 0a |
| RS 7:3 | 0.05 | 50.00 ± 32.40b | 98.00 ± 4.47a | 100 ± 0a |
| | 0.10 | 78.00 ± 4.47a | 100 ± 0a | 100 ± 0a |
| | 0.20 | 92.00 ± 10.95a | 100 ± 0a | 100 ± 0a |
| | 0.40 | 96.00 ± 8.94a | 100 ± 0a | 100 ± 0a |
| | 0.80 | 96.00 ± 8.94a | 100 ± 0a | 100 ± 0a |
| | 1.00 | 96.00 ± 8.94a | 100 ± 0a | 100 ± 0a |

[a] Standard deviation

[b] Percentages of mortality in the same column and the same comparison of extracts followed by the same letter are not significant different based on DMRT at 0.05%

Several members of Piperaceae are toxic to insects. Extracts of *Piper bantamense, P. betle, P. longum* and *P. retrofractum* caused mortality activity against *Callosobruchus chinenchis* while *P. betle* and *P. nigrum* caused mortality activity against *Nephotettix virescens* (Dadang, 1999). It was also reported that extracts of *P. betle* and *P. retrofractum* showed mortality activity against *P. xylostella*, another destructive crucifer insect pest.

Mixing of several plant extracts into one formulation that has high efficacy to produce a botanical insecticide formulation should be efficient particularly for maintaining a sustainable source of plant materials. Our results showed that several plant extract mixtures resulted in high mortality effect against *C. pavonana* larvae at the concentration of 0.05%. The use of plant extracts in pest management will not only reduce the pest population but will also not leave residual effects due to biodegradable compounds, relatively safe for human beings, selectivity in organism target, and relatively safe for natural enemies. So, the use of botanical insecticides should be compatible with other strategies in integrated pest management program. Moreover, the botanical insecticide is a main tool in organic farming system, a farming system which strictly prohibits the use of synthetic pesticides.

Another advantage of using a mixed-extract formulation of botanical insecticide is the delay in the development of insect resistance. The use of several effective plant extracts will slow down the

occurrence of insect resistance (Prijono, 1999) due to the presence of several active compounds that may have multi-bioactivity against the insects at the time of application. Because the rapid development of insect resistance is one important hindrance in using synthetic insecticides, the use of botanical insecticides containing extract mixtures may be able to minimize this problem. So, the prospect for the use of botanical insecticides in the future is great.

**Table 6.** Percent larval mortality of *C. pavonana* treated with extract mixtures of *A. odorata* and *S. mahogani* (OM) at several concentrations.

| **Comparison of Extracts** | **Concentration (%)** | **Larval mortality (%) ± sd[a] (Hrs after treatment)** | | |
|---|---|---|---|---|
| | | **24** | **48** | **72[b]** |
| OM 3:7 | 0.05 | 0.00 ± 0.00c | 12.00 ± 8.37b | 22.00 ± 8.37c |
| | 0.10 | 2.00 ± 4.47c | 22.00 ± 20.49b | 26.00 ± 16.73b |
| | 0.20 | 4.00 ± 5.48c | 28.00 ± 17.89b | 44.00 ± 33.62b |
| | 0.40 | 24.00 ± 19.49b | 96.00 ± 8.94a | 98.00 ± 4.47a |
| | 0.80 | 42.00 ± 16.43a | 100 ± 0a | 100 ± 0a |
| | 1.0 | 44.00 ± 16.73a | 100 ± 0a | 100 ± 0a |
| OM 1:1 | 0.05 | 18.00 ± 10.95bc | 40.00 ± 18.71b | 48.00 ± 20.49b |
| | 0.10 | 8.00 ± 13.04c | 36.00 ± 27.02b | 44.00 ± 32.09b |
| | 0.20 | 28.00 ± 24.90ab | 52.00 ± 26.83b | 58.00 ± 25.88b |
| | 0.40 | 28.00 ± 8.37ab | 84.00 ± 18.17a | 94.00 ± 8.94a |
| | 0.80 | 50.00 ± 27.39a | 92.00 ± 17.89a | 98.00 ± 4.47a |
| | 1.00 | 42.00 ± 10.95a | 100 ± 0a | 100 ± 0a |
| OM 7:3 | 0.05 | 16.00 ± 13.42b | 30.00 ± 21.21c | 36.00 ± 20.74b |
| | 0.10 | 10.00 ± 10.00b | 24.00 ± 8.94c | 42.00 ± 20.49b |
| | 0.20 | 30.00 ± 41.23b | 66.00 ± 24.08b | 94.00 ± 5.48a |
| | 0.40 | 28.00 ± 21.68b | 84.00 ± 15.17ab | 92.00 ± 8.37a |
| | 0.80 | 88.00 ± 13.07a | 100.00 ± 0.00a | 100.00 ± 0.00a |
| | 1.00 | 96.00 ± 8.94a | 100.00 ± 0.00a | 100.00 ± 0.00a |

[a] Standard deviation

[b] Percentage of mortality in the same column and the same comparison of extracts followed by the same letter are not significant different based on DMRT at 0.05%.

## CONCLUSION

Several extract mixtures caused high larval mortality activity against *Crocidolomia pavonana*. Extract mixtures of *Piper retrofractum* and *Swietenia mahogani* (1:1), *S. mahogani* and *Annona squamosa* (3:7), *Aglaia odorata* and *A. squamosa* (3:7 and 1:1), and *P. retrofractum* and *A. squamosa* (3:7, 1:1, and 7:3) at 0.05% gave more than 90% larval mortality at 48 hours after treatment. The presence of *A. squamosa* extract is crucial in each extract mixture in causing high larval mortality. However, aside from the nature of the extract, the comparisons of extracts in the mixture are also responsible for high mortality activity. These results may contribute in cruciferous insect pest management as well as in producing healthy vegetable products.

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# POSTHARVEST STORAGE OF TWO STRAINS OF NAM DOK MAI MANGO FROM NORTHERN THAILAND UNDER DIFFERENT TEMPERATURES USING VARIOUS WRAPPING MATERIALS

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## ABSTRACT

The commercial ripe fruit of two strains of 'Nam Dok Mai' mango (*Mangifera indica* L.), Gold Sritong and Green T4 were stored at 13°C and 20 °C, with various wrapping materials to determine post harvest shelf life and suitable wrapping material. The rate of respiration, ethylene production, and weight loss were significantly decreased at 13 °C storage as compared with those at 20 °C. Regardless of strain and temperature, the lowest rate of respiration and weight loss were observed for the fruit wrapped with ethylene absorbing bag (EAB) and polyethylene plastic bag (PB) while the highest respiration rate and highest weight loss were found in the control fruits followed by the fruits of EABn (ethylene absorbing bag with needle perforated holes). Chilling injury symptoms were not observed in Nam Dok Mai mango, even when stored at 13 °C. The pH increased gradually particularly in control and EABn at 20 °C while in storage, Brix also increased similarly for both strains. Weight loss, skin firmness and pH were highly correlated with the total shelf life and were likewise affected by storage temperature and wrapping treatments. Significant differences were not observed among the treatments with respect to Brix % and total acid content of the fruit. Regardless of storage temperature and wrapping treatments, Green T4 showed the highest respiration rate and reached the ripening stage earlier than Gold Sritong. There were different respiratory and ethylene production patterns observed in the two strains of Thai mango in this experiment. The lower the storage temperature, the lower the rate of respiration and ethylene production, resulting in prolonged shelf life especially in the EAB and PB wrapped fruits. Shelf life and fruit firmness were better at 13 °C than at 20 °C in all the treatments. The infection of anthracnose disease was more rapid in Green T4 than Gold Sritong stored at 20 °C.

The present research indicated that wrapping with EAB and PB kept the Gold Sritong fruits in good condition up to 28 days at 13 °C as evidenced by the lowest weight loss, lowest respiration rate, firmer flesh and longer shelf life with good quality while Green T4 fruit could only be stored up to 15 days. Nam Dok Mai mango may be stored for 10 days without wrapping even at 20 °C regardless of strains. Therefore, mango fruit wrapping fruit with EAB and PB may offer a practical means of prolonging shelf life up to 14 days longer at 13 °C and 2 days longer at 20 °C, depending on the strain or cultivar.

Keywords: Ethylene evolution, respiration rate, shelf life, storage temperature, wrapping material, ethylene absorbing bag

Abbreviations: ethylene absorbing bag (EAB), polyethylene bag (PB), ethylene absorbing bag with needle perforated holes (EABn),

## INTRODUCTION

Mango (*Mangifera indica* L.) is the most popular fruit in the tropics and cv. Nam Dok Mai ranks top among the commercial mango varieties in Thailand. The mango fruit is usually harvested at mature green stage, about 91- 105 days after full bloom (Tungtirmthon., 1998) and the fruit could fully ripen in 4-5 days at ambient temperature (Yantarasri et al., 1994). The climacteric fruit of mango may vary in the respiration pattern and ripening behavior among the varieties. In addition, respiratory pattern has great importance in determining fruit shelf life. All biological processes are more or less controlled by temperature, which is the most important factor affecting fruit weight loss and other physiological changes and disorders which are responsible for visual appearance and shelf life. The application of modified atmosphere packing (MAP) is a versatile technology that is applicable to a wide range of fruits and vegetables by wrapping (Rodov et al., 1997; Wills et al., 1998). It decreases weight loss of fruit by preventing transpiration. It also extends the shelf life of fresh produce by reducing oxygen and increasing carbon-dioxide levels; respiration is decreased and senescence is subsequently postponed (Kader et al., 1989). There is no precise information on the relation of respiration, ethylene production, and softening of the mango fruit which is influenced by pre-harvest factors, cultural practices, fruit maturity and postharvest handling.

In this study, we sought to clarify the difference in postharvest physiology of Thai mango Gold Sritong and Green T4 which are both said to be the strains of Nam Dok Mai. The former is more famous with yellowish skin, and the latter has green skin color but their genetic backgrounds are not clear. The Namdokmai variety is an Indo-Chinese origin cultivar and is poly-embryonic. The present study focused on the determining the effective methods to prolong storage period of Nam Dok Mai mango, strains Gold Sritong and Green T4 harvested at commercial ripe stage in May (mango season) under Thai conditions, with respect to wrapping materials and storage temperature.

## MATERIALS AND METHODS

Freshly harvested commercially ripe 'Nam Dok Mai' mango was obtained from a private local orchard in Phitsanulok province, Wangtong district. The experiments were conducted at the Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok, Northern Thailand from 9th May to 3rd June in 2006. The two strains of cv. Nam Dok Mai, Gold Sritong and Green T4 were transported immediately after harvest by car to the experimental site.

### Postharvest treatments

Fruits were selected for uniform maturity, free from blemishes and defects, and an average weight of 380 g. Fruits were washed and immersed in hot water at $50 \pm 2$ $^{o}$C for about 15 minutes to remove latex and microorganisms but were not treated with chemicals, prior to storage. Individual fruits were then weighed and allowed to air-dry at room temperature.

An equal number of 152 fruits from each strain were divided into 2 groups by storage conditions: $13 \pm 2$ $^{o}$C with 85% RH and $20 \pm 3$ $^{o}$C with 65% RH. Each group (19 fruits) was then divided into 4 subgroups and wrapped with PB (polyethylene plastic bag with a thickness of 0.02 mm) or EAB (ethylene absorbing bag with a thickness of 0.05mm) and EABn (the same ethylene absorbing bag with needle perforated holes of 150 holes on 244.8 $cm^2$ surface area) with unwrapped fruits as a control.

### Weight loss and firmness

The fruit weight loss was noted every other day and the relative weight losses were calculated on eighteen sample fruits as a percent weight loss based on the initial weight. Fruit flesh firmness

($kg/cm^2$) was determined using a universal standard meter (KIYA-5kg UA) from an average of four places at the equatorial portion of the fruit with four replications. The firmness tester is a fruit destructive device that incorporates a penetrometer as a force-sensing component with a tip 3 mm in diameter. The maximum force applied to the fruit is defined as fruit firmness that is measured by the penetrometer gauge.

**Juice quality analysis**

Mango juice was prepared from the fruits using an electric blender and the Brix% (TSS) was measured with a portable refractometer (ATAGO N1). The pH of the squeezed fruit juice was determined using a pH/ ion meter (HORIBA Model F-23).

**Respiration and ethylene gas analysis**

The rates of respiration and ethylene emission were evaluated on the same four sample fruits per treatment at every other day till the end of the experiment. For gas analysis, the fruit was sealed in an isolated plastic container (880 ml volume) covered with silicon cap for an incubation time of 2 hours at 13 $^oC$ and 20 $^oC$, respectively. For ethylene analysis, one ml gas sample was withdrawn with a gas–tight hypodermic syringe and ethylene gas was analyzed by a gas chromatography (Shimadzu GC 8-A) equipped with a flame ionization detector (FID) and Porapack Q aluminum column. The carrier gas was nitrogen with a flow rate of 50 ml $min^{-1}$. The column was operated isothermally at 40 $^oC$ with the flame ionization detector temperature at 60 $^oC$ and the injector temperature at 60 $^oC$. The ethylene gas emission was expressed as µl per kg of fruit per hour.

The respiration rate was measured by evolution of carbon dioxide (ml per kg of fruit per hour). A 5 ml gas sample was withdrawn from the sealed container with a gas-tight hypodermic syringe and was analyzed with an infrared gas analyzer (Komyo Rikagaku Co. Model UR-020S).

**Shelf life**

Total shelf life was taken as the number of days that the fruits remained in a marketable condition judged by visual inspection of appearance and texture using a score system. According to Miller et al. (1983) the shelf life was visually judged by the following score with a slight modification at 3 days intervals. Visual appearance for skin and pulp were measured by using a score from stage 1 to stage 5. Stage 1; green and very hard flesh, stage 2; light green and hard flesh, stage 3; beginning yellow color and slightly hard, stage 4; predominantly yellow and slightly soft, and stage 5; totally yellow and soft. In the case of Green T4, it was determined by finger pressure and visual observation. Stage 4 was evaluated as acceptable and marketable for consumers in Thailand.

## RESULTS

There was no difference in weight loss % among the wrapping treatments of Gold Sritong irrespective of temperature but control fruits showed greater weight loss compared with the wrapped fruits. On the other hand, there was a slight difference among the wrapping treatments in Green T4. Wrapping treatment can prevent fruit weight loss even under a higher temperature of 20 $^oC$. Weight loss of Green T4 fruits in all the treatments was much faster under 20 $^oC$ than under 13 $^oC$ (Fig.1).

The fruit firmness in most treatments was higher at 13 $^oC$ than 20 $^oC$ in Gold Sritong. The fruit of Gold Sritong was firm particularly in EAB at 13 $^oC$ until 3 days after storage but the fruit of Green T4 started to soften. Decrease in firmness was similar in the fruit of Green T4 and Gold Sritong, showing about 3-4 $kg/cm^2$ at 13 $^oC$ and 1-2 $kg/cm^2$ at 20 $^oC$ after 15days storage and there

were not much difference among the treatments (Fig 2).

Generally, fruits of both strains stored at 13 °C and 20 °C showed a clear climacteric pattern of respiration. Control fruits of Gold Sritong and Green T4 at 20 °C reached a climacteric peak at 3 days after storage with a maximum rate of 52.92 $mg^{-1}kg^{-1}$ $h^{-1}$ and 75.3 $mg^{-1}kg^{-1}$ $h^{-1}$, respectively. Gold Sritong fruits of EABn at 20 °C reached a climacteric peak at 3 days after storage similar to

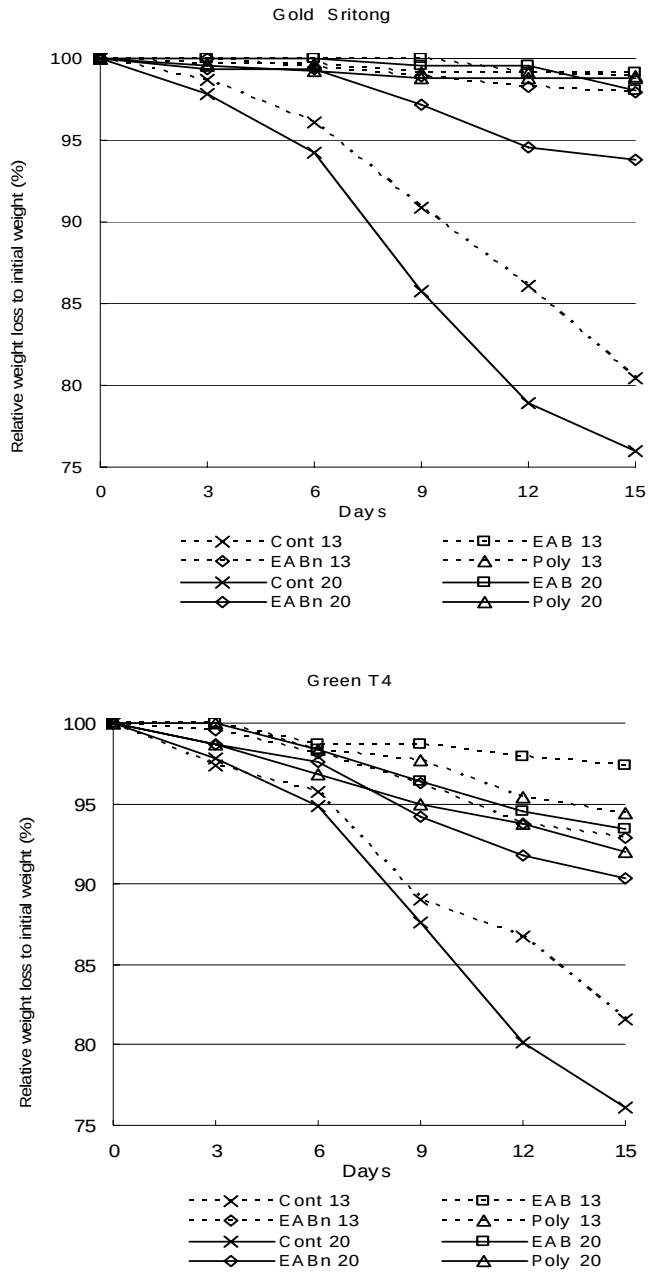


**Fig. 1**. The effects of different temperatures and various wrapping materials on the weight loss of Nam Dok Mai mango, Gold Sritong and Green T4.

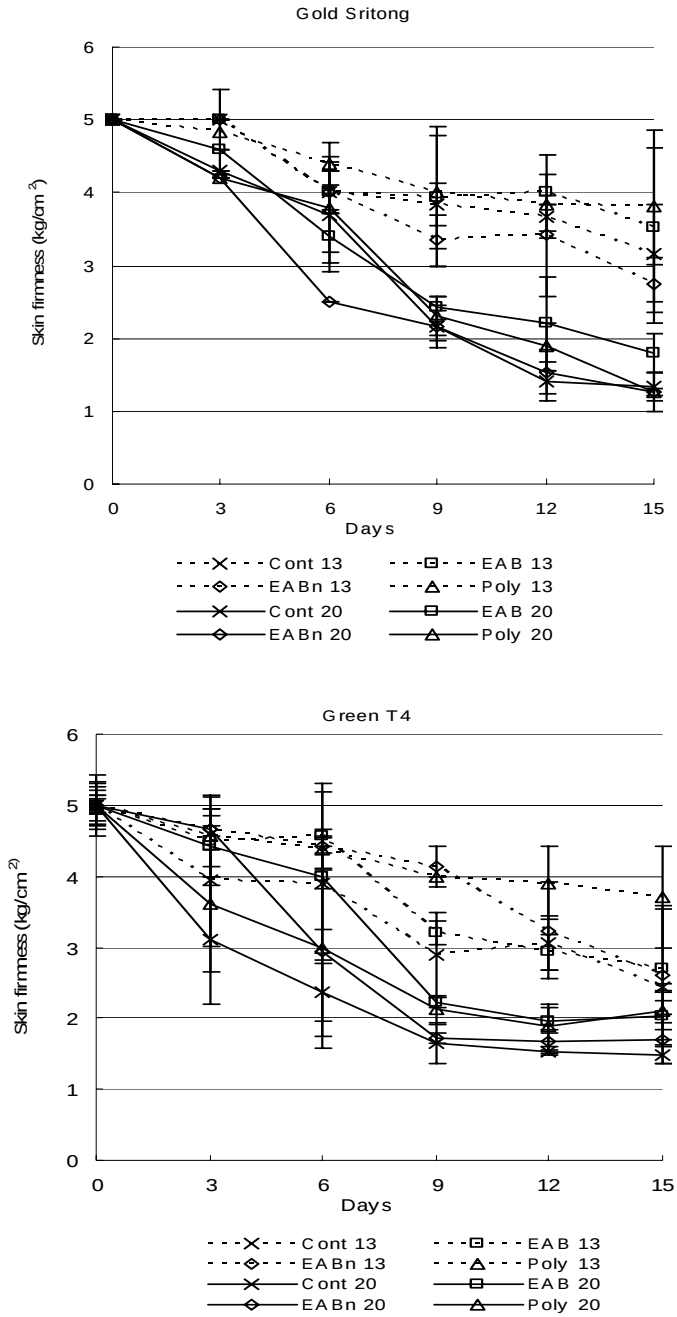


**Fig. 2.** The effects of different temperatures and various wrapping materials on the firmness of Nam Dok Mai mango, Gold Sritong and Green T4. *Average value and standard error bars of three replications.

the control fruits. However, EAB and PB fruits at 20 °C reached a climacteric peak later at 6 days after storage with lower respiration rate which coincided with that of fruits of all the treatments stored at lower temperature of 13 °C (Fig. 3). The ripening of these fruits was retarded. Green T4 fruits in most treatments stored at both temperatures showed the respiratory climacteric peak at 3

days after storage except EAB fruits stored at 13 °C which showed the climacteric peak at 9 days after storage. The peak value of climacteric was lower for the fruits stored at 13 °C than 20 °C. The downward trend after a distinct rise was found in Green T4, however, Gold Sritong showed a different respiratory pattern from Green T4 and the respiration rate increased after 9 days storage (Fig 3.).

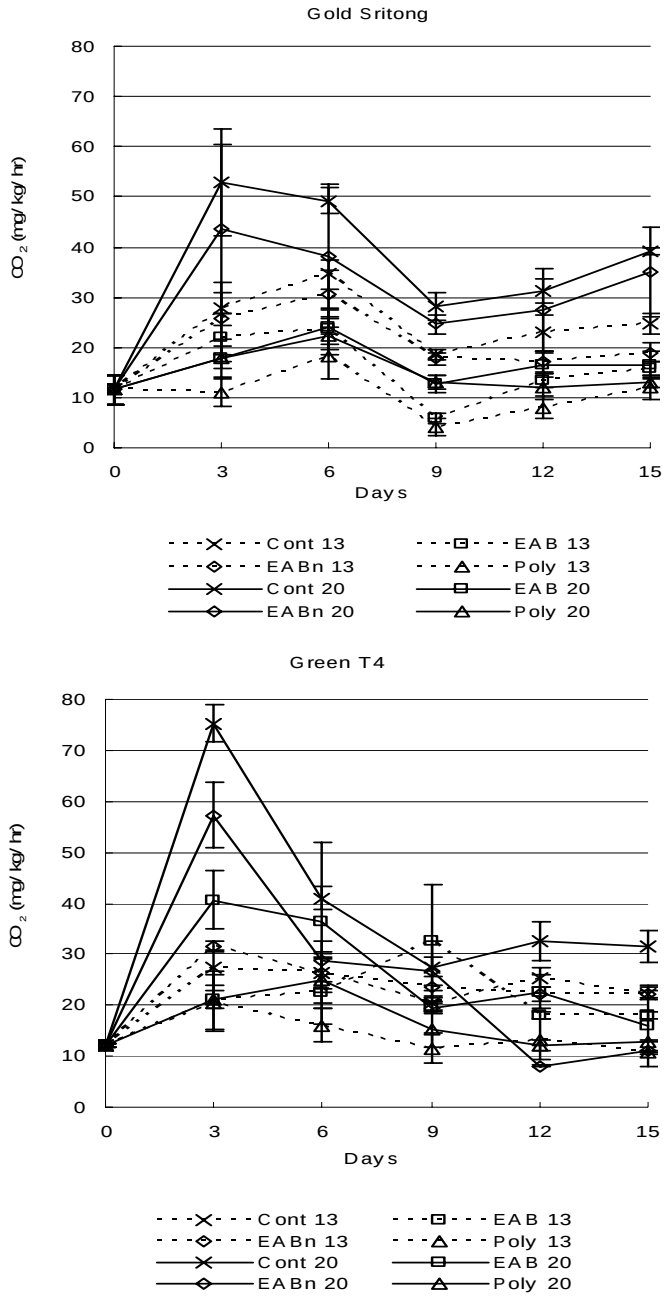


**Fig. 3.** The effects of different temperatures and various wrapping materials on the respiration rate of Nam Dok Mai mango, Gold Sritong and Green T4. *Average value and standard error bars of three replications.

The peak of ethylene production in all treatments of Gold Sritong stored at 20 °C was observed around 3-6 days after storage and then the production gradually decreased along the storage days with fruit ripening. On the other hand, the fruits of Gold Sritong stored at 13 °C showed the peak of ethylene production at 6 days after storage which nearly coincided with respiratory climacteric peak (Fig. 4). Green T4 fruits stored at 20 °C reached a sharp peak of ethylene production with a higher rate either at 3 or 6 days after storage. However, fruits stored at 13 °C showed significantly lower ethylene production at 6 days after storage (Fig. 4).

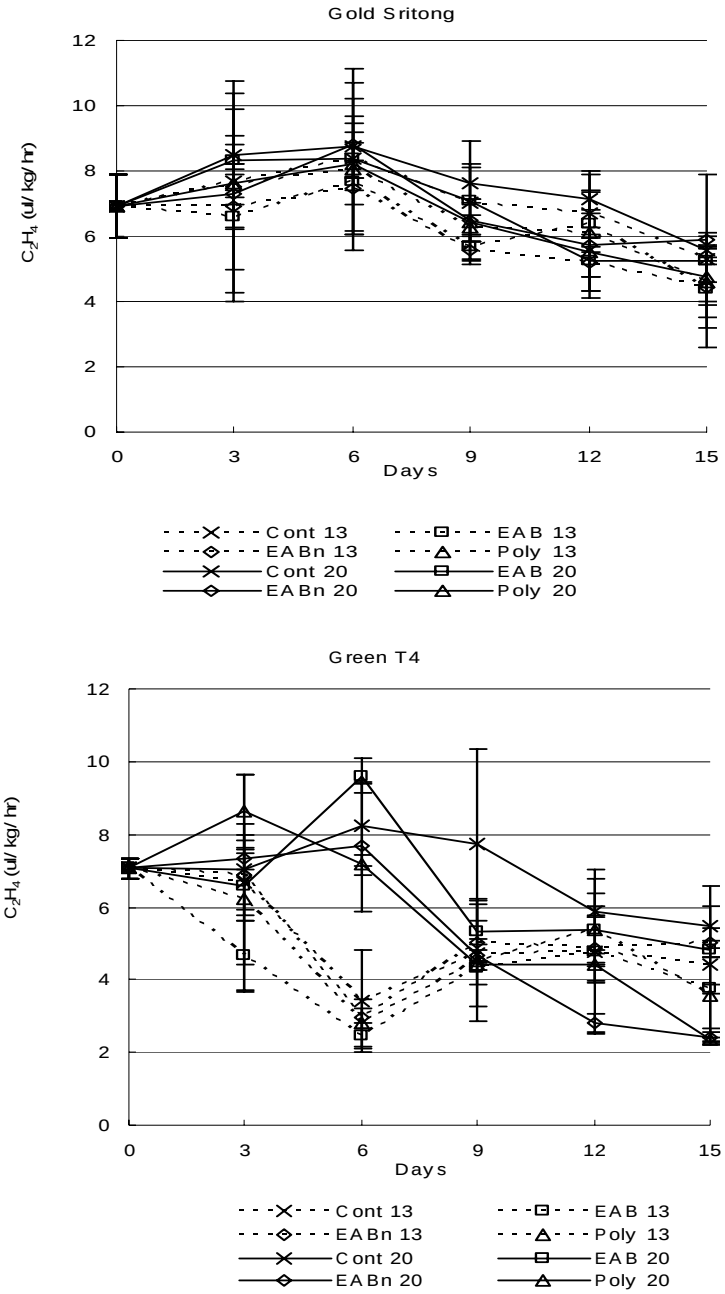


**Fig. 4.** The effects of different temperatures and various wrapping materials on the ethylene emission rate of Nam Dok Mai mango, Gold Sritong and Green T4. *Average value and standard error bars of three replications.

Regardless of the strains, pH was about 3 at harvest time and this gradually increased to 6.5 while in storage for control and EABn fruits stored at 20 °C. However, pH of EAB and PB fruits did not increase much, reaching about 4 to 5 during 15 days storage at 20 °C. All treatments at low temperature of 13 °C did not significantly increase pH during the storage (Fig. 5).

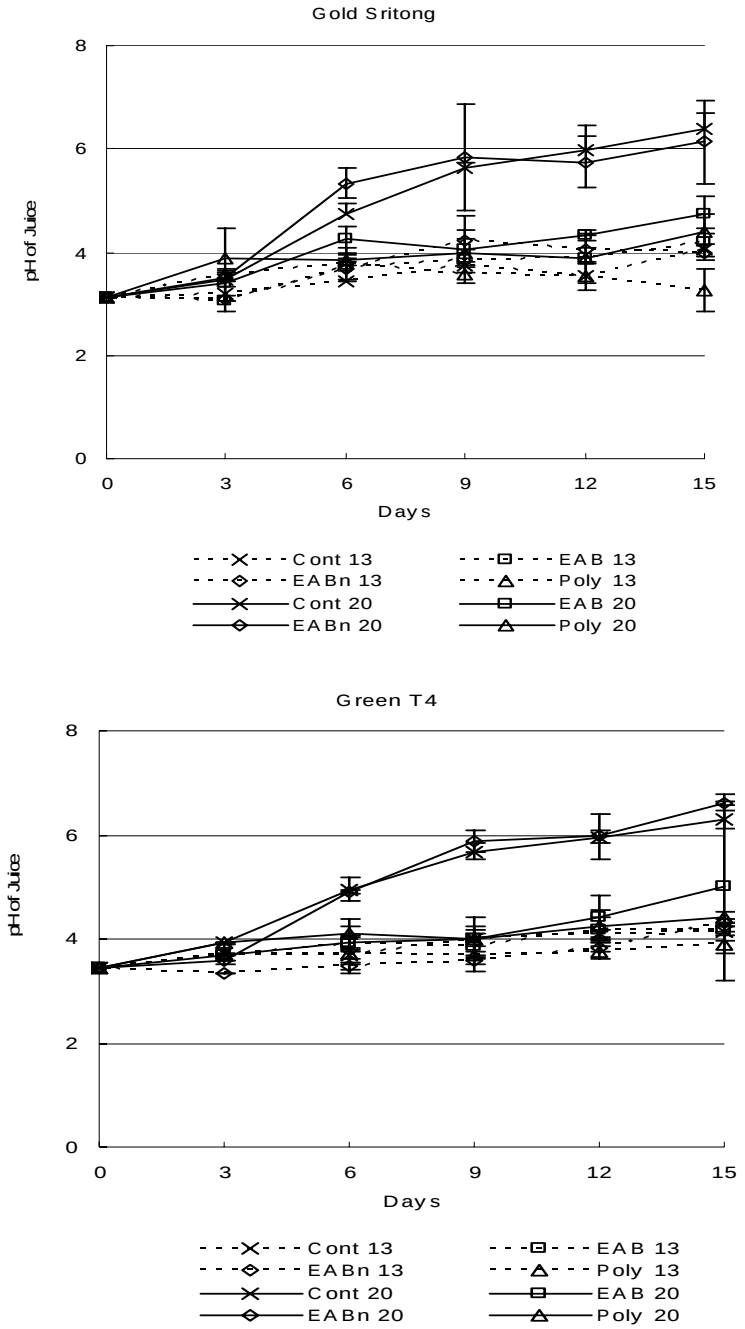


**Fig. 5.** The effects of different temperatures and various wrapping materials on the pH of Nam Dok Mai mango, Gold Sritong and Green T4.

Regardless of strain, control fruit showed the highest Brix% at 9 days and EAB reached the highest Brix % at 12 days after storage at 13 °C. As for temperature, 20 °C showed the highest Brix % a little earlier than that of 13 °C, particularly in control and EABn fruits (Fig. 6). Therefore, Brix % depended on storage temperature and wrapping treatments but did not depend on the strains, showing 6 to 12% throughout the experiment.

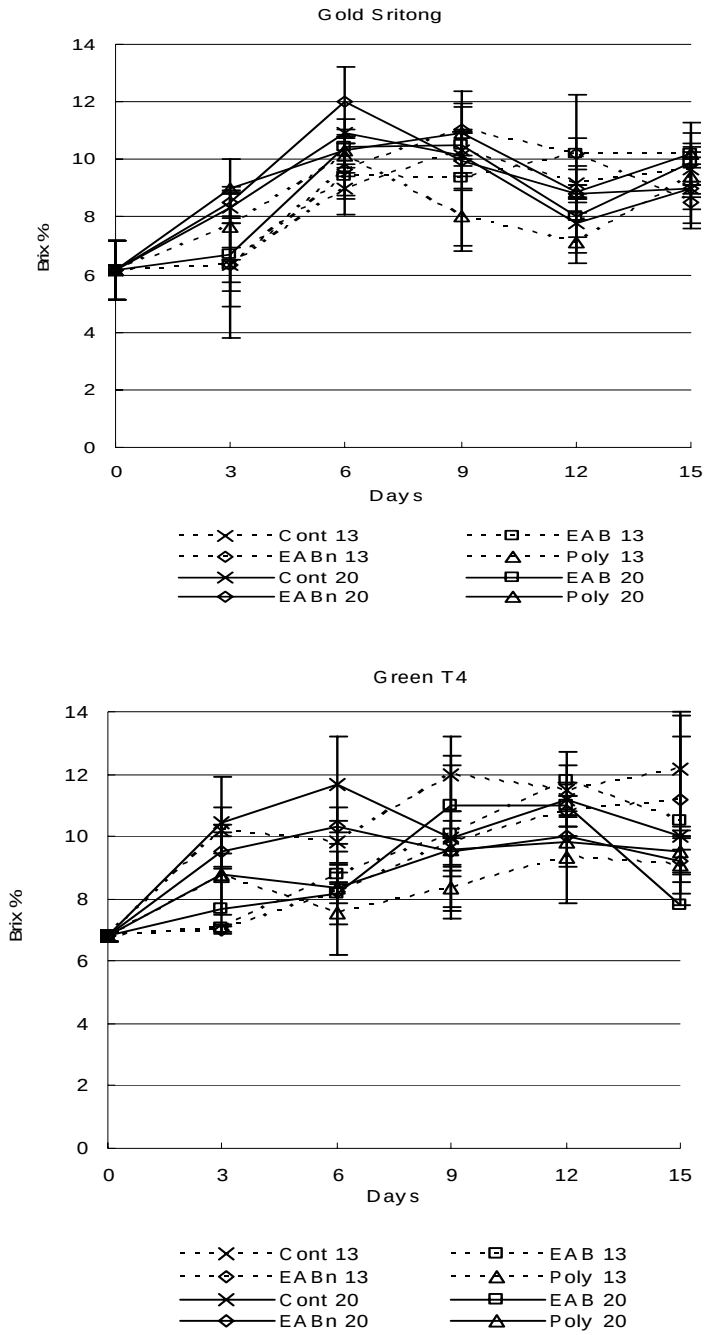


**Fig. 6.** The effects of different temperatures and various wrapping materials on the Brix % of Nam Dok Mai mango, Gold Sritong and Green T4.

Con 13 = Control at 13°C
EAB 13 = Ethylene absorbing bag at 13°C
Poly 13 = Polyethylene plastic bag at 13°C
Con 20 = Control at 20°C
EAB 20 = Ethylene absorbing bag at 20°C
EABn 20 = Ethylene absorbing bag with needle perforated holes at 20°C
Poly 20 = Polyethylene plastic bag at 20°C
*Dotted lines refer to 13°C and the normal line refer to 20°C.

The shelf life of fruits in all treatments at 13 °C were longer than those at 20 °C, particularly in EAB and PB. The shelf life of the control fruits of Gold Sritong and Green T4 were 12 days and 10 days at 20 °C, respectively. EAB and PB treatment prolonged the storage period of both Gold Sritong and Green T4 fruits; the effect was much greater for the fruits stored at 13 °C. Thus, the Gold Sritong fruits wrapped with EAB and PB and stored at 13 °C had a much longer shelf life of 28 days (Fig 7).

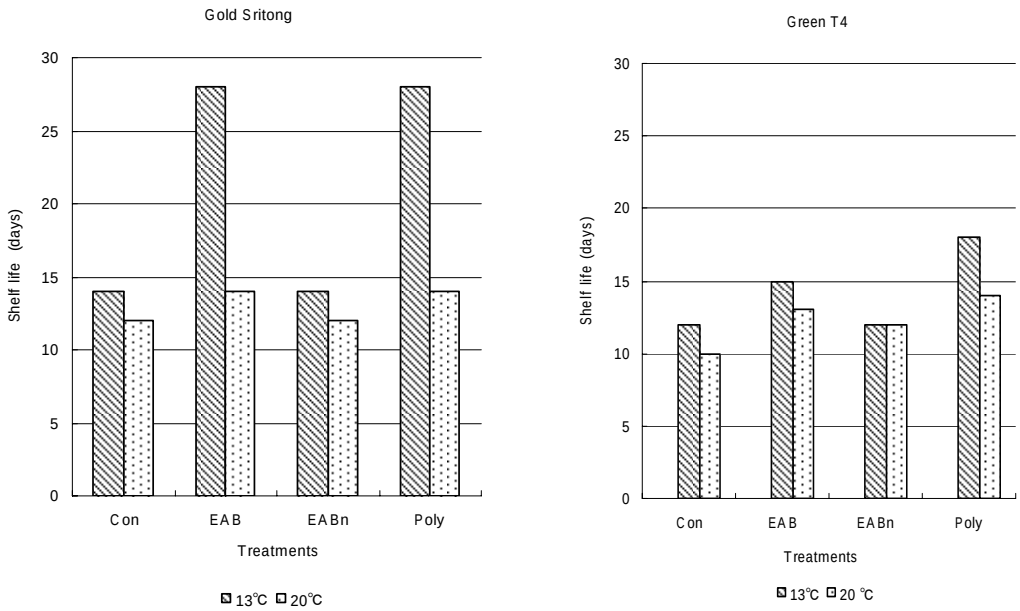


**Fig. 7.** The effects of various temperatures and wrapping materials on the shelf life of Nam Dok Mai mango, Gold Sritong and Green T4.

## DISCUSSION

It is clearly demonstrated that the modified atmosphere (MA) conditions, in particular, enclosing mango fruit in EAB, maintains fruit quality and prolongs the shelf life of commercially ripe fruit of two strains of Nam Dok Mai mango under Thai conditions. PB and EAB treatments delayed the onset of climacteric peak and decreased the respiration rate. The treatment also decreased markedly fruit weight loss which is mainly caused by the loss of water by transpiration, and delayed softening of the fruit as shown by the decrease of fruit firmness. The pH of control and EABn at 20 °C in both varieties increased gradually but the pH of the other treatments did not increase which shows the delay of ripening.

Studies previously conducted in Myanmar showed that EAB mango fruits retained the quality such as preventing shriveling caused by water loss, maintaining the fruit firmness and sugar content, and total appearance of fruits longer than those exposed to open air even under high ambient temperature of more than 30 $^{o}$C (Soe et al., unpublished). Nam Dok Mai mangoes of different maturation were stored well in EAB both at 20 $^{o}$C and 13 $^{o}$C (Soe et al., unpublished). In addition, Soe et al., (2006) showed that 'Irwin' mangoes harvested at full ripe stage had prolonged shelf life by when wrapped in EAB in Japan. Thus, regardless of the cultivars used, the stage of maturation and ripeness of the fruits and ambient conditions such as temperature, the EAB treatment is a very useful means to keep the fruit quality and prolong the shelf life. The main effect of the EAB is to absorb the endogenous ethylene which evolves from the fruits, and this weakens the ethylene effect on respiration and the acceleration of ripening. Likewise, EAB prevents water loss from the fruit by transpiration. Similar results were observed by using polyethylene wrapping (Rodov et al., 1997 and Wills et al., 1998)**.**

One of the shortcomings of EAB is accumulation of excessive water inside the bag which may encourage the proliferation of pathogen fungi, such as anthracnose. The fruits placed inside the perforated EAB showed similar respiration patterns to that of control fruits as well as weight loss pattern. As a result, the shelf life of fruits in the perforated EAB was not different from that of the control fruits.

The present study also showed the importance of storage temperature for the shelf life of mango fruits**.** Fruits, for all the treatment including control, generally had delayed ripening and thus longer shelf life at 13 $^{o}$C than at 20 $^{o}$C. This was evidenced by decreased weight loss caused by transpiration, delayed softening and lower respiration rate. These results are in accordance with the results in Irwin mango (Soe et al., 2006) and Tommy Atkins and Baneshan mangoes (Rodov et al., 1997, Narayana et al., 1996) and Hass avocado (Perez et al., 2004).

Gold Sritong and Green T4 are the strains of cv. Nam Dok Mai mango. The origin of the strains is not clear but probably these originated from natural mutation. Gold Sritong fruits are yellow when ripe and more attractive than Green T4 fruits. There are several varietal differences in ripening pattern and response to EAB treatment. Gold Sritong fruits could be stored for as long as 28 days, when placed in EAB and PB bags at 13 $^{o}$C, about 15 days longer than control fruits at 13 $^{o}$C. On the other hand, Green T4 mangoes may be stored for 12 days at 13 $^{o}$C while only 10 days without wrapping at 20 $^{o}$C and reaches the ripeness earlier with rapid development of softness.

## CONCLUSION

In conclusion, storage of fruit in EAB is a very useful means to prolong the shelf life of mango fruits with good eating quality, particularly in tropical areas where cooling facilities are not common, although the storage of fruits at a lower temperature of 13 $^{o}$C further prolongs the shelf life of EAB enclosed fruits.

## ACKNOWLEDGEMENT

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# ANTIBACTERIAL ACTIVITY OF *Pseudomonas fluorescens* RH4003 AGAINST BACTERIAL WILT OF TOMATO

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## ABSTRACT

Six tomato varieties were tested for resistance against bacterial wilt. Among them, Money Maker and San Marzano were relatively susceptible while TM39 and Ratna were relatively resistant. Application of *Pseudomonas fluorescens* RH4003 reduced the disease intensity on Money Maker and San Marzano up to 39%. The application of *P. fluorescens* produced variable response in peroxidase enzyme activity among the varieties. The highest activity was on Money Maker and the lowest was on TM39. Total colonies of *P. fluorescens* RH4003 $rif^r$ isolated from tomato roots were greater than from the rhizosphere. Population density of *P. fluorescens* on Ratna variety was higher than on the other tomato varieties. Combined application of *P. fluorescens* RH4003 with *Bacillus subtilis* AB89 and *B. cereus* L32 produced antagonistic effects while application with streptomycin sulphate produced synergistic effect at 18 and 25 days after transplanting.

**Key words:** peroxidase, synergy factor, *Bacillus* spp.

**Abbreviation:** PGPR – plant growth promoting rhizobacteria, SDW – sterilized distilled water

## INTRODUCTION

Beneficial plant-associated microorganisms can influence profoundly plant health by suppressing disease, enhancing nutrient uptake, fixing atmospheric nitrogen, and promoting plant growth. Host variation, among cultivars or plant genotypes, for response to beneficial microorganisms suggests that plant genes play a role in supporting these interactions (Smith and Goodman 1999). Between more closely related plants, several studies have reported that both the quantity and quality of root exudates vary between plant species. In addition, it is also recognized that different cultivars of the same species may vary in their root exudation pattern (Brimecombe et al. 2001). Root exudation was believed to have a major influence on the diversity of microorganisms within the rhizosphere (Lemanceau et al. 1995).

There are differences among cultivars in disease suppression caused by certain biocontrol agents (King and Parke 1993; Liu et al. 1995; Smith et al. 1997). Liu et al. (1995) found that there was cultivar specificity in induced resistance to anthracnose of cucumber by plant growth promoting rhizobacteria (PGPR) strains. Host varieties were also affected the expression of *phlA* gene that encoded the biosynthesis of 2,4-diacethylphloroglucinol (Notz et al. 2001).

The majority of microbial interactions considered so far concern a single pathogen and a single biocontrol agent in the rhizosphere. However, one way of improving biocontrol in the

rhizosphere may be to add mixtures or use combinations of biocontrol agents, particularly if these exhibit different or complementary modes of action or abilities to colonize root microsites. Such multiple interactions are the normal situation in the rhizosphere (Whipps 2001; Maurhofer et al. 2004). The effects of combinations of different bacteria, fungi and both bacteria and fungi have been reported. Raupach and Kloepper (1998) reported a seed application of a combination of three PGPR, *Bacillus pumilus* Meyer & Gotheil, *B. subtilis* (Ehrenberg) Cohn and *Curtobacterium flaccumfaciens* (Hedges) Collins and Jones provided greater control of several pathogens on cucumber (*Cucumis sativa* L.) than single inoculations. Combinations of *Paenibacillus* sp. and a *Streptomyces* sp. suppressed *Fusarium* wilt of cucumber better than when each agent was used alone (Singh et al. 1999). A combination of *Pseudomonas fluorescens* and *Stenotrophomonas maltophila* improved protection of sugar beet against *Pythium*-mediated damping-off in comparison with either applied individually (Dunne et al. 1998). When considering the use of mixtures or combinations of strains it is important that inhibition or interference among the components does not occur with the existing, normal and non-pathogenic microbiota associated with the roots (Whipps 2001).

This research sought to investigate the following: 1) the bioefficacies of *P. fluorescens* RH4003 against the bacterial wilt disease on selected tomato varieties, 2) root colonization ability of *P. fluorescens* RH4003 on different tomato varieties, 3) peroxidase activity in the root and stem of the tomato varieties after *P. fluorescens* RH4003 application, and 4) the biological efficacy of *P. fluorescens* RH4003 when applied as a mixture or single formulation.

## MATERIAL AND METHODS

### Isolation of *Ralstonia solanacearum*

The pathogen *Ralstonia solanacearum*, was isolated from tomato plants in Darmaga, Bogor, West Java, Indonesia. The isolate was maintained on Kings B medium for daily use and preserved in -20$^{o}$C using phosphate buffer with 20% glycerol.

### Biocontrol agents and tomato varieties

The biocontrol agents: *Pseudomonas fluorescens* RH4003 and *Bacillus subtilis* AB89 belonged to the collection of The Laboratory of Phytopathology, Tokyo University of Agriculture, Japan. *B. cereus* L32 belonged to the collection of The Laboratory of Plant Bacteriology, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University, Indonesia. One strain of *P. fluorescens* RH4003 used in this experiment was the wild type and another strain was the mutant which was spontaneously resistant to rifampicin, 100 µg/ml.

Five tomato varieties used in this experiment were common varieties in Bogor, West Java. These varieties were: Arthaloka, TM39, Ratna, San Marzano and Money Maker. Ratna is a resistant variety (Jaya 1997), while Arthaloka was stated as a tolerant variety (EWSI 2005). Another variety, AVRDC L390, from AVRDC-Taiwan. AVRDC L390 is a susceptible variety to bacterial wilt (Jaunet and Wang, 1998).

### Inoculation of *P. fluorescens* RH4003 and bacterial wilt assessment

Fourteen-day old tomato seedlings of six varieties were transplanted into polybags (diameter 15 cm) filled with soil and compost, (1:3, w/w). The soil was contaminated (inoculated?) with *R. solanacearum* using $10^7 – 10^8$ cfu/g solution (fresh weight). After transplanting, a 100 ml suspension of *P. fluorescens* RH4003 ($10^8 – 10^9$ cfu/ml) was poured into the contaminated soil. The control treatment used 100 ml sterilized distilled water (SDW). Each treatment had five replications. The experiment was conducted twice using randomized complete design.

Disease classes were determined using the scale: 0 = no symptom, 1 = 0 to 10% leaves wilted, 2 = 10% to 30% leaves wilted, 3 = 30% to 60% leaves wilted, 4 = 60% to 90% leaves wilted, 5 = 90% to 100% leaves wilted, which is a modification of the method developed by Arwiyanto et al. (1994). Disease intensity was calculated using the formula of Townsend & Heuberger (Unterstenhover 1963) as follows:

$$\text{Disease Intensity (\%)} = \frac{\sum_{i=0}^{5} n_i \times v_i}{N \times Z} \times 100\%$$

$n_i$ = number of plant with disease scale-i
$v_i$ = disease scale-i
N = Number of plant on each treatment
Z = The highest value of scales

**Population assessment of *P. fluorescens* RH4003 in the rhizosphere**

Two seeds of six tomato varieties were planted into each plastic pot (5 x 5 x 5 cm). After one week, a 10 ml suspension of *P. fluorescens* RH4004 $rif^r$ ($10^8 – 10^9$ cfu/ml) was poured into these pots. Tomato seedlings were sprinkled with 50 to 100 ml distilled water for daily watering. At one week after treatment, three plants of each variety were over-rooted and the roots were cut off. Two grams root was put into an Erlenmeyer flask with 50 ml sterilized distilled water, and was shaken for five minutes at 150 rpm. After a serial dilution, 100 µl of suspension was poured onto King's B agar plates contained 100 µg/ml of rifampicin. Colonies of *P. fluorescens* RH4003 $rif^r$ were counted at 24 – 48 hours after incubation.

The population of *P. fluorescens* RH4003 $rif^r$ in the soil (rhizosphere) was counted from a suspension of 5 g fresh weight soil in 50 ml sterilized distilled water and shaken for five minutes at 150 rpm. The other manipulation steps were the same as above. The experiments were conducted twice.

**Peroxidase activity in the root and stem**

A 10-ml suspension of *P. fluorescens* RH4003 ($10^8 – 10^9$ cfu/ml) was poured onto the roots and stems of 14-day old tomato seedlings. A week after treatment, tomato plants were over- rooted. Root and 2-cm of stem samples were cut off and weighed. Samples were macerated using a mortar with four volumes of 0.01M phosphate buffer pH 6.0. Macerated samples were centrifuged at 5,000 rpm for 30 minutes at 4°C. The supernatant was used for enzyme analysis. All activities were conducted in cool room (< 5°C) conditions.

Peroxidase activity was determined by mixing 0.2 ml of the diluted supernatant (stock enzyme : phosphate buffer 0.01M pH6.0 = 1 : 3) with a reaction solution containing 5 ml pirogalol 0.5M and 0.5 ml 1% $H_2O_2$ in a cuvette and allowed to become homogeneous. After 5 – 10 seconds the absorbance was measured using a spectrophotometer at 420 nm. Unit Activity Enzyme (UAE) was counted using the formula:

$$\text{Unit Activity Enzyme (UAE)} = \frac{\Delta \text{ OD x stock enzyme (ml)}}{\text{Fresh weight of sample (g)}}$$

$\Delta$ OD = average of the absorbance (b) determined by regression, where $y = a + bx$

**Bioactivity of *P. fluorescens* RH4003 in combination with other biocontrol agents**

Fifteen-day old tomato seedlings (var. Arthaloka) were transplanted into polybags containing 5 kg of soil inoculated with *R. solanacearum* ($10^7$ – $10^8$ cfu/g fresh weight). A 100 ml ($10^8$ – $10^9$ cfu/ml) suspension of a biocontrol agent was poured into the seedlings. When a mixture was used, the volume of each biocontrol agent was 50 ml. SDW was used as control. The experiments were conducted twice using randomized complete design with two replications. Each treatment had seven replications, each consisting of single plant. The codes of the treatments are presented in Table 1.

**Table 1.** Treatment codes for control agents.

| No. | Code | Control agents |
|---|---|---|
| 1 | C | Sterilized distilled water |
| 2 | RH | *P. fluorescens* RH4003 |
| 3 | AB | *B. subtilis* AB89 |
| 4 | L | *B. cereus* L32 |
| 5 | Ss | Streptomycin sulphate 0.2% |
| 6 | RH + AB | *P. fluorescens* RH4003 + *B. subtilis* AB89 |
| 7 | RH + L | *P. fluorescens* RH4003 + *B. cereus* L32 |
| 8 | RH + Ss | *P. fluorescens* RH4003 + Streptomycin sulphate 0.2% |
| 9 | AB + L | *B. subtilis* AB89 + *B. cereus* L32 |
| 10 | AB + Ss | *B. subtilis* AB89 + Streptomycin sulphate 0.2% |
| 11 | L + Ss | *B. cereus* L32 + Streptomycin sulphate 0.2% |

Disease intensity and disease scales were calculated using the formula stated previously. Synergy factors among biocontrol agents were predicted using Abbott's formula (Guetsky et al. 2002) as follows:

$$E_{(exp)} = a + b - a \times b/100 \quad \text{and} \quad SF \text{ (Synergy Factor)} = E_{(obs)}/E_{(exp)}$$

a = the effectiveness of biocontrol agent I
b = the effectiveness of biocontrol agent II
$E_{(exp)}$ = expected effectiveness caused by combination of biocontrol agents
$E_{(obs)}$ = effectiveness caused by combination of biocontrol agents based on the result of the observations

where:
SF = 1; interaction was additive
SF < 1; interaction was antagonistic
SF > 1; interaction was synergistic

## RESULTS AND DISCUSSION

**Bioefficacy of *P. fluorescens* RH4003**

Disease intensity among six varieties was significantly different without application of *P. fluorescens* RH4003 (Fig. 1). On the forty-ninth day, disease intensity on San Marzano and Money Maker without application of *P. fluorescens* RH4003 each was up to 90%, while on Ratna and TM 39 was 10% and 0%, respectively. TM39 and Ratna were relatively resistant to the tomato bacterial wilt disease while San Marzano and Money Maker were relatively susceptible.

The suppressive ability of *P. fluorescens* RH4003 against this disease was different between each variety (Fig. 1). Disease intensity on San Marzano and Money Maker applied with *P. fluorescens* RH4003 each was up to 54%, while on Ratna and TM39 was 0%. Suppression index by

*P. fluorescens* RH4003 on San Marzano and Money Maker were up to 36%. However, the index on Ratna was only 10%. These results suggest that the efficacy of *P. fluorescens* RH4003 against tomato bacterial wilt on susceptible varieties was higher than on the resistant one. The disease can be countered by the resistance of the plant. This result was similar to the previous report of Liu et al (1995).

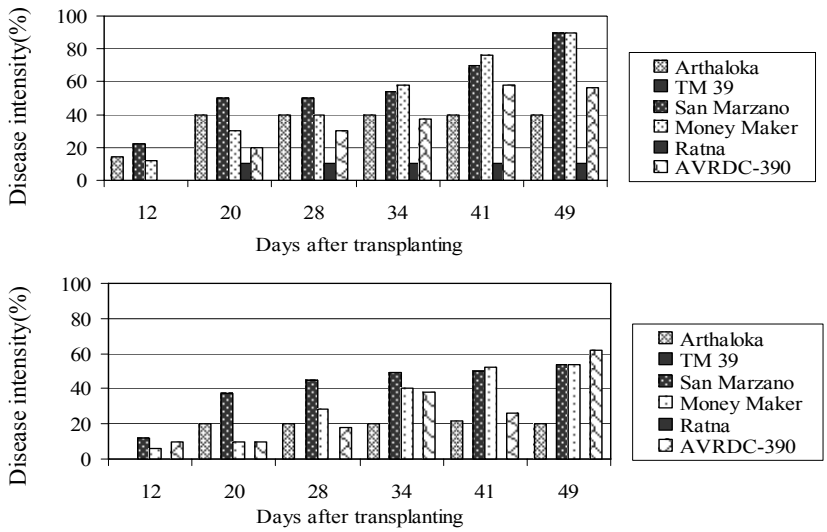


**Fig. 1.** Disease intensity of tomato bacterial wilt on six tomato varieties; without application of *P. fluorescens* RH4003 (above) and with application of *P. fluorescens* RH4003 (below).

### *P. fluorescens* RH4003 population

The *P. fluorescens* RH4003 population in the root surface was the same as that in the soil rhizosphere on all varieties on inoculation. The root surface population decreased similarly at 7 days after inoculation. In the soil, the rate of decrease of *P. fluorescens* RH4003 was higher than the rate of root surface. The decreasing population (Δ log) of *P. fluorescens* RH4003 at 7 days after inoculation was higher than at 14 days (Table 2).

In the soil, the *P. fluorescens* RH4003 population decreased until 14 days after application and was always lower than those in the rhizosphere. The data showed that *P. fluorescens* RH4003 colonizes in the roots and in the rhizosphere than in soil. Similar results were obtained in a previous report (Simon et al. 2001).

In the rhizosphere of Arthaloka, San Marzano and Ratna, the *P. fluorescens* RH4003 population increased from 7 to 14 days after inoculation. *P. fluorescens* RH4003 succeeded to colonize and build up the population in the rhizosphere of Arthaloka (medium resistant), San Marzano (susceptible) and Ratna (resistant) during the two weeks after inoculation. There is no correlation between the level of resistance and the population build up. This result is similar to a previous report that showed the colonization capacity of PGPR strain of rifampicin-resistant mutants had no cultivar specificity on root systems of four cucumber cultivars (Liu et al. 1995).

Root colonization plays a crucial role in biocontrol. Colonization of large parts of the root system will obviously facilitate biocontrol since colonization can be expected to function as the

delivery system for bacterial cells that act as factories for antifungal metabolites. Inadequate colonization leads to decreased biocontrol activity (Lugtenberg et al 2001).

**Table 2.** *P. fluorescens* RH4003 Rif$^r$ population in the root surface and in the rhizosphere soil.

| Tomato varieties | Population of *P. fluorescens* RH4003 Rif$^r$ (log cfu/g fresh weight) | | | | |
|---|---|---|---|---|---|
| | 0 DAT* | 7 DAT | Δ log** | 14 DAT | Δ log*** |
| **Root** | | | | | |
| Arthaloka | 8,19 | 7,28 | - 0.91 | 7,37 | 0.09 |
| TM 39 | 7,95 | 6,71 | - 1.24 | 6,47 | - 0.24 |
| San Marzano | 8,37 | 7,44 | - 0.93 | 7,50 | 0.06 |
| Money Maker | 8,48 | 7,42 | - 1.06 | 7,36 | - 0.06 |
| AVRDC-390 | 8,79 | 7,29 | - 1.50 | 6,79 | - 0.50 |
| Ratna | 8,50 | 7,62 | - 0.88 | 7,73 | 0.11 |
| **Soil** | | | | | |
| Arthaloka | 8,15 | 6,07 | - 2.08 | 6,00 | - 0.07 |
| TM 39 | 8,30 | 6,28 | - 2.02 | 6,26 | - 0.02 |
| San Marzano | 8,08 | 6,23 | - 1.85 | 6,08 | - 0.15 |
| Money Maker | 7,90 | 5,84 | - 2.06 | 5,78 | - 0.06 |
| AVRDC-390 | 8,15 | 6,36 | - 1.79 | 6,19 | - 0.17 |
| Ratna | 8,51 | 6,49 | - 2.02 | 6,48 | - 0.01 |

* days after treatment

** population on 7 DAT compared to the population on 0 DAT

*** population on 14 DAT compared to the population on 7 DAT

**Peroxidase activity**

Peroxidase is one of the enzymes that has a role in defense mechanisms against pathogens. This enzyme is a precursor for lignin development. Root peroxidase activity on relatively susceptible tomato varieties, San Marzano, Money Maker, and AVRDC390 dipped in *P. fluorescens* suspension was higher than on relatively resistant varieties such as Arthaloka, TM39 and Ratna (Fig. 2). This is correlated with disease suppression. It can be said that induction of resistance by *P. fluorescens* RH4003 was more effective in suppressing bacterial wilt disease on relatively susceptible tomato varieties than on the relatively resistant ones.

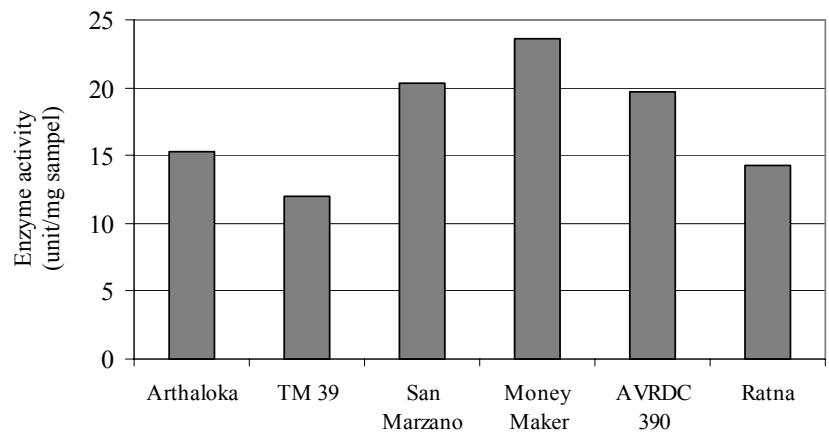


**Fig. 2.** Peroxidase activity of six tomato varieties at 14 hours after root dipping in *P. fluorescens* RH4003 suspension.

**Bioefficacy of *P. fluorescens* RH4003 combined with other agents**

Combination of biocontrol agents with synergistic effect produces higher disease suppression than when the biocontrol agent was applied singly. On the other hand, if the combined biocontrol agentS were antagonistic, the control effect would be worse. At 31 days after seedling transplanting, the disease intensity in the plot treated with *P. fluorescens* RH4003 (RH) only was much lower (29 %) compared with the other treatments, combination of *P. fluorescens* RH4003 with *B. subtilis* AB89 (73 %), *B. cereus* L32 (70 %) and streptomycin sulphate (71 %), (Fig. 3).

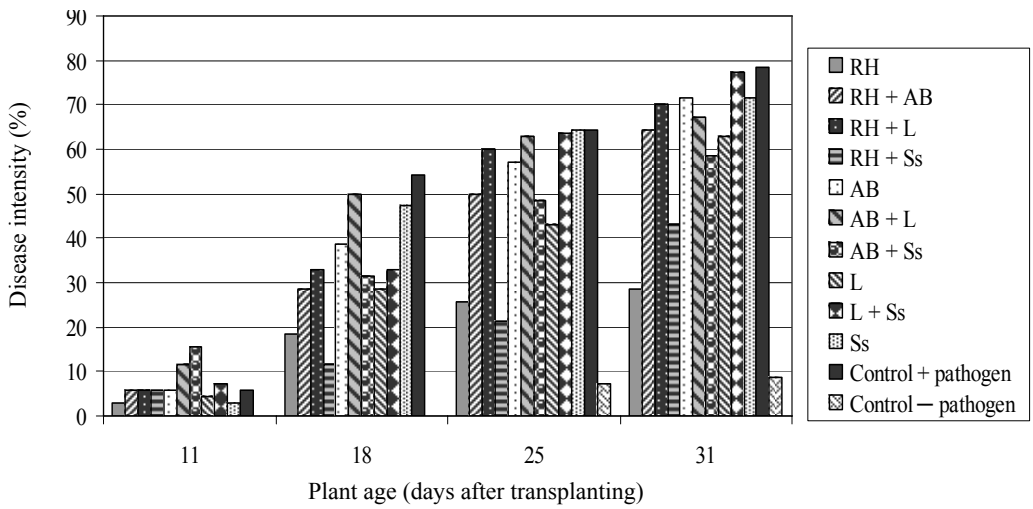


**Fig. 3.** Effects of *P. fluorescens* RH4003 applied as a single and combination biocontrol on the disease intensity of tomato bacterial wilt; RH = *P. fluorescens* RH4003, AB = *B. subtilis* AB89, L = *B. cereus* L32 dan Ss = Streptomycin sulphate 0,02%

At both 18 days and 25 days after seedling transplanting, the RH+ Ss plot combined with *P. fluorescens* RH4003 and streptomycin sulphate had the highest index suppression value compared with the other treatments (Table 3).

**Table 3.** Index suppression of single and combined applications of biocontrol agents to control the tomato bacterial wilt disease

| Combination of biocontrol agents | Index suppression (%)* | | |
|---|---|---|---|
| | 18 DAT | 25 DAT | 31 DAT** |
| RH*** | 65.7 | 59.9 | 63.4 |
| RH + AB | 26.7 | 22.2 | 13.6 |
| RH + L | 16.3 | 6.7 | 17.7 |
| RH + Ss | 72.4 | 62.2 | 16.9 |
| AB | 5.9 | 11.2 | 5.2 |
| AB + L | - 14.6 | 2.3 | 11.5 |
| AB + Ss | 42.0 | 5.1 | 17.9 |
| L | 47.4 | 33.3 | 12.8 |
| L + Ss | 16.3 | 0.2 | - 11.3 |
| Ss | - 9.9 | - 14.3 | 5.2 |

* compared to control treatment

** days after transplanting

*** RH = *P. fluorescens* RH4003, AB = *B. subtilis* AB89, L = *B. cereus* L32 and Ss = Streptomycin sulphate 0,02%

According to the Synergy Factor (SF) value, *P. fluorescens* RH4003 and streptomycin sulphate 0.02% were synergistic at 18 days and 25 days after seedling transplanting but this combination became antagonistic at 31 days after seedling transplanting (Table4). On the other hand, the combination of *B. subtilis* AB89 and streptomycin sulphate 0.02 % was antagonistic at 18 days and 25 days after seedling transplanting but synergistic at 31 days after seedling transplanting. The combination case of *B. subtilis* AB89 and streptomycin sulphate 0.2 % was antagonistic at first but later it became synergistic. It is suggested that streptomycin sulphate precisely suppressed *B. subtilis* activity at first, but later *B. subtilis* started to express its ability to suppress bacterial wilt disease.

**Table 4.** Synergy Factors (SF) and interaction (I) among biocontrol agents

| Combination of biocontrol agents | SF value and interaction (I) | | | | | |
|---|---|---|---|---|---|---|
| | 18 DAT | | 25 DAT | | 31 DAT** | |
| | SF | I | SF | I | SF | I |
| RH + AB** | 0.39 | A*** | 0.35 | A | 0.20 | A |
| RH + L | 0.20 | A | 0.09 | A | 0.25 | A |
| RH + Ss | 1.16 | S | 1.15 | S | 0.25 | A |
| AB + L | - 0.29 | A | 0.06 | A | 0.66 | A |
| AB + Ss | - 12.43 | A | - 3.34 | A | 1.75 | S |
| L + Ss | 0.39 | A | 0.01 | A | - 0.65 | A |

* days after transplanting

** RH = *P. fluorescens* RH4003, AB = *B. subtilis* AB89, L = *B. cereus* L32, Ss = Streptomycin sulphate 0,02%

*** A = antagonistic, S = synergistic

In order to get an effective biocontrol, application of two or more biocontrol agents in one formulation should be based on some requisite. These are: 1) the biocontrol agents have different site or habitat, in the rhizosphere and organic materials, 2) different mechanism of suppression, competition and antibiosis, 3) need different substrate, plant and bacterial mucosa for fungi and root exudates for pseudomonads bacteria, and 4) compatible with the soil environment and the changes caused by increasing breeding system (Graham and Mitchell 1999). The data shows that not all of the biocontrol agents can be combined with *P. fluorescens* RH4003.

## CONCLUSIONS

San Marzano and Money Maker were relatively susceptible to the bacterial wilt disease of tomato. The relatively resistant varieties were TM39 and Ratna. The application of *P. fluorescens* RH4003 to these six varieties was able to suppress the disease intensity of tomato bacterial wilt. Peroxidase activity was higher on the relatively susceptible varieties compared with on the relatively resistant one. The population density of *P. fluorescens* in the rhizosphere of six varieties was higher than in the soil.

The highest suppression index from the combination of *P. fluorescens* RH4003 and 0.02% streptomycin sulphate, at 18 days and 25 days after seedling transplanting, were 72.4 and 62.2 %, respectively. However, the highest suppressive index was attained by the application of *P. fluorescens* alone (63.4 %) at 31 days after seedling transplanting. This combination was synergistic at 18 and 25 days after seedling transplanting but later became antagonistic at 31 days.

The best treatment is the application of *P. fluorescence* RH4003 singly but this can also be applied in combination with streptomycin sulphate, if farmers use this antibiotic in the field.

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# FUNGICIDAL ACTIVITY OF *Piper betle* EXTRACT AGAINST *Fusarium oxysporum* f.sp. *vanillae*

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## ABSTRACT

One of the most destructive diseases of vanilla is stem rot disease caused by the pathogenic fungus, *Fusarium oxysporum* f.sp. *vanillae*. Under favorable conditions for the development of the disease, the vanilla plant may be severely infected and ultimately die.

A previous study found the crude extract of *Alpinia galanga* to possess relatively strong fungicidal activity against *F. oxysporum* f.sp. *vanillae* on PDA and on vanilla seedlings in green house experiments. Five plant species belonging to the family Piperaceae namely *Piper betle*, *P. nigrum*, *P. retrofractum*, *P. cubeba* and *P. decumanum* were studied for their fungicidal activity against *F. oxysporum* f.sp. *vanillae*. The minimum inhibitory concentration (MIC) of the crude leaf extract for each plant was studied on PDA medium to assess its fungicidal activity.

Among the five plant species evaluated, only the 0.15% crude extract of *P. betle* showed strong fungicidal activity against *F. oxysporum* f.sp. *vanillae*, while the other plants did not show fungicidal activity at concentrations from 0.05% to 0.5%. Fungal growth was not observed when *F. oxysporum* f.sp. *vanillae* was grown on PDA amended with 0.4% crude extract of *P. betle*. The 0.5% *P. betle* leaf extract could completely inhibited the fungal growth on PD broth and protected the vanilla stem-cuttings from fungal infection. These results suggest that the *P. betle* crude extract could be used as an alternative natural agent to control stem rot disease on vanilla.

**Key words** : crude extract, minimum inhibitory concentration.

**Abbreviations:** MIC - minimum inhibitory concentration, PDA - potato dextrose agar

## INTRODUCTION

Vanilla (*Vanilla planifolia*) is an as orchid cultivated for its pleasant flavor because it contains natural vanillin. The pods of this plant contain vanillin which produces the specific aroma when subjected to curing process. Vanillin is used largely in the food industry especially in the preparation of ice cream, chocolate, bakery products, pharmaceuticals, perfumes *etc*. The plant is the second most expensive spice after saffron, so it is also known as “green gold” (Anonymous, 2004a). The total global demand for vanilla was estimated about 4,500 tons a year, with the USA accounting for more than 30% of the world imports (Anonymous, 2004b).

In Bali, the total area for vanilla cultivation in 1991 was 4,093 hectares with a production of 279.7 tons of beans. However, the area and production of vanilla in Bali has gradually decreased, and in 2006 the total area of vanilla cultivation was only 820.15 hectares with a production of 14.26 tons of beans (Anonymous, 2007). One of the most important causes for the decrease of both cultivation area and production is stem rot disease caused by the pathogenic fungus *Fusarium oxysporum* f.sp.*vanillae* (Street, 1985; Semangun, 1988, Anonymous, 2006). A survey done in Bali in three main vanilla growing areas indicated that the stem rot disease was the main and the most destructive disease on vanilla, and about 65% of the vanilla trees in these areas died because of the disease (Anonymous, 2006). Several measures have been implemented to control the disease such as improvement of cultural practices, sanitation of the vanilla orchard, use of fungicides, crop rotation, mixed cropping *etc*., but the disease still can not be managed properly.

Several higher plants of Bali origin which possess antifungal activity include *Pometia pinnata* which is active against *Pythophthora infestans* the causal agent of potato late blight (Suprapta *et al*., 2002). *Carica papaya* leaf extract is active against *Ceratocystis* sp. (Suprapta *et al*., 2001), *Piper betle*, *Alpinia galanga* and *Carica papaya* extracts were found to be effective in suppressing banana wilt disease in the field (Arya *et al*., 2001). The 0.5% (w/v) crude extract of *Alpinia galanga* inhibited significantly the growth of *F. oxysporum* f.sp. *vanillae* on PDA and reduced the incidence of stem rot disease on vanilla seedlings (Suprapta *et al*. 2005a). In addition, the crude extract of *Alpinia galanga* in combination with the extract of *Piper betle* suppressed effectively the development of wilt disease on banana seedlings caused by *Fusarium oxysporum* f.sp. *cubense* (Suprapta *et al*., 2005b).

As alternatives to synthetic fungicides, several plants belonging to the family Piperaceae were evaluated for their fungicidal activity against *F. oxysporum* f.sp. *vanillae*.

## MATERIALS AND METHODS

### Preparation of plant extract

Five species of Piperaceae plants namely: *Piper betle*, *P. nigrum*, *P. retrofractum*, *P. cubeba* and *P. decumanum* were studied. *P. cubeba*, *P. decumanum* and the other three species were collected in Central Java, Maluku and Bali, respectively. The leaves were washed in tap water and distilled water to remove all of the surface contamination before chopping into small pieces using a sharp knife. The chopped leaves were air dried under room temperature for about 3 days. This material was then extracted with PA grade methanol (1:10, w/v) for 48 h under room temperature in the dark. The extraction procedure was repeated three times and all of the filtrates obtained were evaporated until dryness using vacuum rotary evaporator (Iwaki, Tokyo). The crude extract was dissolved in acetone before use.

### Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was determined against *Fusarium oxyspo*rum f.s. *vanillae* that was received from the Laboratory of Microbiology Faculty of Agriculture, Udayana University. The fungus was grown on PDA slant medium for 4 days to stimulate spore formation. The spores were harvested in sterile distilled water and the spore density was adjusted to $10^5$ spores/ml. A 200-µl spore suspension was poured and mixed into melted PDA medium in a Petri dish.

After the medium became solid, two absorbance discs (4 mm diam.) loaded with crude extract of each plant and its respective concentration were placed side by side on the Petri dish. Concentrations tested were from 0.05% to 0.5% (w/v). Ten Petri dishes were prepared for each concentration and plant species. These cultures were incubated in the dark at 25$^o$C for 48 hours. The

formation of inhibition zone around the disc was observed to evaluate the fungicidal activity of the extract.

**Spore formation in Potato Dextrose (PD) Broth**

The fungal isolate of *F. oxysporum* f.sp. *vanillae* was grown on slant PDA medium for 3 days to allow the fungus to form spores. The spores were then harvested in sterile distilled water. The spore density was measured and then adjusted to $10^5$ spores/ml. Sterile PD broth medium (50 ml) was prepared in 100-ml Erlenmeyer flasks to which *P. betle* crude extract was added to make several concentrations ranging from 0.05% to 0.5% (w/v). Flasks without extract were prepared as controls. Three flasks were prepared for each concentration. Each flask was then inoculated with a 500 µl spore suspension ($10^5$ spores/ml). The cultures were incubated in the dark at room temperature for 24 h to determine the spore density under the microscope.

The inhibitory activity of each plant extract was determined using the following formula:

$$\text{Inhibitory activity} = \frac{\text{Spore formation of control} - \text{Spore formation of treatment}}{\text{Spore formation of control}} \times 100\%$$

Two sets of experiments of the same treatment and procedure were performed in this study.

**Radial Growth on Potato Dextrose Agar (PDA)**

Each plant extract at a specific concentration was amended into melted PDA medium in a Petri dish. The Petri dish was shaken gently horizontally to allow the extract to distribute evenly on the PDA medium. Mycelial plugs ( 4mm-diam.), taken from the edge of an actively growing colony of *F. oxysporum* f.sp. *vanillae*, were inoculated on the PDA medium. These cultures were then incubated in the dark under room temperature for 5 days. The PDA amended with 200 µl acetone was used as a control. Five Petri dishes were prepared for each concentration evaluated. The diameter of the colony was measured on the fifth day of incubation to determine the inhibitory activity of the extract. The inhibitory activity was calculated using the following formula :

$$\text{Inhibitory activity} = \frac{\text{Colony diam. of control} - \text{Colony diam. of treatment}}{\text{Colony diam. of control}} \times 100\%$$

Two sets of experiments of the same treatment and procedure were performed in this study.

**Application of Extract on Seedlings**

Vanilla stems were kindly supplied by the Bali Government Corporation located at Jembrana Regency. The stems obtained from the field were washed with tap water and then with distilled water to remove all surface contaminants. Stem-cuttings with two nodes (approximately 20 cm length) were prepared by using sharp scissors prior to the treatment with the plant extract. The stem-cuttings were then soaked in extract suspension at concentrations 0.15%, 0.2%, 0.25, 0.3%, 0.35%, 0.4%, 0.45% and 0.5% (w/v) for one hour. Stem-cuttings soaked with distilled water were prepared for controls. These stem cuttings were planted in polyethylene bags containing sterilized fertile soil and cow manure and inoculated with 10 ml spore suspension of *F. oxysporum* f.sp. *vanillae* ($10^6$ spores/ml). The average spore density was $2 \times 10^3$ spores/gram of soil. The subsequent treatments were done at three, six and nine days after planting by dressing a 50-ml extract formulation into the soil at the bottom of each stem-cutting in polyethylene bags. Three replicates for each extract concentration were prepared for this experiment. These cultures were maintained in a screen house for 70 days.

Observation of the first disease symptom was done every day, while the observation and determination of disease incidence was done weekly. Disease incidence at 70 days after planting was used to determine the inhibitory activity using the following formula :

$$\text{Inhibitory activity} = \frac{\text{Disease incidence of control} - \text{Disease incidence of treatment}}{\text{Disease incidence of control}} \times 100\%$$

Treatments in this study were allocated according to the completely randomized design. Two sets of experiments with the same treatment and procedure namely experiment 1 (June to August 2006) and experiment 2 (October to December 2006) were conducted in this study.

## RESULTS AND DISCUSSION

The crude leaf extracts of several plants belonging to the family Piperaceae were studied for their bioactivity against *Fusarium oxysporum* f.sp. *vanillae* on PDA medium. Among the five plant species tested, only *Piper betle* showed fungicidal activity against *F. oxysporum* f.sp. *vanillae*, with an MIC at 0.15%. The rest of the four plant species namely *P. nigrum*, *P. retrofractum*, *P. cubeba* and *P. decumanum* did not show inhibitory activity against *F. oxysorum* f.sp. *vanillae* up to a concentration of 0.5% (w/v).

The *P. betle* crude extract affected significantly the spore formation of *F. oxysporum* f.sp. *vanillae* in PD broth medium (Table 1). The spore formation was inhibited by the *P. betle* crude extract as low as 0.1% with inhibitory activity of 84.41%. Spore formation was completely inhibited when 0.3% to 0.5% *P. betle* crude extract was used. This result suggests that inhibition of spore formation is one of the mechanisms by which the crude extract can inhibit the fungal growth and development.

**Table 1.** Inhibitory activity of *Piper betle* leaf extract to the spore formation of *Fusarium oxysporum* f.sp. *vanillae* in potato dextrose broth.

| No. | Extract concentration (%: w/v) | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|---|
| | | Spore formation ($x10^5$/ml)* | % Inhibitory activity | Spore formation ($x10^5$/ml)* | % Inhibitory activity |
| 1 | Control | 5.71 a** | - | 6.22 a | - |
| 2 | 0.15 | 0.89 b | 84.41 | 1.21 b | 80.54 |
| 3 | 0.20 | 0.21 c | 96.32 | 0.28 c | 95.49 |
| 4 | 0.25 | 0 d | 100 | 0.02 d | 99.68 |
| 5 | 0.30 | 0 d | 100 | 0 d | 100 |
| 6 | 0.35 | 0 d | 100 | 0 d | 100 |
| 7 | 0.40 | 0 d | 100 | 0 d | 100 |
| 8 | 0.45 | 0 d | 100 | 0 d | 100 |
| 9 | 0.50 | 0 d | 100 | 0 d | 100 |

* Spore density at the beginning of experiment was $10^3$ spores/ml.

** Values followed by the same letters in the same column are not significantly different (P>0.05) as determined by the Least Significant Difference (LSD).

In addition to spore formation, the *P. betle* crude extract also affected the radial growth of *F. oxysporum* f.sp. *vanillae* on PDA medium. The radial growth of *F .oxysporum* f.sp. *vanillae* was inhibited significantly by the *P. betle* crude extract at concentrations as low as 0.15% with an inhibitory activity of 20.23%. The more the concentration of the extract increased, the more the inhibitory activity observed within the tested concentrations (Table 2). Fungal growth was not

observed when the PDA medium was amended with 0.4% of crude extract. This indicates that the *P. betle* crude extract not only inhibited the spore formation, but also the radial growth of the fungus on PDA.

A previous study showed that the extract of red ginger (*Alpinia galanga* L.) possesed strong fungicidal activity against *F. batatatis* (*F. oxysporum* f.sp. *vanillae*). The methanol phase of the red ginger extract at 0.10% (w/v) totally inhibited the radial growth of *F. oxysporum* f.sp. *vanillae* on PDA medium (Wibawa and Suprapta, 2007).

**Table 2.** Inhibitory activity of *Piper betle* leaf extract on the radial growth of *Fusarium oxysporum* f.sp. *vanillae* on potato dextrose agar plate

| No. | Extract concentration (%: w/v) | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|---|
| | | Radial Growth of fungal colony (mm)* | % Inhibitory activity | Growth of fungal colony (mm)* | % Inhibitory activity** |
| 1 | Control | 84.45 a** | - | 86.03 a | - |
| 2 | 0.15 | 67.36 b | 20.23 | 64.88 b | 24.58 |
| 3 | 0.20 | 38.71 c | 54.16 | 41.28 c | 52.01 |
| 4 | 0.25 | 28.55 d | 66.19 | 27.34 d | 68.22 |
| 5 | 0.30 | 21.44 e | 74.61 | 19.27 e | 77.60 |
| 6 | 0.35 | 14.52 f | 82.80 | 15.09 e | 82.45 |
| 7 | 0.40 | 0 g | 100 | 0 f | 100 |
| 8 | 0.45 | 0 g | 100 | 0 f | 100 |
| 9 | 0.50 | 0 g | 100 | 0 f | 100 |

* Diameter of mycelial plug used at the beginning of experiment was 4 mm.

** Values followed by the same letters in the same column are not significantly different (P>0.05) as determined by the Least Significant Difference (LSD).

The application of the *P. betle* crude extract into the soil that was previously inoculated with *F. oxysporum* f.sp. *vanillae* could protect significantly the vanilla seedlings from infection (Table 3).

**Table 3.** Inhibitory activity of *Piper betle* extract against stem rot disease on vanilla seedlings.

| No. | Extract concentration (%: w/v) | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|---|
| | | Disease incidence (%) | % Inhibitory activity | Disease incidence (%) | % Inhibitory activity |
| 1 | Control | 95.94 a* | - | 97.03 a* | - |
| 2 | 0.15 | 77.23 b | 18.94 | 81.37 b | 16.49 |
| 3 | 0.20 | 38.42 c | 60.00 | 37.46 c | 61.85 |
| 4 | 0.25 | 9.72 d | 90.52 | 4.78 d | 95.87 |
| 5 | 0.30 | 0 e | 100 | 0 e | 100 |
| 6 | 0.35 | 0 e | 100 | 0 e | 100 |
| 7 | 0.40 | 0 e | 100 | 0 e | 100 |
| 8 | 0.45 | 0 e | 100 | 0 e | 100 |
| 9 | 0.50 | 0 e | 100 | 0 e | 100 |

* Values followed by the same letters in the same column are not significantly different (P>0.05) as determined by the Least Significant Difference (LSD).

In field experiments, *P. betle* has been proven to possess fungicidal activity against *Fusarium oxysporum* f.sp. *cubense*, the causal agent of banana wilt disease (Arya *et al.*, 2001). Two applications of a 500 ml water extract of *P. betle* with one month interval at a concentration of 5% (w/v) reduced significantly the wilt disease incidence (Arya *et al*., 2001). Another study showed that the use of a mixture of *P. betle* and *A. galanga* extracts (1:1) inhibited significantly the development of wilt disease caused by *F. oxysporum* f.sp. *cubense* on banana seedling in comparison with control and treatment with the synthetic fungicide, chlorothalonil (Suprapta *et al*., 2005b).

## CONCLUSION

The *P. betle* leaf extract possesses potential antifungal activity against *F. oxysporum* f.sp. *vanillae* both on PDA and on vanilla seedlings. A 0.4% leaf extract completely inhibited spore formation in PD broth medium, radial growth of the fungus on PDA medium, and suppressed the development of stem rot disease on vanilla seedlings. Further studies on the elucidation of substances responsible for the antifungal activity of *A. galanga* as well as the field experiment to determine the effects of the extract on to the development of stem rot disease on vanilla seedlings are necessary.

## ACKNOWLEDGEMENT

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# EFFICACY OF HYDRATED SODIUM CALCIUM ALUMINOSILICATE AND VERMICULITE FOR AFLATOXIN $B_1$ ADSORPTION IN BLACK TIGER SHRIMP (*Penaeus monodon*) DIETS

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## ABSTRACT

The effects of hydrated sodium calcium aluminosilicate (HSCAS) and vermiculite minerals as binders were evaluated when added to black tiger shrimp (*Penaeus monodon*) diets contaminated with aflatoxin $B_1$. Four treatments consisting of balanced diets were as follows: diet 1 as a control diet, free of the toxin and without a binder, diet 2 containing 1% of HSCAS and 500 ppb of aflatoxin $B_1$, diet 3 containing 1% of vermiculite and 500 ppb of aflatoxin $B_1$, and diet 4 containing 500 ppb of aflatoxin $B_1$ without a binder. Shrimps weighing 0.500 g were fed with these diets for a period of eight weeks. The shrimps were stocked in a closed sea water system inside a fiberglass tank. It was observed that the weight gain, average daily gain, feed conversion ratio, protein efficiency ratio, survival rate and aflatoxin $B_1$ residue in tissues were significantly superior in shrimps fed with diet 2 and diet 3, compared to those with diet 4 ($P<0.05$). These indicators showed lower performance than those obtained from diet 1. Overall, diet 1 had the best performance and a significant difference ($P<0.05$) when compared with other diets. This indicated that aflatoxin $B_1$ can adversely affect shrimp health and growth. In addition the aflatoxin $B_1$ residue level in shrimp head plus shell was higher than in shrimp muscle, but was below the FAO/WHO standard of 20.0 ppb. HSCAS and vermiculite were therefore capable of binding some part of the aflatoxin $B_1$ dose and the use of mycotoxin-binding substances could be beneficial in shrimp raising. The toxin-binding efficacy of the HSCAS was similar to that of the vermiculite.

**Key words :** mycotoxin, binder, growth, survivability, aflatoxin, residue

**Abbreviations:** ADG – average daily gain, FCR – feed conversion ratio, HSCAS, PER – protein efficiency ratio

## INTRODUCTION

The increasing demand for black tiger shrimps (*Penaeus monodon*) in overseas markets has given rise to the increase in production of this kind of shrimp. Thailand has become the world's leading shrimp exporter. This is mainly because farmers receive high profits for *P. monodon*, due to a strong market abroad. This has led to the rapid growth of their farms (Suppadit *et al*., 2005). But in the last few years, shrimp farms have been attacked by infectious disease outbreaks, followed by the restrictions on export due to residual antibiotics in the edible tissue (Soonngam, 2005). Apart from infections, a more consistent and frequent problem relates to poor nutrition due to the inability to achieve satisfactory growth.

Mycotoxins are a group of structurally diverse secondary metabolites of fungi that occur as contaminants of grains worldwide (Watts *et al*., 2003). Improper storage conditions further aggravate this vulnerability. Various species of *Fusarium* and *Aspergillus* fungi are often found to contaminate aquaculture feed raw materials (Lightner, 1993; Lumlertdacha and Lowell, 1995; Tepsic *et al*., 1997). The two most encountered species are *Aspergillus flavus* and *Aspergillus parasiticus*, and one of the

most potent types of toxin produced is aflatoxin. Aflatoxins are toxic metabolites of fungi which belong to the difurocumarocyclopentanon series, called difurocumarolactons (Betina, 1989). These toxins are grouped into 4 major classes: $B_1$, $B_2$, $G_1$ and $G_2$ (Bintvihok *et al*., 2003). Ingestion of aflatoxin at very low doses for short periods leads to some adverse effects, such as liver damage and mutagenic effects. (Smith *et al*., 1975; Maurice *et al*., 1983; Robens and Richard, 1992; Smith *et al*., 1992; Eraslan *et al*., 2003). The investigations into the mechanism of aflatoxin $B_1$ toxicity have revealed that after entering the body, this mycotoxin assumes an epoxide that is capable of binding with bio-molecules of vital significance such as DNA, RNA and proteins (Colvin *et al*., 1989). While the effects of aflatoxin in various farm animal species have been investigated (Scheidler, 1993; Watts *et al*., 2003; Eraslan *et al*., 2004; Suppadit *et al*., 2006), there is limited published information available on the effects of aflatoxins on the *P. monodon*.

A number of approaches to deal with mycotoxin contaminated shrimp feeds have been evaluated. One of these approaches is the addition of adsorbent materials to the diet (Watts *et al*., 2003). The present research therefore sought to develop a procedure to minimize the aflatoxin exposure of *P. monodon* through the contaminated feed. This can be accomplished by adding feed mycotoxin-adsorbent silicate minerals such as hydrated sodium calcium aluminosilicate (HSCAS) and vermiculite. These chemicals belong to the group of chemically heterogeneous non-nutritive adsorptive minerals commercially available either in an entirely natural form or in the chemically modified form prepared from the natural silicate minerals. These have been shown to bind to aflatoxin *in vivo* and *in vitro* (Phillips *et al*., 1988; Kubena *et al*., 1990; Huff *et al*., 1992; Ledoux *et al*., 1999) due to a very large surface area within the crystal lattice of these minerals which have electrochemical properties to absorb aflatoxin (Mumton and Fishman, 1977; Erasmus and Prinsloo, 1989).

This study sought to determine the beneficial effects of the HSCAS and vermiculite minerals as aflatoxin $B_1$ binders when added to mycotoxin-contaminated shrimp diets. The effectiveness was indirectly reflected in improvements in growth performance, survivability, and feed efficiency.

## MATERIALS AND METHODS

### Experimental Animals

The experiment was conducted from December, 2005 to January, 2006 using 6,000 shrimps *P. monodon* in the post-larval stage 15, obtained from a commercial hatchery in Chantaburi Province. These shrimps when fed with control diets (Table 1) attained the weight of around 0.500 g/ease. The animals (640) were randomly selected for 4 treatments and 4 replications (40 shrimps per replication) in a completely randomized design (Chanthalukana, 1980). The shrimps were then stocked in four fiberglass tanks (10 tons) located in Siriwan Farm, Kaeng Khoi District, Saraburi Province at a density of 160 shrimps per tank in one replication with 4 treatments, by stocking one shrimp per 25.0 cm diameter basket. All shrimps were fed experimental diets three times per day (06:00 am, 02:00 pm and 10:00 pm) at the set levels, depending on the shrimp's body weight of each tank according to Robertson *et al*. (1993)'s suggestion. These were reared for 8 weeks in a flow-through sea water system with a flow rate at 6.00 l/min, common biological filter, pressurized sand filter and circulation pump.

### Experimental Diets

Four experimental diets (4 treatments) were formulated at least cost to contain an equal amount of all ingredients, except for the level of aflatoxin $B_1$ and type of adsorbent as follows: diet 1 contained 0 ppb of aflatoxin $B_1$ as the control diet; diet 2 contained 500 ppb of aflatoxin $B_1$ with 1 kg of HSCAS/100 kg diet; diet 3 contained 500 ppb of aflatoxin $B_1$ with 1.00 kg of vermiculite/100 kg

diet; and diet 4 contained 500 ppb of aflatoxin $B_1$. The appropriate ratio to reduce the aflatoxin $B_1$ introduced by Soonngam (2005) was 1% of HSCAS and vermiculite. This can efficiently reduce 88.5% and 89.4% of toxin, respectively. All diets were mixed in a mixer with about 28.0% moisture and pelleted through a 2.00 mm die. This process was followed by 8 hours of drying in an air flow oven at 90.0ด C until the moisture content was lower than 10.0%. The dry pellets were kept in plastic bag in a refrigerator prior to feeding. The experimental diets were randomly selected for chemical analysis following procedures by AOAC (1992) and aflatoxin $B_1$ by a high performance liquid chromatography (HPLC) method (Soonngam, 2005). Three replicates per sample were analyzed. The composition of experimental diets and chemical composition, as well as aflatoxin $B_1$ accumulated analysis in the diets, are shown in Table 1.

**Table 1.** Feed composition, nutrient content, and aflatoxin $B_1$ level accumulated in experimental diets

| Feed composition[1/] | Experimental diets | | | |
|---|---|---|---|---|
| | **1** | **2** | **3** | **4** |
| Fish meal (68.0 % protein) | 28.0 | 28.0 | 28.0 | 28.0 |
| Shrimp head meal | 10.0 | 10.0 | 10.0 | 10.0 |
| Soybean meal (48.0 % protein) | 12.0 | 12.0 | 12.0 | 12.0 |
| Squid meal | 5.00 | 5.00 | 5.00 | 5.00 |
| Broken rice | 14.0 | 13.0 | 13.0 | 14.0 |
| Wheat gluten | 7.00 | 7.00 | 7.00 | 7.00 |
| Wheat flour | 10.0 | 10.0 | 10.0 | 10.0 |
| Fish oil | 2.00 | 2.00 | 2.00 | 2.00 |
| Lecithin | 2.00 | 2.00 | 2.00 | 2.00 |
| Vitamin mix[2/] | 0.300 | 0.300 | 0.300 | 0.300 |
| Cellulose | 0.500 | 0.500 | 0.500 | 0.500 |
| Vitamin C | 0.100 | 0.100 | 0.100 | 0.100 |
| Vitamin E | 0.150 | 0.150 | 0.150 | 0.150 |
| Choline chloride | 0.300 | 0.300 | 0.300 | 0.300 |
| Ca – lactate | 0.800 | 0.800 | 0.800 | 0.800 |
| Trace mineral[3/] | 0.280 | 0.280 | 0.280 | 0.280 |
| Yeast | 5.00 | 5.00 | 5.00 | 5.00 |
| Mono sodium phosphate | 1.80 | 1.80 | 1.80 | 1.80 |
| Mono potassium phosphate | 0.500 | 0.500 | 0.500 | 0.500 |
| BHT | 0.020 | 0.020 | 0.020 | 0.020 |
| KCl | 0.250 | 0.250 | 0.250 | 0.250 |
| HSCAS | - | 1 | - | - |
| Vermiculite | - | - | 1 | - |
| Total weight (kg) | 100 | 100 | 100 | 100 |
| Aflatoxin $B_1$ (ppb) | - | 500 | 500 | 500 |
| **Nutrient content (dry matter basis)[4/]** | | | | |
| Protein (%) | 40.2 | 39.5 | 39.8 | 40.5 |
| Fat (%) | 5.50 | 4.74 | 4.66 | 5.32 |
| Fiber (%) | 4.81 | 4.90 | 4.92 | 4.84 |
| Ash (%) | 15.1 | 15.4 | 15.3 | 14.8 |
| Moisture (%) | 8.00 | 7.80 | 7.60 | 8.10 |
| NFE[5/] | 26.4 | 27.7 | 27.7 | 26.4 |
| Energy (kcal/100 g) | 387 | 381 | 382 | 387 |
| **Aflatoxin $B_1$ accumulated in diets[6/]** | | | | |
| Average aflatoxin $B_1$ (ppb) | 0.100 | 273 | 270 | 269 |

**Note :** [1/] Least cost calculation by Feed Live program according to feed ingredient price in November 9, 2005 (Live Informatics Company Limited, 2005)

[2/] Vitamin mix per kg: thiamine HCl 0.500 g, riboflavin 3.00 g, pyridoxine HCl 1.00, Dl Ca-pantothenate 5.00 g, nicotinic acid 5.00 g, biotin 0.050 g, folic acid 0.180 g, vitamin $B_{12}$ 0.002 g, inositol 5.00 g, menadione 2.00 g, vitamin A acetate (20,000 IU/g) 5.00 g, vitamin $D_3$ (400,000 IU/g) 0.002 g, dL-alpha-tocopherol acetate (250 IU/g) 8.00 g, food preservative 4.00 g, and carriers : add wholely 1.00 kg

[3/] Trace mineral per kg: $ZnSO_4$ 0.145 g, $CoCl_2$ 0.044 g, $MnSO_4.4H_2O$ 0.007 g, $KIO_3$ 0.003 g, $CuCl_2$ 0.085 g, and carriers: add wholely 1.00 kg

[4/] Results of fed proximate analyses of the experimental diets in laboratory

[5/] NFE = 100 - (% protein + % fat + % fiber + % moisture + % ash)

[6/] Results of aflatoxin $B_1$ accumulated in experimental diets from analysis in laboratory after diets were transformed by mixing, pelleting, and refrigeration processes.

**Data Record and Data Analysis**

Shrimps weight, feed consumption, and survival rate were recorded every two weeks. The number, body weight of shrimps and death date throughout the study were likewise recorded. Dissolved oxygen and temperature were measured daily, while pH, salinity and ammonia-nitrogen were measured biweekly, according to Spotte (1979). At the end of the feeding phase, aflatoxin $B_1$ residue (ppb) in head plus shell and muscle were analyzed by the HPLC method (Soonngam, 2005). The productive performance mean for each size of shrimps were compared for significant differences in weight gain, average daily gain (ADG), feed conversion ratio (FCR), protein efficiency ratio (PER), survival rate and aflatoxin $B_1$ residue in shrimps. Data were analyzed by the analysis of variance (ANOVA). When significant differences were observed, the Duncan New Multiple Range Tests (DNMRT) of the Statistical Analysis System (SAS version 6.12) were used to test for the differences among the treatment means at $P<0.05$ (SAS, 1996).

**RESULTS**

All growth trials were conducted without interruption, water quality problems, or disease problems. For the preparation of the experimental diets in 4 formulae and the test diet for shrimps, aflatoxin $B_1$ levels accumulated in the experiment after processing were 0.100 ppb (diet 1), 273 ppb (diet 2), 270 ppb (diet 3), and 269 ppb (diet 4) (Table 1). The observed water quality in each treatment was suitable for uninterrupted growth of *P. monodon*. During the 8-week experiment, water temperature was 27.0-29.0 C, pH 8.00-8.40, dissolved oxygen concentration 5.50-6.50 mg/l, and salinity and ammonia-nitrogen 30.0-33.0 mg/l and 0.060-0.200 mg/l, respectively.

The initial weight for shrimps averaged 0.500 g/each. After feeding for 8 weeks, the final weight gain was 2.44, 2.35, 2.32 and 2.01 g (Fig. 1), the final ADG were 34.6, 33.0, 32.5 and 27.0 mg/day (Fig. 2), the final FCR 2.68, 2.84, 2.88 and 3.20 (Fig. 3), the final PER 1.04, 0.950, 0.930 and 0.760 (Fig. 4), and the final survival rate 78.7, 78.4, 77.9 and 67.4% (Fig. 5) for diets 1, 2, 3 and 4, respectively. The shrimp fed control diet had the best productive performance, whereas diet 4 without adsorbent had the lowest compared to the other diet formulae. All results were also significantly ($P<0.05$) related to the use of HSCAS and vermiculite to absorb aflatoxin $B_1$ in diets. But, no significant differences ($P>0.05$) were observed in all productive performances between the 2 treatments, which received diet with different types of binding agent. However, diet 2, which added aflatoxin $B_1$ with HSCAS, was slightly better in productive performance than the diet 3 with vermiculite, but this was not significantly different.

The average concentration of aflatoxin $B_1$ remaining in the head plus shell was 0.010, 0.040, 0.052 and 0.084 ppb and in muscle was 0.010, 0.026, 0.030 and 0.065 ppb for the diet 1, 2, 3, 4, respectively (Fig. 6). Shrimps fed with diet 1 had the lowest aflatoxin $B_1$ residues whereas shrimps fed with 500 ppb aflatoxin B had the highest residues. All treatments were significantly different ($P<0.05$). In addition, head plus shell had a higher level of aflatoxin $B_1$ residue than muscle (Fig. 6).

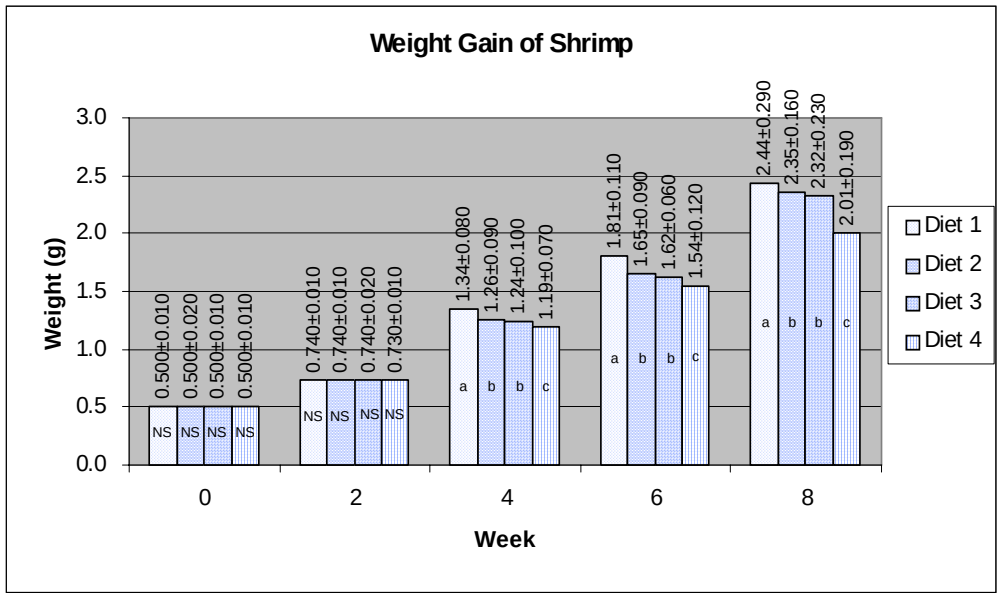


**Fig. 1.** Weight gain of shrimps fed with experimental diets
[a, b, c] Values without common script within bar graphs in the same week differ significantly (*P<0.05*)

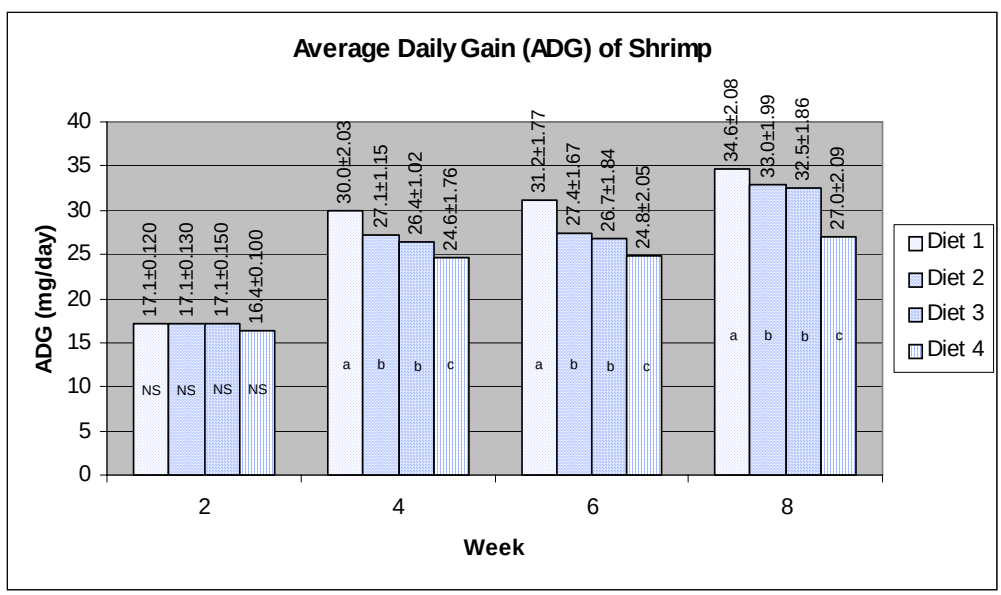


**Fig. 2.** Average daily gain of shrimps fed with experimental diets
[a, b, c] Values without common script within bar graphs in the same week differ significantly (*P<0.05*)

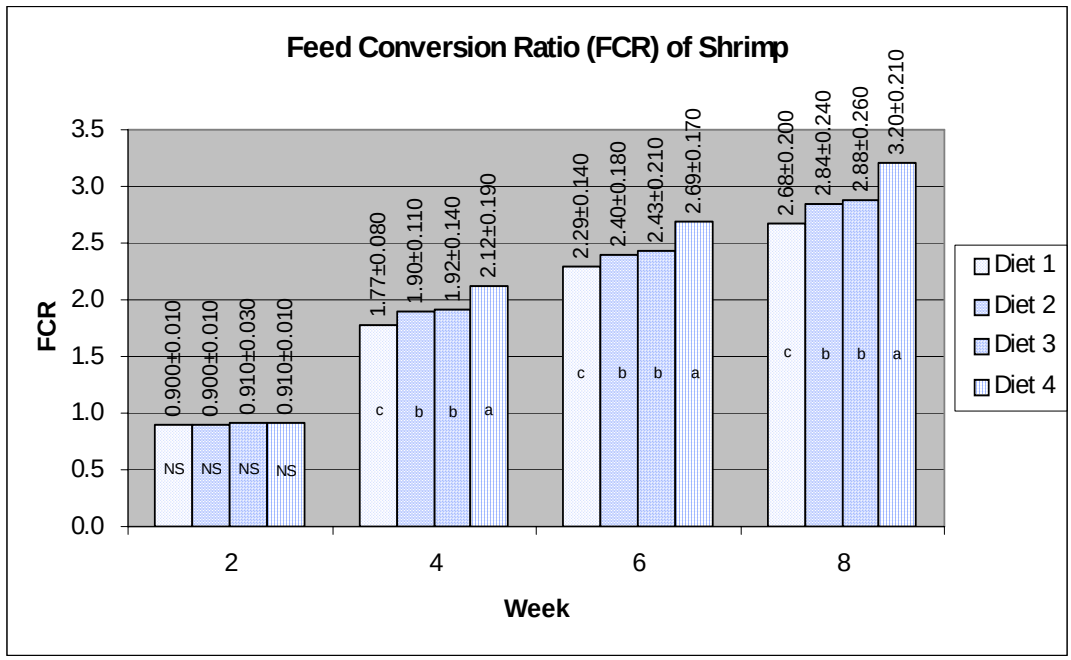


**Fig. 3.** Feed conversion ratio of shrimps fed with experimental diets
[a, b, c] Values without common script within bar graphs in the same week differ significantly (*P<0.05*).

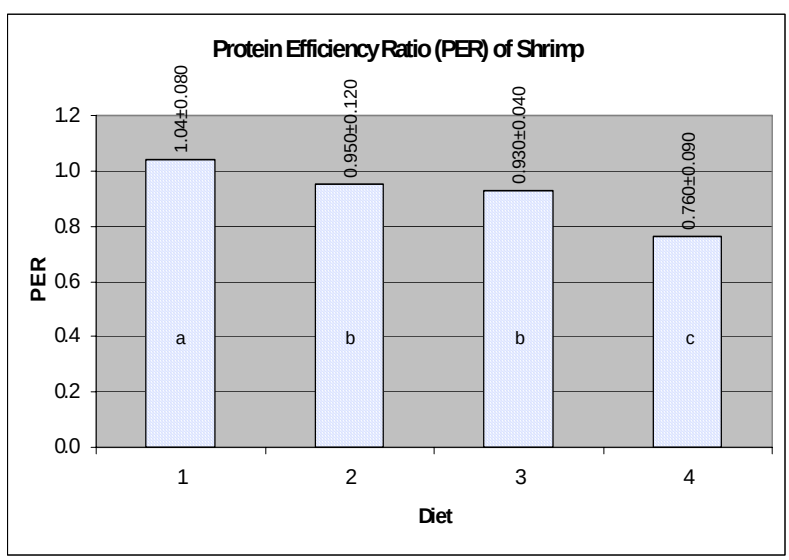


**Fig. 4.** Protein efficiency ratio of shrimps fed with experimental diets
[a, b, c] Values without common script within bar graphs differ significantly (*P<0.05*)

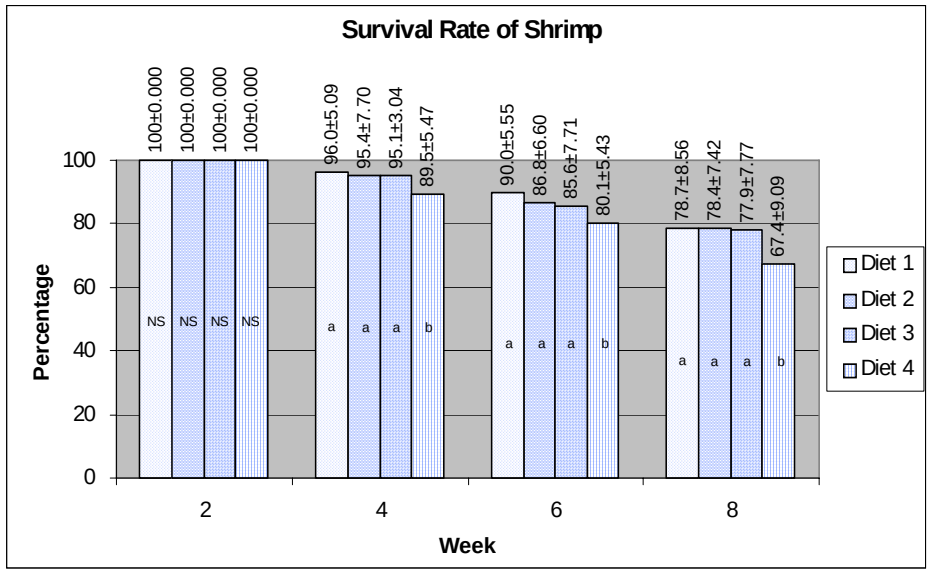


**Fig. 5.** Survival rate of shrimps fed with experimental diets

[a, b] Values without common script within bar graphs in a same week differ significantly (*P<0.05*).

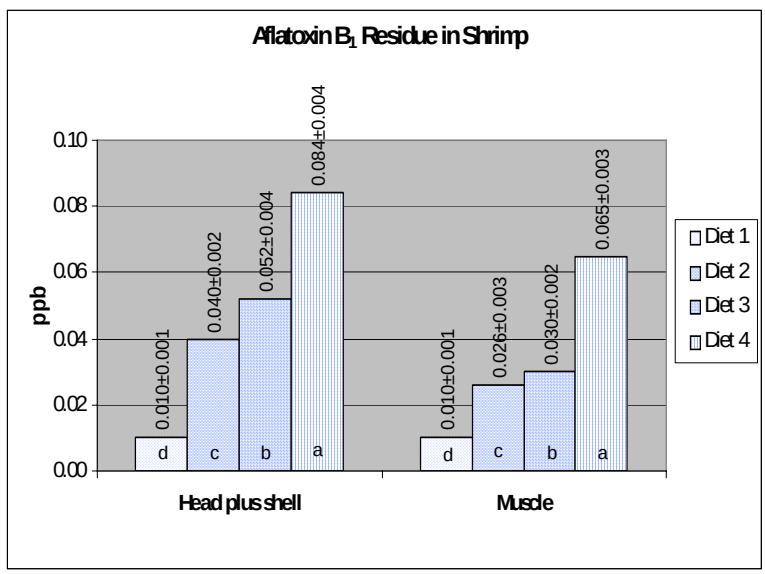


**Fig. 6.** Aflatoxin $B_1$ residue in shrimp

[a, b, c] Values without common letters within bar graphs in the same tissue differ significantly (*P<0.05*)

## DISCUSSION

*P. monodon* were fed with aflatoxin $B_1$ with adsorbent added, HSCAS (diet 2) and vermiculite (diet 3), and without adsorbent (diet 4). The shrimps fed with diet 2 and diet 3 had significantly higher weight gain, ADG and PER than shrimps fed with diet 4. The shrimps fed with

aflatoxin $B_1$ diet without adsorbent had the lowest weight gain, ADG and PER compared to those fed with the other diet formulae because aflatoxin $B_1$ can inhibit growth rate of shrimp (Ostrowski *et al*., 1995; Suppadit *et al*., 2006 in *Penaeus vannamei*, and De la Cruz *et al*., 1989; Soonngam, 2005 in *Penaeus monodon*). HSCAS and vermiculite have the absorption binding properties to aflatoxin $B_1$ *in vivo* and *in vitro* (Phillips *et al*., 1988; Ledoux *et al*., 1999). However, the results showed that *P. monodon* fed with aflatoxin $B_1$ with adsorbent HSCAS added had slightly better productive performance than shrimp fed with aflatoxin $B_1$ with adsorbent vermiculite added because HSCAS has a property of aluminosilicate to reduce the toxin of aflatoxin $B_1$ more than vermiculite (Kubena *et al*., 1990). HSCAS are chemically complex materials exhibiting a variety of functional properties like sizeable areas of high porosity and variable cation exchange activities. HSCAS forms a slightly more stable complex with aflatoxin $B_1$ than other adsorbents such as vermiculite (Jensen, 2005). Vermiculite is the mineralogical name given to hydrated laminar magnesium-aluminum-ironsilicate which preserves feed integrity and promotes livestock health. Both adsorbents have absorption properties similar to a tetrahedral framework where there is ease in exchange of its ion with other $H_2O$ and oxygen molecules while allowing other organic molecules to pass through (Harvey *et al*., 1994). The absorption ability is dependent on the structure and chemical components (Mumton and Fishman, 1977; Schell, 1993; Jensen, 2005). HACAS and vermiculite *in vitro* can reduce aflatoxin $B_1$ by about 90.0% (Phillips *et al*., 1988; Soonngam, 2005). Broilers fed with aflatoxin $B_1$ contaminated diet supplemented with three types of aluminosilicate (natural zeolite, HSCAS and sodium bentonite) had higher protein digestibility, net protein utilization, and digestible than the non-supplemented group (Scheidler,1993; Charoonkiatkamchorn, 1996).

The FCRs of shrimp fed with diets 1, 2 and 3 had a better value than diet 4 with a statistical significance. But no statistically significant difference was found between shrimps fed with supplemented HSCAS (diet 2) and vermiculite (diet 3). These results were similar to the study of Miazzo *et al*. (2000) and Suppadit *et al*. (2006). The FCR of *P. monodon* increased at the same rate as aflatoxin $B_1$, but PER decreased with increase of aflatoxin $B_1$ (Boonyaratpalin *et al*., 2000). Shrimps fed with more than 200 ppb aflatoxin $B_1$ showed decreasing FCR efficiency (Soonngam, 2005). The survival rate of shrimps fed with aflatoxin $B_1$ (diet 4) showed a statistically significant difference compared with shrimps fed without aflatoxin $B_1$ in diet (diet 1) and those fed with diets supplemented by adsorbents (diet 2 and 3). Aflatoxin $B_1$ is absorbed by the adsorbents in the gastrointestinal tract and the aflatoxin $B_1$ excreted resulting in less aflatoxin $B_1$ to form aflatoxin $B_1$-epoxide in livers (Soonngam, 2005), thus increasing shrimp survival rate.

Aflatoxin $B_1$ levels in shrimp head and shell were higher than in muscle, but less than the FAO/WHO standard of 20.0 ppb (FAO, 1990). The aflatoxin $B_1$ level found in muscle at 4 weeks, would decrease after 6 weeks (Boonyaratpalin *et al*., 2001). The residues found in the head and shell were less and then stable throughout the feeding period. The movement cannot be explained clearly. Bintvihok *et al*. (2003) studied shrimp fed a diet containing 0-20.0 ppb of aflatoxin $B_1$ for 10 days. The aflatoxin $B_1$ residues in shrimp muscle were not detected so it may pose a very low risk, if any, to human health, but this may cause economical losses due to reduction in shrimp growth and survival rate. However, Chou *et al*. (1994) and Soonngam (2005) reported that the absence of aflatoxin $B_1$ in shrimp tissues after the experiment was attributed to either the low amount of incorporation which did not cause retention in the body of the animal or aflatoxin $B_1$ conversion to other metabolites which later were excreted by the animal. Ostrowski *et al*. (1995) also observed histopathological change in *P. vannamei* fed diets with 25.0-2,500 ppb aflatoxin $B_1$, but aflatoxin $B_1$ residues were not found in the tissues at the end of the experiment. This indicated a very low potential for aflatoxin $B_1$ transmission from edible tissues to humans and the environment.

## CONCLUSIONS

The processing of shrimp feed involving mixing, pelleting and refrigeration can reduce aflatoxin $B_1$ levels from 500 ppb to about 270 ppb. The use of mycotoxin-binding substances could be beneficial in shrimp culture. The toxin-binding efficacy of HSCAS was similar to that of vermiculite. These had the ability to reduce the toxicity of aflatoxin $B_1$ as shown by increased weight gain, ADG, PER and survival rate of shrimps, but these decreased FCR including aflatoxin $B_1$ residues. However, contaminated diets were still less than control diet without aflatoxin $B_1$. The two types of adsorbents did not cause a significant difference in weight gain, ADG, FCR, PER and the survival rate, but contributed to differences in aflatoxin $B_1$ residues in the tissues. Aflatoxin $B_1$ residues in shrimp tissues in all treatments were below the FAO/WHO standard of 20.0 ppb.

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# SURVEY OF APHIDS (HOMOPTERA: APHIDIDAE) AND THEIR NATURAL ENEMIES IN NORTH VIETNAM

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## ABSTRACT

A survey of aphids and their natural enemies was conducted in 12 localities of agricultural fields and national parks in north Vietnam from March to May 2006. Collections included 16 species of aphids and were identified to belong to three subfamilies: 11 species in Aphidinae, 3 species in Greenideinae, and 2 species in Hormaphidinae. Natural enemies of aphids were found to belong to three groups including 2 species of pathogenic entomophagous fungi, 1 species of parasitoid, and 10 species of predator.

**Key words**: biological control, pest

## INTRODUCTION

Aphids are small, soft-bodied insects with long, slender mouthparts with which they pierce stems, leaves, and other tender plant parts to suck out plant fluids. Almost every plant has one or more aphid species which occasionally feed on it. The economic importance of aphids was outlined in three ways: (1) by sucking the sap of plants, (2) by the toxic action of their salivary secretions injected during feeding, thus causing stunting of growth, deformation of leaves and fruits or causing galls on leaves, stems and roots, and (3) by acting as vectors of viruses which cause many plant diseases (Chapman and Eastop, 1984). In Vietnam, the outbreak of aphids is often found in several places and is reported as one of the major pests that cause damage to many agricultural plants. The control methods are based exclusively on the use of insecticides. Indiscriminate use of insecticides leads to several undesirable consequences, such as destruction of natural enemies, development of resistant pest species and secondary pest problems (Pham Van lam 2005, Quy and Chi 2005).

Natural enemies of aphids play a big role in reducing their numbers. Among the most important natural enemies are various species of parasitic wasps that lay their eggs inside aphids and predators including lady bird beetle adults and larvae, lacewing larvae, and syrphid fly larvae which feed on aphids (Pham Van Lam, 2005).

This study is concentrated only on the aphids and their natural enemies found in north Vietnam, an area of extensive plant cultivation. The preliminary information from this study will, hopefully, be utilized as the basis for further studies, especially biodiversity and fields related to aphid transmitted viruses and application for biological control.

## MATERIALS AND METHODS

Aphids and their natural enemies were collected in north Vietnam from ten localities of agricultural fields in Gialam and Tay Ho, Hanoi, Bavi, Ha Tay and two national parks including Cat Ba and Cuc Phuong from March to May 2006.

Aphids were collected by removing the part of the host plant where the insects aggregated. The insect samples were kept in a plastic box until examination in the laboratory. Natural enemies were collected by sweeping or by hand. Both collected specimens were brought to the laboratory. Natural enemies were found to be growing inside the host insect. Immature stages were reared to mature stage. For morphological examination, the mature aphids and their natural enemies were preserved in small vials filled with 70 % ethyl alcohol. The aphid slide preparation and measurement technique was basically after Blackman and Eastop (1994). Ten specimens of each species were measured for the indicated features.

The identification was carried out by the authors and by using classification keys by Chapman and Eastop (1984, 1994), Raychaudhuri (1980), Sirikajornjaru (2002), and Chanram (2002).

## RESULTS AND DISCUSSION

In this study, apterous and alate viviparous females of aphids were collected from host plants. About 16 species of aphids were identified belonging to three subfamilies, 11 species including *Aphis citricola*, *A. craccivora*, *A. glycines*, *A. gossypii*, *Lipaphis erysimi*, *Myzus persicae*, *Rhopalosiphum maidis*, *R. padi*, *Toxoptera aurantii*, *T. citricidus*, and *T. odinae* in subfamily Aphidinae; *Cervaphis rappadi*, *Greenidea ficicola* and *Schoutedenia* sp. in subfamily Greenideinae; *Astegopteryx* sp. and *Ceratovacuna lanigera* in subfamily Hormaphidinae.

The collections showed that aphid species in subfamilies Aphidinae appeared to be the most interesting groups. It was frequently found on a wide variety of host plants. In this study, 11 species out of 16 were recorded whereas 14 species out of 15 and all of 7 species were reported by Pham Van Lam (2005) and Quy and Chi (2005), respectively.

In the survey for natural enemies of aphids, three groups including pathogenic entomophagous fungi, parasitoids, and predators were found (Table 1). Two strains of entomophagous fungi were *Beauveria brassiana* and *Metarhizium* sp. One species of parasitoid was *Aphidius* sp. Naturally-occurring predators were classified from three orders, namely Coleoptera, Diptera, and Neuroptera. In Coleoptera, five species of Coccinellidae including *Coccinella transversalis*, *Menochilus sexmaculatus*, *Micraspis discolor*, *Propylia japonica*, and *Scymnus* sp.; and one species of Staphylinidae: *Paederus fuscipes* Curtis were found. For Diptera, about four species of Syrphidae including *Episyrphus balteatus*, *Ichyrosyrphus glaucica*, *Paragus quadrifaciatus*, and *Syrphus ribesii* were found on fauna of aphids in vegetable fields. One species of lacewing was found on *Greenidea ficicola* colonies on lychee. Among the predators, lady bird beetles have a more important role in controlling aphid populations.

Natural enemies of aphids are abundant in Vietnam. Pham Van Lam (2005) found that 52 species of insects have been recorded as natural enemies of aphids feeding on agricultural plants, whereas 13 species was noticed in this study. For syrphid flies, 4 species were recorded whereas 7 species were reported by Hung and Hong (2004)

Among the lady bird beetles collected, *Micraspis discolor* and *Menochilus sexmaculatus* were common species which were found to be closely associated with many aphids in the field crop plants.

**Table 1.** Aphids and their natural enemies collected from fields of crop plants and national parks in north Vietnam.

| Host Plants | Aphid species | Natural enemy species |
|---|---|---|
| *Alstonia scholaris* (L.) | *Toxoptera odinae* (van de Goot) | *Coccinella transversalis* Fabricius |
| *Bambusa* sp. | *Astegopteryx* sp. | *Scymnus* sp. |
| *Brassica chinensis* L. | *Lipaphis erysimi* (Kaltenbach) | *Beauveria brassiana* (Bals.) Vuill. |
| *Brassica oleracea* L. var. botrytis | *Myzus persicae* (Sulzer) | *Metarhizium* sp.<br>*Aphidius* sp.<br>*Coccinella transversalis* Fabricius<br>*Menochilus sexmaculatus* (Fabricius)<br>*Micrapis discolor* (Fabricius)<br>*Propylia japonica* (Thunberg)<br>*Paederus fuscipes* Curtis<br>*Episyrphus balteatus* de Geer<br>*Ischyrosyrphus glaucica* L.<br>*Syrphus ribesii* L. |
| *Camellia sinensis* (L.) Kuntze | *Toxoptera aurantii* (Boyer de Fonscolombe) | *Scymnus* sp. |
| *Chrysanthemum* spp. | *Aphis gossypii* Glover | *Menochilus sexmaculatus* (Fabricius)<br>*Micrapis discolor* (Fabricius) |
| *Citrus grandis* L. | *Aphis citricola* van der Goot<br>*Toxoptera citricidus* (Kirkaldy) | *Menochilus sexmaculatus* (Fabricius)<br>*Micrapis discolor* (Fabricius) |
| *Coffea* spp. | *Toxoptera aurantii* (Boyer de Fonscolombe) | *Menochilus sexmaculatus* (Fabricius)<br>*Micrapis discolor* (Fabricius)<br>*Scymnus* sp. |
| *Cucurbita moschata* Decn | *Aphis gossypii* Glover | *Micrapis discolor* (Fabricius) |
| *Curcumis sativus* L. | *Aphis gossypii* Glover<br>*Myzus persicae* (Sulzer) | *Coccinella transversalis* Fabricius<br>*Menochilus sexmaculatus* (Fabricius)<br>*Micrapis discolor* (Fabricius)<br>*Propylia japonica* (Thunberg)<br>*Paederus fuscipes* Curtis<br>*Menochilus sexmaculatus* (Fabricius)<br>*Episyrphus balteatus* de Geer<br>*Ischyrosyrphus glaucica* L.<br>*Syrphus ribesii* L. |
| *Glycine max* (L.) | *Aphis glycines* Matsumura | *Coccinella transversalis* Fabricius<br>*Menochilus sexmaculatus* (Fabricius)<br>*Micrapis discolor* (Fabricius) |
| *Hippeastrum* sp. | *Aphis gossypii* Glover | *Menochilus sexmaculatus* (Fabricius)<br>*Micrapis discolor* (Fabricius) |

| **Host Plants** | **Aphid species** | **Natural enemy species** |
|---|---|---|
| *Lagerstroemia* sp. | *Scheutidinia* sp. | *Stethorus* sp. |
| *Litchi chinensis* Somn. | *Cervaphis rappadi* Hille Ris Lambers<br>*Greenidea ficicola* Takahashi | Lacewing larvae<br>Syrphid fly larvae |
| *Lycopersicon esculentum* Mill | *Myzus persicae* (Sulzer) | *Micrapis discolor* (Fabricius) |
| *Manilkara zapota* (L.) | *Aphis gossypii* Glover | *Micrapis discolor* (Fabricius) |
| *Nicotiana tabacum* L. | *Aphis gossypii* Glover<br>*Myzus persicae* (Sulzer) | *Micrapis discolor* (Fabricius) |
| *Solanum tuberosum* L. | *Aphis gossypii* Glover<br>*Myzus persicae* (Sulzer) | *Micrapis discolor* (Fabricius) |
| *Saccharum officinale* Salisb. | *Ceratovacuna lanigera* Zehntner | *Menochilus sexmaculatus* (Fabricius)<br>*Syrphus ribesii* L. |
| *Vigna radiata* (L.) R. Wilcz | *Aphis craccivora* Koch | *Coccinella transversalis* Fabricius<br>*Micrapis discolor* (Fabricius)<br>*Paederus fuscipes* Curtis |
| *Vigna unguiculata* (L.) | *Aphis craccivora* Koch | *Coccinella transversalis* Fabricius<br>*Micrapis discolor* (Fabricius) |

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# NUTRIENT RECOVERY IN AN ARTIFICIAL INTEGRATED CULTURE SYSTEM BASED ON FRESHWATER PRAWNS ( *MACROBRACHIUM ROSENBERGII)*, LETTUCE (*Lactuca sativa*) AND FERTILIZERS

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## ABSTRACT

The concentration and recovery rate of nutrients in different compartments of an integrated culture system were evaluated during a 60-day culture period. *Macrobrachium rosenbergii* were placed in tanks at a stocking rate of 380-400 $m^{-3}$ and all tanks were aerated to stabilize dissolved oxygen. N, P, K, Mg, Zn, Mn, Cu and Ca in water demonstrated a significant quadratic response and increased within the culture period. The accumulated range and biological availability of macro and microelements in lettuce, root, freshwater prawn (*M. rosenbergii*) tissue and sediments indicated that N accumulated more in lettuce and prawn tissue rather than sediments. P does not get accumulated, however its recovery rate was high in sediments > prawn > lettuce tissue. The highest recovery rate of K was observed in lettuce > prawn > sediment (mobile). The retention rate of Mg was equal in lettuce and prawn tissue and that is higher than sediments . Minor and trace elements (Fe, Zn, Mn and Cu) were accumulated mainly in sediments followed by lettuce and prawn tissues (immobile). Ca levels were higher in lettuce and prawn tissues when compared to sediments. The input, constant concentration and removal of nutrients by lettuce, prawn and sediment in different media were considered as an artificial model to obtain the most sustainable culture system and fate of nutrients in aquatic species and environment.

**Key words:** lettuce, integrated culture; nutrient recovery, recirculating system

**Abreviation**: FW – fresh water, LF- liquid fertilizer, CM – chicken manure

## INTRODUCTION

An integrated culture system is simply a combination of aquaculture and agriculture production system and has been examined over the past three decades with a wide variety of system designs, plant and fish species. Water recirculation would be a substantial part in an integrated culture system. The large volumes of wastewater discharged from aquaculture sites can become a serious source of pollution. These raw and untreated effluents may damage natural ecosystem and influence aquaculture activities (Rakocy *et al*., 1991; Seawright *et al*., 1998; Brown *et al*., 1999). Nutrient recycling (converting nitrogen back to protein) through integrated culture systems could be more practical and efficient than controlling or treating the effluents associated with traditional, intensive monoculture practices. Research has been carried out in order to reduce mineral content in water by production of edible plants in polyculture trials (McMurtry *et al*., 1997). There are some major advantages of this integrated recirculating system, which includes reduced land and water requirements, the ability to control water quality parameters and environmental conservation through the reduction of nutrient and organic pollutant in the ecosystem. The excretion, absorption and use of

nutrients, by species in polyculture system are affected by many factors such as stocking density, feeding, climate (light, temperature) and management techniques (Rakocy and Hargreaves, 1993; Quillere *et al*., 1995). The relative advantages of organic and inorganic minerals and their effectiveness in fish and prawn production has been significantly documented. A wide variety of organic and inorganic materials can be used as fertilizers for fish and prawn ponds, such as animal manures, agricultural by-products and processing wastes, green manures, domestic wastes and industrial wastes (New, 1995; Das and Jana, 1996). Accordingly, many studies have been conducted on the application of minerals and fertilizers in pond and culture tanks. Conflicting and inconsistent results were reported due to different types of fertilizer, rates of input, application methods and frequency. The essential concentrations of nutrient, available source and accumulation rates played a major role in selection and optimization of desirable nutrients in the production systems (Rakocy *et al*., 1993; Maclean and Ang, 1994; Tidwell *et al*., 1995; Rakocy, 1999).

Apparently, there has been no report on the nutrient dynamics and their recovery rate in prawn, lettuce and sediment in integrated culture system. The series of experiments were adapted to compare nutrient dynamics and retention rate of macro and micro elements in an artificial polyculture system.

## MATERIALS AND METHODS

Rectangular fiber glass tanks (1 $m^{-3}$) were arranged to evaluate nutrient recovery in an artificial recirculated polyculture system. Juvenile of *M. rosenbergii* was stocked at a density of 380-400 individual per $m^{-3}$ in 500 liters of clean and aerated tap water. Different treatments include, freshwater (unfertilized), organic fertilizer (chicken manure) and formulated inorganic minerals were arranged for the study. All tanks were provided with artificial substrate (polyethylene net) to increase available surface area (50%). Water from each rearing tank was transferred to a second aeration tank to stabilize dissolved oxygen during the culture period. A hydroponics system based on nutrient film technique (NFT) was installed over the fiberglass tanks and the water from freshwater prawn was filtered (bio-physical) and recirculated continuously through the hydroponics troughs for vegetable production (*Lactuca sativa*, 45 plants $m^{-2}$). Different levels of formulated liquid fertilizer (LF) and chicken manure (CM) were inoculated weekly to the treatment.

To create the optimum interface and manage the requirements of both *M. rosenbergii* and lettuce, the estimated range of minerals considered are as followed: calcium nitrate (68.8 mg $l^{-1}$), EDTA iron (3.5 mg $l^{-1}$), potassium dihydrogen phosphate (18.1 mg $l^{-1}$), potassium nitrate (21.9 mg $l^{-1}$), magnesium sulphate (41.4 mg $l^{-1}$), manganous sulphate (0.4 mg $l^{-1}$), boric acid (0.1 mg $l^{-1}$), copper sulphate (0.02 mg $l^{-1}$), ammonium molybdate (0.023 mg $l^{-1}$) and zinc sulphate (0.03 mg $l^{-1}$). By increasing the rate of chicken manure (g), the level of supplemental liquid fertilizer (mg $l^{-1}$) was reduced in different treatments, LFCM15: LF (100%) + CM (15g/week), LFCM30: LF (50%) + CM (30g/week), LFCM50: LF (25%) + CM (50g/week), FW: Freshwater without any fertilizer and CM70: LF (0%) + CM (70g/week). Juveniles of *M. rosenbergii* were fed with a commercial prawn diet two times $d^{-1}$ (0900h, 1700h) respectively. The feeding rate was estimated according to average body weight of prawn, which was gradually reduced from to 20-30 % (Pb1) to 10-15 % (Pb2).

Important chemical and physical characteristics of recirculating water such as: temperature, pH, dissolved oxygen (DO), specific conductivity (SPC), turbidity, total dissolved solid (TDS) and ammonia ($NH_3$) were monitored weekly. Water samples were collected 40 cm below the surface from each rearing tank. The electrical conductivity (mS/cm) as EC was recorded with a HANNA conductivity meter (Dist WP 4). Water temperatures (°C) were measured using YSI model (60-10 FT). Ammonia ($NH_3$ mg $l^{-1}$), nitrate ($NO_3$ mg $l^{-1}$), as well as turbidity (NTU) were all determined using HYDROLAB (DATASONDE$^R$ 4a) and HACH spectrophotometer (DREL 12400). Fresh samples of vegetable, prawn, solid materials (sediment) were collected at the end of the experiment and oven

dried at 65-70 °C. The solid and dried samples (leaf tissue and sediments) were squashed and digested with pure $H_2SO_4$. Available nitrogen, phosphorus and potassium were determined by the auto-analyzer (LACHAT instrument, 8000 Series), Ca, Mg, K, Cu, Fe and Zn by atomic absorption spectrometry (Perkin Elmer 350).

### Statistical Analysis

Experiment units were arranged in a completely randomized design (CRD). Significant difference in the mean number of water quality and growth rate variables between control and experimental groups were determined by one-way analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (DNMRT). In all analysis, p-values less than 0.05 were interpreted as being statistically significant.

## RESULTS AND DISCUSSION

### Water Quality

There were no significant differences in the measured parameters (water quality) between the treatments. Range of dissolved oxygen (DO), pH and temperature during study were approximately constant in the culture tanks. Overall range for water quality variables in integrated system were 25.5-28.7°C for temperature, 6.4-7.8 mg $l^{-1}$ for DO, 0.11-0.44 mS/cm for SPC, 0.04-0.22 ppt for salinity, 0.0-5.3 NTU for turbidity, 6.6-8.2 for pH, 0.07-0.28 mg $l^{-1}$ for TDS and 0.12-0.58 mg $l^{-1}$ for ammonia. These values represent suitable condition for *M. rosenbergii* culture, however higher concentration of ammonia was recorded in the freshwater (FW) treatment.

### Lettuce and *M. rosenbergii* growth

The greatest level of lettuce and *M. rosenbergii* yields were obtained from LFCM15 rearing tanks (Table 1). Survival (87.9%), average daily growth (ADG: 0.07 $d^{-1}$), feed conversion ratio (FCR: 0.42), yield of *M. rosenbergii* (1343 g $tank^{-1}$) and lettuce (1783.4 g $tank^{-1}$) also indicated a desirable prototype system for *M. rosenbergii* production in integrated culture system. This media was inoculated with a formulated supplemental liquid fertilizer for prawn and lettuce polyculture system. The application and recommended value of dry chicken manure (15 g/week) was equal to 1000-1200 birds $ha^{-1}$ in a natural integrated poultry-fish production system.

**Table 1**. Percent survival, average daily growth (ADG), feed conversion ratio (FCR), yield of *M. rosenbergii* and lettuce in a polyculture system (mean ± se).

| Treatment | Survival (%) | ADG ($d^{-1}$) | FCR | Prawn Net Yield (g/tank) | Lettuce Yield (g/tank) |
|---|---|---|---|---|---|
| LFCM15 | $87.9\pm0.8^{c}$ | $0.07\pm0.001^{e}$ | $0.42\pm0.004^{a}$ | $1343.0\pm11^{d}$ | $1783.4\pm325^{b}$ |
| LFCM30 | $90.0\pm0.7^{c}$ | $0.04\pm0.001^{b}$ | $0.67\pm0.010^{b}$ | $840.1\pm13^{bc}$ | $1086.3\pm410^{ab}$ |
| LFCM50 | $93.8\pm1.4^{c}$ | $0.04\pm0.001^{ab}$ | $0.65\pm0.010^{b}$ | $869.5\pm14^{c}$ | $791.6\pm90^{ab}$ |
| FW | $41.0\pm1.7^{a}$ | $0.05\pm0.001^{d}$ | $1.18\pm0.035^{c}$ | $481.1\pm15^{a}$ | $84.2\pm28^{a}$ |
| CM70 | $75.0\pm3.5^{b}$ | $0.05\pm0.002^{c}$ | $0.69\pm0.012^{b}$ | $820.5\pm14^{b}$ | $753.1\pm465^{ab}$ |

Means within a column followed by the same letter are not significantly different ($P<0.05$) as determined using DNMRT.
LFCM15: LF (100%) + CM (15g/week), LFCM30: LF (50%) + CM (30g/week), LFCM50: LF (25%) + CM (50g/week), FW: Freshwater without any fertilizer, CM70: LF (0%) + CM (70g/week)

**Nutrients in an Integrated Culture System**

The current study focuses on the effects of exogenous concentration and recovery rate of minerals (N, P, K, Mg, Fe, Zn, Mn, Cu and Ca) on lettuce, prawn and sediment during culture period. All terrestrial plants and aquatic organisms need elements to grow. These elements are C, H, O, N, P, K, Ca, Mg, S, Zn, Cu, Fe, Mn, B, Mo, Cl, Na, Ni, Co, Mo, Se, Cr, I, F, Sn, Si, Va and As. In nature, the organisms derive these elements from the atmosphere or minerals from the soil or sediments (Wheaton, 1977; FAO, 1983 and 1984; Singer and Munns, 1992). Regardless of form (liquid or powder) and brand, all nutrient solutions should contain the essential nutrient elements needed for both plant and aquatic animal growth. The composition of all nutrients depends on the raw materials and the intended purpose (Lægreid *et al*., 1999).

Mineral elements are important in many aspects of fish and prawn metabolism. These elements are chemically combined in the organism's body to form the complex molecules and allow the conversion of food to energy or to build organic molecules and provide strength and rigidity to bones in fish and exoskeleton in crustaceans (Wheaton, 1977). In body fluids, elements are involved mainly in the maintenance of osmotic equilibrium with the aquatic environment. Research has been carried out for several decades to determine which of the 105 chemical elements are necessary for aquatic animal life and terrestrial plant (FAO 1987). In a polyculture system, the concentration of minerals and nitrogenous compound (e.g. $NO_3$, $NH_3$) increased frequently through application of commercial feed and fertilizers during production cycle. The abnormal accumulation rate of nutrients may adversely affect the aquatic organisms and ecosystem. Few studies have focused on nutrient accumulation rate in integrated culture of fish and plant (Rakocy and Hargreaves, 1993; Palada et al., 1999). The results of nutrient recovery in different compartments of current integrated culture system may offer an optimum and suitable production design of *M. rosenbergii*, lettuce and poultry manure.

Differences in soluble nutrient (mean) was not consistent during the 60 days production cycle except N and K in the treatments (Table 2). The highest N, P, K, Mg, Zn, Ca were detected in LFCM15 treatment. In all treatments the concentration of recorded minerals, in descending order of magnitude, were N > Ca > K > P ~ Mg > Fe > Zn > Mn > Cu. Regression analysis of recorded minerals showed significant quadratic response versus time in fertilized treatments (LFCM15, LFCM30, LFCM50, and CM70) except Mn in LFCM30, Zn, Fe in LFCM50 and CM70 treatment. In FW culture tank the polynomial regression of Ca, K, P, Mg and Mn showed significant relationship with time (Fig. 1).

**Table 2.** Evaluation of nutrient concentration (mg $l^{-1}$) in water of *M. rosenbergii* culture tanks (mean ±se).

| Media | N | P | K | Mg | Fe | Zn | Mn | Cu | Ca |
|---|---|---|---|---|---|---|---|---|---|
| LFCM15 | 26.79± 5.75$^{c}$ | 2.16± 0.69$^{a}$ | 12.32± 2.89$^{b}$ | 2.26± 0.40$^{a}$ | 0.14± 0.02$^{a}$ | 0.10± 0.03$^{a}$ | 0.010± 0.002$^{a}$ | 0.006± 0.001$^{a}$ | 24.33± 3.98$^{b}$ |
| LFCM30 | 25.10± 5.05$^{bc}$ | 2.27± 0.75$^{a}$ | 11.79± 2.70$^{b}$ | 2.22± 0.39$^{a}$ | 0.14± 0.02$^{a}$ | 0.10± 0.03$^{a}$ | 0.016± 0.006$^{a}$ | 0.005± 0.001$^{a}$ | 23.66± 3.95$^{b}$ |
| LFCM50 | 24.58± 5.01$^{bc}$ | 2.08± 0.73$^{a}$ | 10.81± 2.61$^{b}$ | 1.92± 0.43$^{a}$ | 0.12± 0.02$^{a}$ | 0.08± 0.02$^{a}$ | 0.009± 0.002$^{a}$ | 0.005± 0.001$^{a}$ | 20.71± 4.29$^{b}$ |
| FW | 10.97± 2.14$^{ab}$ | 0.91± 0.23$^{a}$ | 6.21± 0.69$^{ab}$ | 1.37± 0.15$^{a}$ | 0.15± 0.03$^{a}$ | 0.06± 0.02$^{a}$ | 0.016± 0.006$^{a}$ | 0.005± 0.002$^{a}$ | 18.10± 3.04$^{ab}$ |
| CM70 | 10.72± 2.22$^{ab}$ | 2.19± 0.82$^{a}$ | 10.69± 2.62$^{b}$ | 1.95± 0.36$^{a}$ | 0.11± 0.01$^{a}$ | 0.10± 0.03$^{a}$ | 0.017± 0.006$^{a}$ | 0.008± 0.003$^{a}$ | 22.99± 3.35$^{b}$ |

Means within a column followed by the same letter are not significantly different ($P<0.05$) as determined using DNMRT.

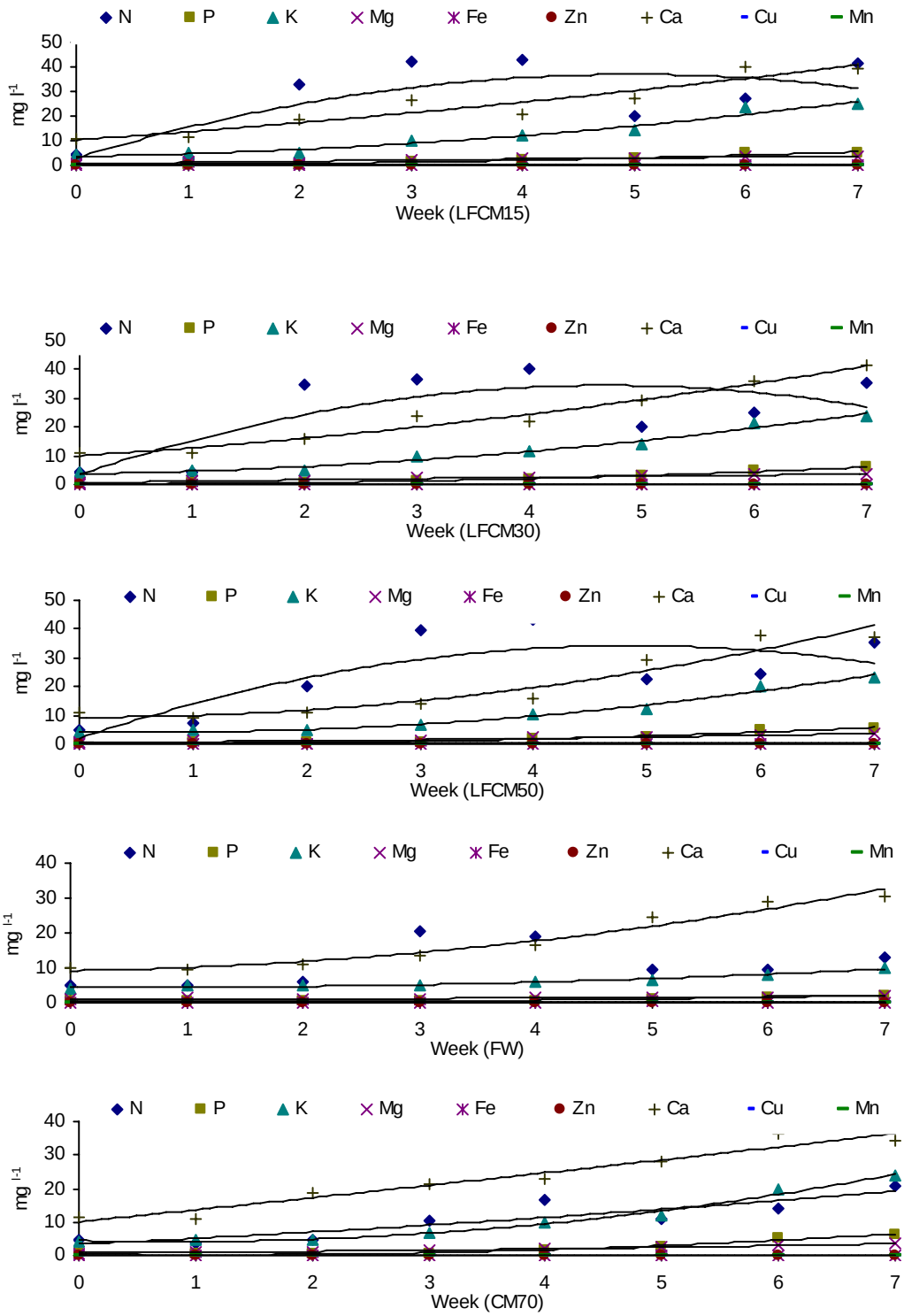


**Fig. 1**. Polynomial regression of nutrient concentration in the integrated culture system.

**Table 3**. Recovery of nutrients in lettuce, root, sediment and prawn tissue as percent (%) of *M. rosenbergii* diet, CM and supplemental liquid fertilizer.

| Lettuce | N | P | K | Mg | Fe | Zn | Mn | Cu | Ca |
|---|---|---|---|---|---|---|---|---|---|
| LFCM15 | 11.43 | 4.35 | 59.18 | 6.96 | 4.42 | 4.44 | 11.18 | 5.17 | 9.70 |
| LFCM30 | 7.54 | 2.68 | 32.16 | 3.17 | 3.03 | 2.64 | 4.85 | 2.52 | 6.09 |
| LFCM50 | 5.38 | 1.53 | 19.65 | 1.54 | 1.82 | 1.70 | 2.50 | 1.42 | 3.97 |
| FW | 1.79 | 0.69 | 9.58 | 2.08 | 0.92 | 1.19 | 2.73 | 0.81 | 2.34 |
| CM70 | 3.84 | 1.10 | 12.83 | 1.16 | 1.61 | 1.56 | 1.38 | 0.92 | 2.77 |
| Root | | | | | | | | | |
| LFCM15 | 2.49 | 0.33 | 3.75 | 0.54 | 9.92 | 4.13 | 10.68 | 5.55 | 2.36 |
| LFCM30 | 1.21 | 0.15 | 1.54 | 0.12 | 4.72 | 2.24 | 7.65 | 3.23 | 0.94 |
| LFCM50 | 0.88 | 0.10 | 1.01 | 0.09 | 2.80 | 1.24 | 1.98 | 1.23 | 0.45 |
| FW | 0.37 | 0.04 | 0.32 | 0.05 | 0.72 | 0.58 | 1.99 | 0.58 | 0.11 |
| CM70 | 0.72 | 0.05 | 0.50 | 0.06 | 1.50 | 1.00 | 1.03 | 0.88 | 0.32 |
| Sediment | | | | | | | | | |
| LFCM15 | 9.46 | 23.00 | 2.55 | 5.07 | 142.10 | 34.26 | 221.2 | 44.97 | 15.40 |
| LFCM30 | 7.63 | 17.07 | 1.88 | 3.08 | 89.54 | 27.87 | 127.9 | 28.04 | 9.49 |
| LFCM50 | 3.31 | 6.83 | 0.72 | 1.03 | 31.50 | 13.55 | 36.8 | 10.02 | 4.42 |
| FW | 4.30 | 10.82 | 1.84 | 2.10 | 61.63 | 24.63 | 149.5 | 16.13 | 6.90 |
| CM70 | 9.12 | 16.93 | 1.78 | 2.72 | 85.26 | 39.34 | 54.5 | 27.66 | 10.82 |
| Prawn | | | | | | | | | |
| LFCM15 | 39.97 | 30.49 | 25.77 | 11.37 | 8.13 | 13.76 | 5.01 | 47.53 | 43.79 |
| LFCM30 | 23.36 | 13.75 | 10.60 | 4.82 | 4.39 | 7.05 | 2.07 | 26.78 | 25.18 |
| LFCM50 | 27.80 | 17.61 | 12.59 | 4.52 | 4.94 | 7.65 | 1.75 | 31.37 | 26.18 |
| FW | 16.38 | 10.08 | 10.76 | 6.76 | 3.28 | 6.46 | 2.75 | 44.25 | 22.18 |
| CM70 | 24.18 | 13.49 | 9.80 | 3.14 | 4.45 | 5.93 | 1.32 | 19.18 | 21.02 |
| Total (T) | | | | | | | | | |
| LFCM15 | 63.36 | 58.17 | 91.25 | 23.94 | 164.57 | 56.58 | 248.03 | 103.22 | 71.24 |
| LFCM30 | 39.75 | 33.65 | 46.18 | 11.19 | 101.67 | 39.80 | 142.48 | 60.58 | 41.70 |
| LFCM50 | 37.37 | 26.07 | 33.97 | 7.18 | 41.06 | 24.14 | 43.01 | 44.05 | 35.03 |
| FW | 22.84 | 21.63 | 22.50 | 10.99 | 66.55 | 32.87 | 156.94 | 61.78 | 31.52 |
| CM70 | 37.86 | 31.58 | 24.92 | 7.08 | 92.83 | 47.82 | 58.26 | 48.63 | 34.92 |
| Solution (S) | | | | | | | | | |
| LFCM15 | 133.59 | 40.00 | 135.41 | 66.83 | 40.16 | 68.95 | 20.70 | 45.76 | 221.24 |
| LFCM30 | 115.64 | 36.57 | 102.52 | 44.33 | 36.31 | 58.24 | 22.70 | 30.32 | 177.94 |
| LFCM50 | 104.00 | 27.77 | 71.29 | 24.52 | 25.06 | 38.44 | 8.94 | 24.08 | 113.80 |
| FW | 40.58 | 14.42 | 44.74 | 48.42 | 49.91 | 50.10 | 61.96 | 49.60 | 145.94 |
| CM70 | 29.20 | 25.57 | 58.22 | 19.41 | 19.31 | 41.19 | 13.08 | 32.92 | 119.62 |
| Total T and S | | | | | | | | | |
| LFCM15 | 196.95 | 98.17 | 226.66 | 90.76 | 204.74 | 125.53 | 268.74 | 148.98 | 292.48 |
| LFCM30 | 155.39 | 70.22 | 148.70 | 55.52 | 137.98 | 98.05 | 165.18 | 90.90 | 219.65 |
| LFCM50 | 141.37 | 53.84 | 105.26 | 31.70 | 66.13 | 62.57 | 51.95 | 68.13 | 148.84 |
| FW | 63.42 | 36.05 | 67.24 | 59.41 | 116.46 | 82.97 | 218.90 | 111.37 | 177.46 |
| CM70 | 67.07 | 57.14 | 83.14 | 26.49 | 112.14 | 89.01 | 71.34 | 81.55 | 154.54 |

(T= tissue of lettuce, sediment and prawn, S= Solution (soluble nutrients in recirculated water)

**Nitrogen (N)**

The N levels in all treatments increased between week 3-5 and the highest peak was observed in LCM15 (Fig. 1). Nitrogen uptake by lettuce was estimated from 11.4% in LFCM15 to 1.79% in FW and N recovery of roots ranged from 2.5% in LFCM15 to 0.4% in FW of the quantity provided in the prawn feed and fertilizers. Quantities of N recovered in the sediments ranged from 9.5% in LFCM15 to 3.3% in LFCM50 of the N provided in the experiment and were significantly different among the treatments except in LFCM15 and CM70. The apparent retention value of N by the prawn ranged from 40.0% in LFCM15 to 16.4% in FW of the N provided in the experiment. The

total N recovered in lettuce, roots, sediments, prawn and water tanks exceeded the quantity prepared in the prawn diet, CM and supplemental nutrient solution for all treatments except for FW and CM70 (Table 3). N adsorption by *M. rosenbergii* tissue was significantly higher when compared to lettuce, root and sediment compartments (Table 4).

Nitrogen accumulated in the treatments during the production cycle. The total yields and different concentration of N in rearing tanks indicated that the optimum range of N should be about 25-26 mg $l^{-1}$ in *M. rosenbergii* polyculture system (Table 2). However the low concentration of N in FW and CM70 treatments demonstrated that the sufficient growth of lettuce might be influenced by the N concentration (Table 1). The highest recovery rate of N was observed in prawn followed by lettuce roots and sediments (Tables 3 and 4). The low recovery rate of N in sediments and total accumulation rate (%) of lettuce, roots, sediment, prawn and rearing water indicated that nitrogen from prawn diet, CM and liquid fertilizer was highly available in LFCM15, LFCM30 and LFCM50 treatments. The higher rates of N (over 100%) in fertilized treatments were caused by *M. rosenbergii* excretion, biological availability and nitrification processes in polyculture system (Chen and Kou, 1996a; 1996b). On the basis of the results by Quillere *et al*. (1993) it appeared that the essential N for plant growth can be obtained from fish excretion and nitrogen was not a limiting factor in intensive integrated culture system.

**Table 4.** Recovery of nutrients (g/tank) in different compartments of the recirculated polyculture system (mean ± se).

| Tissue | N | P | K | Mg | Fe | Zn | Mn | Cu | Ca |
|---|---|---|---|---|---|---|---|---|---|
| Lettuce | 1.43± | 0.14± | 2.62± | 0.11± | 0.01± | 0.005± | 0.004± | 0.0004± | 0.48± |
| | 0.36$^{a}$ | 0.04$^{a}$ | 0.70$^{c}$ | 0.033$^{b}$ | 0.002$^{a}$ | 0.002$^{a}$ | 0.001$^{a}$ | 0.0001$^{a}$ | 0.16$^{ab}$ |
| Root | 0.27± | 0.01± | 0.14± | 0.01± | 0.01± | 0.004± | 0.004± | 0.0005± | 0.08± |
| | 0.08$^{a}$ | 0.003$^{a}$ | 0.05$^{a}$ | 0.001$^{a}$ | 0.002$^{a}$ | 0.001$^{a}$ | 0.001$^{a}$ | 0.0001$^{a}$ | 0.01$^{a}$ |
| Sediment | 1.64± | 1.07± | 0.17± | 0.12± | 0.28± | 0.066± | 0.092± | 0.0053± | 0.94± |
| | 0.33$^{a}$ | 0.22$^{b}$ | 0.03$^{a}$ | 0.033$^{b}$ | 0.119$^{b}$ | 0.037$^{b}$ | 0.05$^{b}$ | 0.002$^{b}$ | 0.47$^{b}$ |
| Prawn | 6.36± | 1.22± | 1.38± | 0.23± | 0.02± | 0.018± | 0.002± | 0.0066± | 2.71± |
| | 0.91$^{b}$ | 0.23$^{b}$ | 0.24$^{b}$ | 0.106$^{a}$ | 0.009$^{a}$ | 0.01$^{a}$ | 0.001$^{a}$ | 0.0056$^{b}$ | 1.50$^{c}$ |

Means within a column followed by the same letter are not significantly different ($P<0.05$) as determined by DNMRT.

**Phosphorus (P)**

The phosphorus (P) levels in the water demonstrated a significant quadratic response and increased within culture period (Fig. 1). The quantity of P in lettuce tissue ranged from 4.4% in LFCM15 to 0.7% in FW treatments. P uptake by root was evaluated from 0.3% in LFCM15 treatment to 0.04% in FW media. The rate of P in sediment ranged from 23% in LFCM15 to 6.8% in LFCM50 treatment. The retention value of P in prawn tissue ranged from 30.5% in LFCM15 to 10.1% in FW of the P provided in the *M. rosenbergii* diet, chicken manure and liquid fertilizer. The total quantity of P recovered in lettuce, roots, sediments, prawn tissue and water did not exceed the quantity prepared in the prawn diet and fertilizers for all treatments (Table 3). The lowest recovery of P was shown in lettuce and root tissue when compared to the others (Table 4).

P accumulated in treatments during production cycle. The soluble P ranged from 2.08 to 2.27 mg $l^{-1}$ in fertilized treatments, indicated a suitable range for polyculture system (Table 2). Unlike nitrogen the recovery rate of P was significantly lower in lettuce and roots tissue when compared to sediments (Tables 3 and 4). The highest and lowest rate of P in sediments was probably due to the different fertilization rate and P uptake by lettuce. P is commonly known as a limiting nutrient for algal growth in natural ecosystem. This nutrient is mainly available to lettuces as orthophosphate

($PO4^{3-}$) and its deficiency leads to slower, uneven growth, poorer stands and delayed maturity in many crops. The concentration of orthophosphate in the pond increases almost instantly after ponds were fertilized and then declined sharply to pretreatment levels (Boyd, 1981). The rapid decrease of phosphate in ponds is caused by dilution, assimilation and adsorption to the sediments bottom through precipitation. Precipitation is the removal of two dissolved substances from solution within chemical reaction (Wahby, 1974; Das and Jana, 1996). Cooper (1988) and Seawright *et al*. (1998) pointed out that P, Fe and Ca tend to precipitate in hydroponic nutrient solutions and the chemical composition of the precipitate is similar to that of calcium phosphate $Ca_3(PO4)_2$ found in integrated fish-lettuce system. The total recovery rate (%) of P in lettuce, root, sediment, prawn tissue and water demonstrated that the availability of P was sufficient for LFCM15 but low for LFCM30, LFCM50, FW and CM70 treatments.

**Potassium (K)**

The K levels increased in all the treatments during production cycle and water of rearing tanks demonstrated a significant quadratic response of K with time (Fig. 1). Potassium uptake by lettuces were estimated from 59.2% (LFCM15) to 9.6% (FW) and K recovery of root ranged from 3.8% in LFCM15 to 0.3% in FW of the quantity provided in the prawn feed and fertilizers. Quantities of N recorded in the sediments ranged from 2.6% (LFCM15) to 0.7% (LFCM50). The apparent retention value of K by the prawn tissue ranged from 25.8% (LFCM15) to 9.8% (CM70) of the N supplied in the experiment. The total quantity of K in lettuce, roots, sediments, prawn tissue and rearing water exceeded the quantity evaluated in the prawn diet and fertilizers for all treatments except FW and CM70 (Table 3). The highest retention of K was recorded in lettuce tissue followed by prawn, sediment and root (Table 4).

The K levels demonstrated a significant accumulation rate in all treatments during the production period. However dissolved K did not show any significant differences among the treatments (Table 2). The optimum rate of K was observed in LFCM15 treatment (12.32 mg $l^{-1}$) while the maximum recovery rate of K was observed in lettuce tissue ($P<0.05$) followed by prawn tissue, root and sediment (Tables 3 and 4). The higher total quantities of retention K (over 100%) in LFCM15, LFCM30 and LFCM50 culture tanks and minimum recovery rate in sediments indicated the desired availability of soluble forms for K in the current integrated culture system (Table 3). Normally, nutrient solution for vegetative growth will have more nitrogen and less potassium and those for flowering and fruiting phase will have less nitrogen and more potassium. According to Rakocy *et al*. (1993) the plant requirement for K is beyond the quantity for K provided in the diet and nutrient supplement ($KNO_3$) that would be essential in a recirculating aquaculture system integrated with hydroponic vegetable production.

**Magnesium (Mg)**

The Mg levels in water demonstrated a significant quadratic response and increased within culture period (Fig. 1). Quantities of Mg recovered in the lettuce ranged from 7.0% (LFCM15) to 1.2% (CM70) of the Mg estimated in the diet, chicken manure and liquid fertilizer. The quantity of Mg in lettuce roots ranged from 0.5% (LFCM15) to 0.05% (FW). Quantities of Mg recovered in the sediments ranged from 5.1% (LFCM15) to 1.03% (LFCM50) of the Mg provided in the diet and fertilizers. The apparent retention value of Mg by the prawn ranged from 11.4% (LFCM15) to 3.1% (CM70) of the Mg provided in the system. The total quantity of Mg recovered in lettuce, roots, sediments, prawn and water did not exceed the quantity of prepared nutrients for all treatments (Table 3). The maximum recovery rate of Mg was shown in prawn tissue. Root indicated minimum accumulation rate of Mg among the different groups (Table 4).

The significant accumulation rate of Mg was evidence in prawn rearing water of polyculture system. Results from total yields of *M. rosenbergii* and lettuce (LFCM15) showed that the 2.2 to 2.3 mg $l^{-1}$ should be the optimum range for Mg concentration in culture tanks (Table 2). The pervious studies demonstrated that the Mg deficiency should be apparent in fish-plant culture system (Pierce, 1980; Zweig, 1986, and Rakocy *et al*., 1993). However Quillere *et al*. (1993) suggested that Mg requirements is sufficient from tap water and animal excretion in a recirculated fish-plant system. The results of this study demonstrated the maximum recovery rate (%) of Mg was recorded in prawn tissue rather than lettuce-root and sediment (Table 3). The approximately equal quantity of Mg in lettuce tissue and sediment was related to biological availability of Mg in the system. LFCM15 rearing tank present a desirable relationship of Mg input and yields (uptake) during production cycle (Tables 3 and 4).

**Iron (Fe)**

The Fe levels increased in all treatments and Fe demonstrated a significant quadratic response within the trial period in the water of rearing tanks (Fig. 1). The quantity of Fe in lettuce tissue ranged from 4.4% (LFCM15) to 0.9% (FW). Fe uptake by root was evaluated from 9.9% (LFCM15) to 0.7% (FW). The Fe concentration in sediment ranged from 142.1% (LFCM15) to 31.5% (LFCM50) of the Fe provided in the *M. rosenbergii* diet, chicken manure and liquid fertilizers. The retention value of Fe in prawn tissue ranged from 8.1% (LFCM15) to 3.3% (FW). The total quantity of Fe recovered in lettuces, roots, sediments, prawn tissue and rearing water exceeded the quantity of nutrients prepared in the system for all treatments except LFCM50 (Table 3). Sediment showed the highest recovery of Fe when compared ~~to~~ with other compartment in the polyculture system (Table 4).

The Fe levels increased significantly in LFCM15 and LFCM30 treatments. The recovery rate of Fe was significantly higher in sediment followed by tissue of prawn, root and lettuce. The massive precipitation of Fe occurred during culture period (Tables 3 and 4). It is believed that the sedimentation and microbial uptake reduce the availability of Fe to plants. According to Alt (1980) Fe commonly precipitates as hydrous ferric oxide at pH above 6.5. The stability of dissolved Fe was increased by periodic additions of liquid chelated iron (FeEDTA) to the aeration tanks (Fischer, 1984). Rakocy *et al*. (1993) suggested that the concentration of 2-3 mg $l^{-1}$ Fe is sufficient in an integrated fish-plant system however current experiment demonstrated that the optimum growth rate of plants (lettuce) and *M. rosenbergii* was related to the maintenance of 0.14 mg $l^{-1}$ Fe concentration in rearing tanks.

**Zinc (Zn)**

The Zn levels increased during the production cycle and CM70, LFCM30 and LFCM15 treatments demonstrated a significant quadratic response of Zn with time (Fig. 1). Zinc uptake by lettuces was estimated from 4.4% (LFCM15) to 1.2% (FW) and Zn recovery in root ranged from 4.13% (LFCM15) to 0.6% (FW) of the quantity provided in the prawn feed and fertilizers. Zn quantities recorded in the sediments ranged from 34.3% (LFCM15) to 13.6% (LFCM50). The apparent retention value of Zn by the prawn tissue ranged from 13.8% (LFCM15) to 5.9% (CM70) from the N supplied in the system. The total quantity of Zn in lettuce, roots, sediments, prawn tissue and water did not exceed the quantity evaluated in the prawn diet and fertilizers for all treatments except LFCM15 (Table 3). The recovery rate of Zn was not significantly different among the compartments except sediment (Table 4).

The significant accumulation rate of Zn was monitored in LCM15, LFCM30 and CM70 treatments. The highest quantity of Zn (%) in sediment rather than prawn, root and lettuce tissue was related to low availability of this nutrient in integrated culture system (Tables 3 and 4). The total

retention rate of Zn was about equal to total quantity of prepared nutrients (input) in LFCM15 and LFCM30 culture tanks. Therefore the suitable level of Zn would be 0.10 mg $l^{-1}$ in rearing water system.

**Manganese (Mn)**

The manganese (Mn) levels in LFCM50, LFCM15 and CM70 demonstrated a significant quadratic response and increased within culture period (Fig. 1). The quantities of Mn estimated in the lettuce ranged from 11.2% (LFCM15) to 1.4% (CM70) of the Mn in the diet and fertilizers. The quantity of Mn in roots was recorded from 10.7% (LFCM15) to 1.03% (CM70). Mn recovered in the sediments ranged from 221.2% (LFCM15) to 36.8% (LFCM50) of the Mn provided in the system. The apparent retention value of Mn by the prawns ranged from 5.01% (LFCM15) to 1.3% (CM70) of the N provided in the diet and fertilizers. The total quantity of Mn recovered in lettuce, roots, sediments, prawns and rearing water exceeded the quantity of nutrient prepared in the diet and fertilizers for all treatments except LFCM50 and CM70 (Table 3). The retention of Mn did not differ significantly among the groups except sediment (Table 4).

Mn concentration accumulated significantly in LFCM15, LFCM50 and CM70 of *M. rosenbergii* tanks. To obtain sufficient yields of freshwater prawn and lettuce in polyculture system the optimum range of Mn concentration should be about 0.010 mg $l^{-1}$ in culture tanks (Table 2). The recovery rate of Mn was significantly higher in sediment when compared to lettuce, root and prawn tissues. This result showed that the biological availability of Mn is low and a supplement of liquid fertilizer ($MnSO_4$) would be necessary during the production cycle (Graves, 1983; Rakocy and Hargreaves, 1993).

**Copper (Cu)**

The copper (Cu) levels in the water demonstrated a significant quadratic response and increased within cultural period in LFCM30, LFCM15 and LFCM50 treatments (Fig. 1). Cu uptake by lettuce was estimated from 5.2% (LFCM15) to 0.8% (FW) and Cu recovery of root ranged from 5.6% (LFCM15) to 0.6% (FW) of the quantity provided in the prawn feed and fertilizers. Quantities of Cu recovered in the sediments ranged from 45.0% (LFCM15) to 10.02% (LFCM50) of the Cu provided in the system. The apparent retention value of Cu by the prawn ranged from 47.5% (LFCM15) to 19.2% (CM70) of the Cu provided in the diet and fertilizers. The total quantity of Cu recovered in lettuces, roots, sediments and prawn exceeded the quantity prepared in the diet and fertilizers for all treatments (Table 3). The highest recovery of Cu was in prawn tissue followed by sediments, root and lettuce tissue (Table 4).

The Cu levels showed a significant accumulation rate in LFCM15, LFCM30, LFCM50 and CM70 treatments during the production period. Based on the maximum yields of freshwater prawns and lettuce it seems that the optimum range of Cu should be from 0.005 to 0.006 mg $l^{-1}$ in the water of the rearing tanks. The maximum retention quantity of Cu (%) was recorded in *M. rosenbergii and* approximately equal to sediment (Tables 3 and 4). This finding was similar to the work of Seawright and co-workers (1998) where the maximum recovery rate of Cu was observed in *M. rosenbergii* followed by sediment and lettuce tissue. However the high accumulation rate of Cu in sediment was caused by low biological availability and chemical reaction of some compound present in feed.

**Calcium (Ca)**

Calcium (Ca) levels increased in all treatments and illustrates a significant quadratic response within trial period in the water of the rearing tanks (Fig. 1). Ca recorded in the lettuce ranged from 9.7% (LFCM15) to 2.3% (FW) of the Ca prepared in the diet and fertilizers (organic and inorganic).

Ca in the roots ranged from 2.4% (LFCM15) to 0.1% (FW). Ca recovered in the sediments ranged from 15.4% (LFCM15) to 4.4% (LFCM50). The apparent retention value of Ca by the prawn ranged from 43.8% (LFCM15) to 21.02% (CM70). Total Ca recovered in lettuce, roots, sediments, prawn tissue and rearing water exceeded the quantity prepared in the prawn diet, chicken manure and liquid fertilizer for all treatments (Table 3). The highest accumulation rate of Ca was evident ($P<0.05$) in *M. rosenbergii* tissue followed by sediment, lettuce and root compartment (Table 4).

The significant accumulation of Ca concentration was observed in all treatments during the production cycle. The total *M. rosenbergii* and lettuce yields indicated that the Ca concentration range from 23.66 to 24.33 mg $l^{-1}$ would be the optimum range for the current polyculture system. Generally Ca is an essential nutrient in the biological processes of plants and aquatic animals. It is also important in the molting process of *M. rosenbergii* and all crustaceans. The limitation of Ca can affect the hardening process of the newly formed shell. It has been reported that *M. rosenbergii* can tolerate a wide range of calcium hardness concentration (25-150 mg $l^{-1}$) in the ecosystem (Brown *et al*., 1991). The highest recovery rate of Ca was recorded in prawn tissue rather than sediment, lettuce and root tissues (Tables 3 and 4). In the study by Seawright *et al*. (1998) the maximum recovery rate of Ca was found in solids (sediment). The presence of snails in solids had a significant effect on the evaluation rate of Ca quantity in sediments. Under natural conditions and high pH levels the abundance of Ca is often bound to phosphorus leading to the precipitation of calcium phosphates (insoluble form). Optimum pH, absence of snails and utilization of shell as substrate in biological filters may encourage the availability and retention of Ca in *M. rosenbergii* tissue in the polyculture system (Rakocy *et al*., 1993).

## CONCLUSIONS

The results of this study suggested that in a polyculture system the fluctuation of minerals cannot be in an equal range. Therefore chemical reaction, mineralization, nutrient retention by live organisms and sedimentation are the main source of nutrient diversity in the current integrated culture system. A substantial increase of nutrients in LFCM15 and LFCM30 were attributed to *M. rosenbergii* excretion, feces, revenue of nutrient by bacterial activity and mineralization of organic compounds. The relative precipitation rates were Cu > Mn > Fe > Cu > Zn > P > Ca >N > Mg > K which indicated the highest biological availability of K and the same trend for Fe Cu, Zn and P in the previous results of polyculture trials (Rakocy and Hargreaves, 1993; Quillere *et al*., 1993). The lower percentage rate of nutrients recovered from prawn, lettuce root and sediment demonstrated insufficient minerals input through fertilization and low biological availability from chemical reactions and precipitation. The application of *M. rosenbergii* diet partially increased the concentration of essential nutrients required by the lettuce in the system. However, inoculation of formulated liquid fertilizer and CM would be necessary during the production cycle in order to achieve optimal effectiveness.

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# STAKEHOLDER ANALYSIS APPROACH TO NATURAL RESOURCE MANAGEMENT PLANNING

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## ABSTRACT

The competing nature of resource uses and the multiplicity of resource users make natural resource management (NRM) planning complicated. The utility of a particular resource to the multi-stakeholders vary depending on the resource component that is of interest to them and the social, economic and environmental value that such resource generates. For instance, the priority concern of upland farmers is to draw economic value from the forest lands for subsistence while others who are not economically dependent on the forest would prefer to preserve its use for its aesthetic value Informed decision on the appropriate NRM strategy should be based on the nature and the extent of stakeholders' dependence on the resource, the state of the resource that is most useful to them and should take into consideration the social, economic and environmental implications of alternative programs that will be introduced. Participatory rural appraisal (PRA), generally done through public consultation, is the methodology commonly used to generate such information. An alternative approach that was recently found to have greater application in NRM planning is Stakeholder Analysis.

This paper presents the results of stakeholder analysis conducted in the upland and coastal zones of Sibonga, Cebu for agroforestry and coastal resources management. It also shows the potentials of stakeholder analysis as a tool for soliciting stakeholders' perception about the utility of the resource and their perceived roles and responsibilities in NRM as well as the importance of multi-stakeholder participation in making decisions about appropriate NRM strategies.

**Key words:** Stakeholders, community-based resource management, Sibonga, Cebu

**Abbreviations:** NRM - natural resource management, PRA - participatory rural appraisal

## INTRODUCTION

The competing nature of resource uses and the multiplicity of resource users make natural resource management (NRM) planning complicated. The utility of a particular resource to the multi-stakeholders vary depending on the resource component that is of interest to them and the social, economic, and environmental value that such resource generates. Informed decision on the appropriate NRM strategy should be based on the nature and the extent of stakeholders' dependence on the resource, the state of the resource that is most useful to them and should take into consideration the social, economic, and environmental implications of alternative programs that will be introduced.

Program success is attained when the key stakeholders' interests converge with the program objectives. For an NRM plan to be effectively implemented, a group or an individual should claim

ownership or identify themselves with that plan and should harmonize potential conflicts of interest. Hence, it is important that all key stakeholders participate in the formulation of the plan to ensure that their particular concerns are integrated and their roles in its implementation and sustainability are clearly defined, understood and appreciated.

Participatory rural appraisal (PRA), generally done through public consultation, is the methodology commonly used to generate such information. An alternative approach that was recently found to have greater application in NRM planning is stakeholder analysis. This paper: a) describes the Stakeholder Analysis as a method for engaging stakeholders' participation in NRM planning; and b) presents the results of stakeholder analysis conducted in the upland and coastal zones of Sibonga, Cebu for agroforestry and coastal resources management.

## METHODOLOGY

Information on the Stakeholder Analysis process that were obtained from the literature were applied on the empirical data gathered from the six (two coastal and four upland) barangays of Sibonga, Cebu. Sibonga is a third class coastal municipality located on the eastern side of the province of Cebu, 50 kilometers south of Cebu City (Fig. 1). The Municipality of Sibonga is divided into 25 component barangays covering a total land area of 13,345 hectares with a 2006-projected total population of 41,874. Approximately 11,343 hectares are agricultural lands while 1,849 hectares constitute the municipal waters (Sibonga CLUP, 2003).

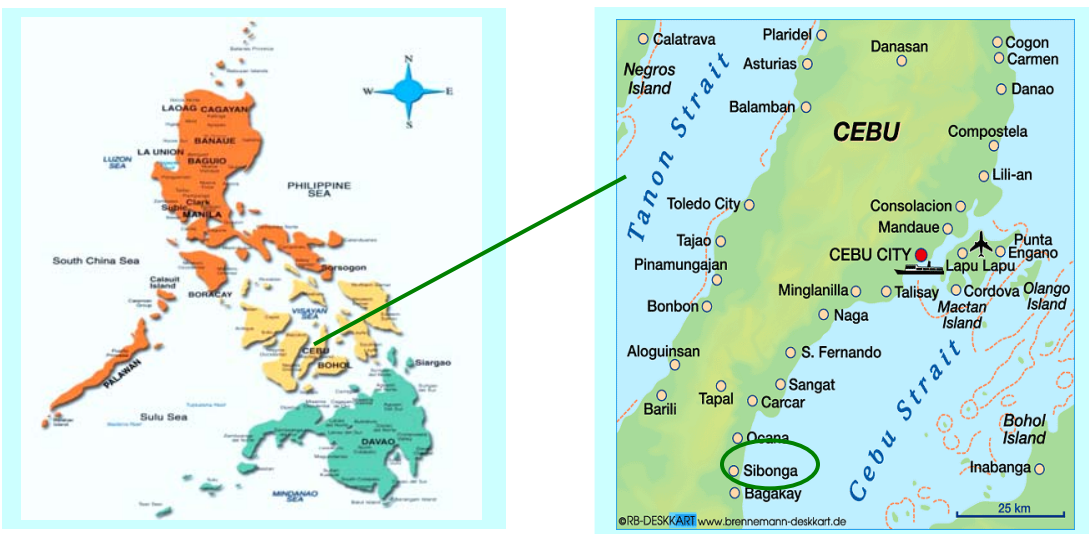


**Fig. 1.** Map of the Philippines showing Sibonga, Cebu.

Photos from www.welt-atlas.de and www.brennemon-deskkart.de

Empirical data were obtained through interview of key informants and focused-group discussion (FGD). Separate FGDs were conducted for each barangay and participated by past and current PO officers especially NRM committee chair, barangay officials and other community members. Equal number of males and females were targeted to participate in the discussions to

minimize gender bias and elicit more objective responses, opinions and experiences regarding the use and management of the resource.

## RESULTS AND DISCUSSION

### Definition of Stakeholders

A stakeholder is an individual, group or organization with interests in an issue pertaining to a particular natural resource, has power and influence to control the use of the resource or whose livelihoods are affected by changing the resource-use policies (Brown et.al., 2001). Proper identification and classification of stakeholders is a necessary element of Stakeholder Analysis.

Stakeholders may be classified into three types depending on their relative influence and importance in resource management: (i) primary stakeholders are those who have low influence in decision-making but whose welfare is important to the decision-makers and stand to lose the most from a decision; (ii) secondary stakeholders can influence decisions being made because they are predominantly the decision-makers and are engaged in implementing decisions but since they are not dependent on the resource for livelihood, their welfare is not at stake, and, (iii) external stakeholders (e.g. NGOs, civil society organizations) can exert influence over the outcome of a decision-making process through lobbying with the decision-makers, but whose interests are not directly affected by whatever management decision is made. Stakeholders can also include the more nebulous categories of 'future generations', the 'national interest' and 'wider society' (Grimble et.al., 1995).

### Steps in the Stakeholder Analysis Process

This process, called the Stakeholder Analysis, is a system for collecting information about groups or individuals who are affected by decisions, categorizing that information, and explaining the possible conflicts that may exist between important groups, and areas where trade-offs may be possible. It can be undertaken simply to identify stakeholders, or to explore opportunities for getting groups or individuals to work together (Brown et.al., 2001).

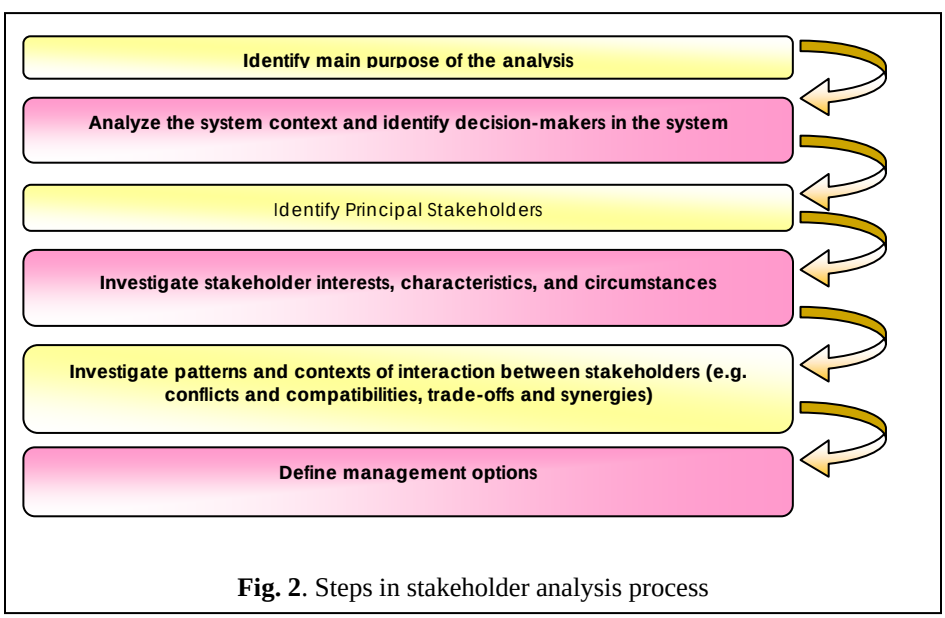


**Fig. 2**. Steps in stakeholder analysis process

The Stakeholder Analysis process begins with the identification of stakeholders. This is usually done through literature search and discussions with informants, and can be guided by analyzing the macro to micro level continuum which includes the global and international, national, regional, local off-site and local on-site stakeholders (Matsaert, 2002; Grimble, et.al 1995). It is critical to ensure that all relevant stakeholders are included and have equal participation in the process because they are supposed to identify the specific management problem and decision criteria that could address their concerns (Brown, et. al., 2001). However, stakeholder identification is complicated by the fact that stakeholders tend to fall under more than one category.

**Relevance of Stakeholder Analysis for Natural Resource Management Planning**

Stakeholder Analysis is particularly relevant for NRM planning and takes into account NRM's attributes (Grimble et.al., 1995) such as: a) management of soil and water regimes cut across social, economic and political units and is likely to involve different stakeholders – local communities, commercial interests, and government departments; b) environmental problems are frequently associated with externalities that are characterized by competing interests; c) many natural resources are common or public resources with multiple and competitive users and stakeholders; d) there are temporal issues associated with the appropriation of natural resources (e.g. affects future availability and productivity); and e) natural resources tend to have multiple uses which are often not compatible. Forests and tree resources may have both productive and environmental benefits but the land on which the forest trees stands can also be used for subsistence farming.

Stakeholder Analysis complements and builds on quantitative (e.g. Cost-Benefit Analysis (CBA)) and qualitative (e.g. PRA) methods but takes the diverse interests of the various stakeholders as its starting point, takes into account the interests of the whole range of stakeholders and seek compromises between potentially conflicting public and private stakeholder objectives. Other participatory approaches have generally given less emphasis to factors that give rise to conflicts of interest (Brown, et.al., 2001).

Stakeholder Analysis can also be a useful tool for policy makers at several levels such as: a) national level policy formulation; b) local planning to integrate and harmonize the interest of stakeholders and national plan; and c) project analysis to take into consideration the effect of the project on the various stakeholder's interests. Stakeholder Analysis can also be used for different purposes, such as *ex ante* appraisal and *ex post* evaluation of projects and policies (Grimble, et.al. 1995).

**Limitations of Stakeholder Analysis**

In the application of Stakeholder Analysis as a planning tool, the analyst should be conscious of its limitations. For instance, Stakeholder Analysis tends to treat different stakeholder groups as distinct entities, whereas in reality, social groupings are not entirely distinct. Therefore, analysts should be conscious of the overlaps between defined groupings as well as unequal levels of influence among stakeholders.

To overcome these limitations, Stakeholder Analysis should: a) critically assess the typology of stakeholders; b) assess stakeholders' level of understanding of issues; and c) employ various modes of information elicitation (FGD, public consultation) to ensure equal opportunity for free expression of preferences particularly when some stakeholder groups that have socio-cultural, economic and intellectual advantage over the others tend to dominate the discussions.

**NRM Goals Identified through PRA**

During the earlier stage of NRM project planning, the project staff conducted PRA to assess the state of the upland and coastal resources, the people's perception and level of awareness about the resource degradation problems in their communities, and to identify the activities that have to be carried-out to address these problems. It was participated by local community leaders and local government officials. One of the outputs of the PRA sessions was a set of NRM goals and activities.

However, the NRM goals and activities identified by the PRA participants did not provide concrete basis for sound NRM planning and reflect their limited awareness and appreciation of the scope of the problem, the interdependence between the upland and coastal ecosystems and the role that the various stakeholders can take to contribute to NRM.

Moreover, the information generated through the PRA was not thoroughly processed such that some of the identified "goals" were not clearly differentiated from "activities". For instance, "soil analysis", "planting of trees", and "implement ordinance for illegal fishing", which can be considered as activities were listed as goals (CBRMP, n.d.). The limitations of the PRA process that was undertaken prompted the conduct of a more thorough Stakeholder Analysis.

## RESULTS OF STAKEHOLDER ANALYSIS

**Characterization of Stakeholders**

Stakeholder Analysis was conducted to enhance and supplement the PRA that was conducted earlier. Guided by the Stakeholder Analysis procedure, the study generated more detailed and more comprehensive information on stakeholders' interest and their perception about the utility of the resource, their access and use rights, and their roles/responsibility in NRM.

Several types of stakeholders in the upland and coastal areas of Sibonga were identified and characterized based on key informant interviews and FGD results (Table 1). The primary stakeholders include the:

a) subsistence farmers and fisherfolks who largely depended on the resource for livelihood and have limited livelihood skills and options but can be mobilized as partners in the community-based resource management projects;

b) a plastic wares firm that used the resource as harvesting ground for its raw materials (e.g. carrageenan from seaweeds), provided financial assistance to upland farmers for tree plantation establishment, reforestation and assisted natural regeneration and to coastal farmers for mangrove rehabilitation and reforestation as well as reef check and mobilized its employees for coastal clean-up; and

c) a group of commercial fishing vessel operators who oftentimes undermined the conservation efforts of the other stakeholders.

The secondary stakeholders composed of the Sibonga LGU, and the national government agencies (NGAs) such as the departments of Agriculture (DA) and Environment and Natural Resources (DENR) as well as the Bureau of Fisheries and Aquatic Resources (BFAR) together with the external stakeholders (e.g. NGOs, CSOs, academic institutions and other LGUs) promoted community participation in NRM, provided technical assistance to create farmers' and fisherfolks' awareness, developed their capability in NRM and helped them identify non-resource-extractive livelihood options.

**Table 1**. Characterization of stakeholders in Sibonga, Cebu.

| Stakeholders | Characteristics, interests and circumstances |
|---|---|
| **Primary Stakeholders** | |
| Subsistence Farmers/ Fisherfolks (legal and illegal) | • resource poor<br>• limited skills/capability<br>• subsistence farming/fishing<br>• limited livelihood options<br>• fragile upland and coastal ecosystems |
| CPKELCO | • used seaweeds as raw materials for manufacturing plastic wares<br>• extended livelihood and financial assistance to upland and coastal communities<br>• provided funds for ANR, reef check and tree plantation establishment<br>• mobilized its employees for coastal clean-up |
| Commercial Fishers | • big business<br>• can strongly influence law enforcement and local policies<br>• profit-oriented<br>• huge fish catch<br>• compete with small and subsistence fisherfolks |
| **Secondary Stakeholders** | |
| Sibonga LGU | • gave high priority to NRM and people's well-being<br>• supported community-based resource management strategy<br>• showed political will for environmental conservation/protection<br>• passed and imposed resolutions that ban illegal logging and illegal fishing<br>• imposed fines to violators<br>• organized environmental law enforcement groups<br>• limited technical and financial capability |
| NGAs (DA, DENR, BFAR) | • national regulatory agencies that enforced national environmental laws and policies<br>• worked with LGUs in law enforcement<br>• provided technical assistance to LGUs<br>• implemented NRM programs<br>• monitored LGU compliance with NRM programs and policies |
| **External Stakeholders** | |
| NGO | • pursued programs on environmental protection, people empowerment and community education<br>• monitored status of fish sanctuaries<br>• have technical and financial capability<br>• highly driven and motivated NGO workers<br>• extended technical assistance to LGU and community |
| Academic Institutions | • technical and financial capability<br>• scientific knowledge on forest and marine ecosystem<br>• provided technical assistance to LGUs |
| CSOs | • not economically dependent on the resource<br>• occasional resource users<br>• willing to pay for aesthetic resource value and environmental protection |
| Other LGUs | • concerned with NRM information sharing<br>• interested to draw lessons and acquire additional knowledge on NRM for replication in their respective communities |

**Patterns of interaction and NRM options identified by the stakeholders**.

Further extension of stakeholder analysis motivated stakeholders to identify patterns of interaction. Various modes of resource utilization were also highlighted and NRM options were discussed during the stakeholder analysis process to minimize conflicts, enhance synergy and explore areas of compatibility (Table 2). Conflicts among the resource users particularly the resource extractors and the regulators were discussed and areas of cooperation and compatibility were fleshed-out during the discussions.

**Table 2.** Patterns of interaction and NRM options identified by the stakeholders.

| Interactions and Options | Stakeholders Involved |
|---|---|
| Conflicts | - subsistence fishers vs. commercial fishers vs. BFAR<br>- illegal loggers/upland farmers vs. DENR and LGU<br>- upland farmers vs. subsistence fishers<br>- illegal fishers vs. DENR, BFAR and LGU |
| Compatibilities | - DENR, LGU, NGOs, CSOs, DA, communities involved in CBRM |
| Trade-offs<br>- food production/income vs. resource protection/conservation | - upland farmers and fisherfolks vs. DENR, DA, BFAR, LGU |
| Synergies in resource use—regulated NRM | - LGU, DENR, BFAR, NGO, tourists/enlightened farmers and fisherfolks |

Through the use of the Stakeholder Analysis approach, interaction and exchange of ideas between the different stakeholders were facilitated and compromises to minimize resource use conflicts were arrived at. After the dialogues, conflicts between the resource users were minimized, agreements between the communities and the regulators to work as partners in law enforcement were forged, and alternative NRM options that take into consideration environmental conservation and people's well-being were identified. Active and balanced participation of the different stakeholders in the open discussion of NRM issues, responsibilities and accountabilities created awareness and an environment for cooperation among the different stakeholders.

**NRM Imperatives and Stakeholder Analysis Results**

In the subsequent consultations and FGDs involving the various stakeholders, the facilitator presented the NRM imperatives that the stakeholders reviewed to determine their applicability to local conditions. The stakeholders concurred on the suggested NRM imperatives and the need for immediate action to address the resource degradation problem. Further probing by analysts revealed that heavy encroachment and extraction of forest and coastal resources are rooted on deeper social and economic problems such as limited access to livelihood opportunities by the resource poor farmers and fisher folks.

To arrest further resource degradation, the stakeholders agreed to: a) find a comprehensive solution that will provide alternative and non-resource extractive livelihood sources for the primary stakeholders; b) solicit the active participation of resource users in environmental law enforcement; c) conduct information, education and communication programs to raise environmental awareness and develop the primary stakeholders' knowledge and skills on NRM, and d) pursue long-term NRM

options under the leadership of the LGU through the formulation of policies and programs to sustain NRM activities.

Among the NRM options that were identified by the stakeholders were: a) agroforestry; b) assisted natural regeneration; c) establishment of tree plantations; d) establishment of fish sanctuary and marine reserve; and e) mangrove reforestation. The result of the Stakeholder Analysis is a comprehensive plan that integrates local knowledge with science-based NRM imperatives.

The stakeholder analysis approach enabled the stakeholders to thoroughly discuss their respective priority concerns, identify areas of support and complementation, and possible options as well as the role that each of them can play to ensure that sustainability and welfare goals can be attained. The stakeholder analysis process helped the subsistence farmers and fisherfolks appreciate the essence of NRM and elicit their cooperation in the NRM conservation efforts of the secondary and tertiary stakeholders.

A follow-up on the NRM efforts of the Sibonga LGU showed indications of sustainability as former illegal loggers and illegal fisherfolks who were enlightened on NRM principles became LGU and NGA partners in environmental law enforcement.

## CONCLUSIONS

The Sibonga, Cebu experience showed that the Stakeholder Analysis approach is an appropriate and effective tool for NRM Planning. Stakeholder Analysis is more structured and the prescribed procedures guided the analysts in the identification and characterization of stakeholders and in helping the stakeholders resolve resource use conflicts and identify NRM options.

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# AGRICULTURAL INSURANCE IN VIETNAM: SITUATION AND RECOMMENDATIONS

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## ABSTRACT

Insurance is one of the financial system areas, of managing risks. Basically insurance is divided into social and commercial insurance which covers agricultural insurance. Up to now, Vietnam is still an agricultural country with some 14 million farm households living in rural areas. Their livelihood is based on agricultural production. The agricultural production in Vietnam is faced with difficulties and risks, therefore, insurance is one strong solution to help farm households to overcome these. Although insurance, in general, has improved in Vietnam, the agricultural insurance system is still weak.

This research sought to review the current situation of insurance and propose some solutions to develop the agricultural insurance system in Vietnam. This paper covers (i) description of the three main periods of agricultural insurance development in Vietnam. (ii) analysis of the reasons for success and failure of the agricultural insurance from the perspective of the Vietnamese Government, company and farming system; and (iii) proposals to improve agricultural insurance in order to reduce risks for farm households and contribute to the development of the rural financial market in Vietnam.

**Key words:** insurance product, risk management, mutual and voluntary insurance.

## INTRODUCTION

There are many definitions of insurance, but basically it is divided into (1) social insurance which serves society without any profit and (2) commercial insurance, which operates on the basis of profit. Social insurance is protected by the government to ensure social security. This kind is followed by the Insurance Business Law. Although the insurance system of Vietnam was established late, the two types of insurance mentioned above are adequate.

Vietnam is still an agricultural country with approximately 14 million farm households living in rural areas. Their livelihood is dependent on agricultural production which is faced with difficulties and risks, of which natural risks is the heaviest. In the period from 1986 - 2003, natural hazards caused a full loss of 1.4 million ha of rice and this led to a loss of nearly 6 million tons of grain (Pham Thi My Dung, 2007). Insurance is one of the important tools to reduce the losses of agricultural production. Therefore, agricultural insurance is very important and needs to be developed in Vietnam.

This research sought to analyze the situation of agricultural insurance and propose some solutions to develop agricultural insurance in Vietnam.

## METHODOLOGY

Data for the research is derived from two main sources. Primary data came from interviews with staff of the Vietnamese Farmer Association and the Policy Department of the Ministry of Agriculture and Rural Development. Structural and semi-structural questionnaires were used for these interviews. In addition, case surveys with agricultural producers in some pilot areas and Participatory Rural Appraisals (PRAs) with key persons were used to collect more data. Secondary data included published reports, documents etc. in the Department of Financial Ministry, the Vietnam Insurance Corporation Bao Viet, the Financial Journals, and other researches.

Descriptive, comparative, expert and reference methods were adopted to analyze the current situation and the characteristics of insurance, in general and agricultural insurance, in particular.

## RESULTS AND DISCUSSION

### Development and characteristics of insurance in Vietnam

#### Social Insurance

Social insurance in Vietnam was established in August 1945 and provided insurance only for civil servants for health, labor accident and retirement until 1960. The Vietnamese Government subsidized 100% of insurance payment fees through the state budget. From 1961 to January 1995, however, some changes occurred for the insurance policy such as scope for insurance concerning state enterprises. The enterprises only contributed 13% from the salary enterprise fund and the rest was subsidized by the government.

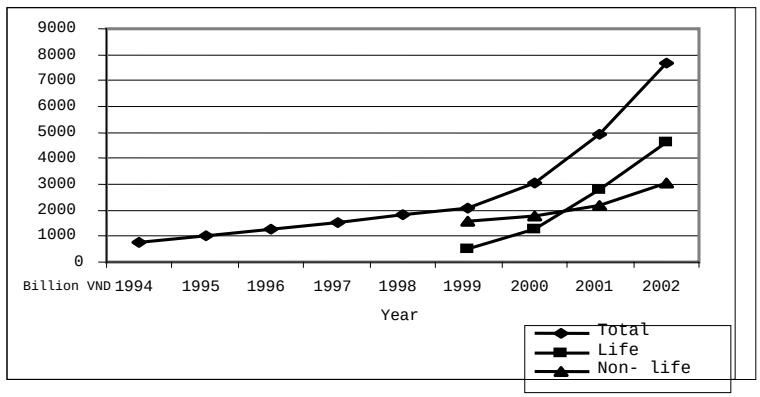
From 1995 to the present, the scope for insurance has been expanded widely. This includes insurance for civil servants, laborers for foreign investment enterprises and laborers for private enterprises with more than 10 laborers. The insurance fund is derived mainly from two major sources: employers and employees. The fund has been increased sharply from VND 1,530 billion in 1995 to VND 5,008 billion in 2000 (Dung, 2002). However, the social insurance only provided the social insurance book for over 3 million insurers until 2000. Most of the laborers have not been insured yet. Thus, the Social Insurance Law of Vietnam was prepared in 2001 and approved in 2006 by the National Assembly.

In principle, health insurance is one part of social insurance. Before 1992 the social insurance was divided in two types: (i) social insurance managed by the Ministry of Labor, Invalid and Social Affairs and (ii) health insurance was under the management of the Ministry of Health. In 2002, the Government decided to merge the two into the social insurance. The Ministry of Labor, Invalid and Social Affairs is responsible mainly for policies, institutions, laws and others, while the Ministry of Health is an associated institution. The insurance fund is managed by Vietnam Social Insurance.

#### Commercial Insurance

The first commercial insurance with contracts was made in Italy in 1182, while the first insurance company was established in UK in 1667. In Vietnam, the commercial insurance was really put into operation in 1965 and underwent two phases. From 1965 - 1992 a monopolized insurance period existed with a unique state insurance company called 'Bao Viet' operating in Vietnam. From 1993 to the present, there are some improvements in the insurance polices and institutions such as the Directive 100/CP issued in 1993, the Insurance Association of Vietnam established in 1999, and the Insurance Business Law passed in 2000 which was placed into operation in April 2001. The Insurance Law highlighted the special points of the commercial insurance of Vietnam (Thanh, 2002). The above events have helped in the rapid development of insurance. These include: (i) more diversity of ownerships: state enterprises, share enterprises, joint - venture enterprises and foreign enterprises. (ii)

number of insurance enterprises increased rapidly, from only two in 1993 up to 17 in 2001 and 26 in 2005, and (iii) turnover increased sharply in ten years, from VND 741 billion in 1993 to 7, 685 billion in 2003 (Fig. 1)




Source: Information on Insurance Market 2003

**Fig. 1**. Insurance turnover in Vietnam

**Agricultural insurance in Vietnam**

Because agriculture in always faced with high risks such as diseases, typhoons and flooding, commercial insurance companies do not want to cover agricultural production (Dung 1994). The first agricultural insurance in the world was in Germany in 1893 in the form of the Mutual Insurance Company but this collapsed afterward. In Vietnam, agricultural insurance was established in 1981 and until 2001 only Bao Viet had agricultural insurance activities which can be divided into stages:

**From 1981 to 1991:** Bao Viet implemented pilot agricultural insurance in Vu Ban and Nam Ninh districts of Nam Dinh province. Insurance contracts were signed with agricultural cooperatives, and compensation money was paid directly to the cooperatives (Table 1).

**Table 1.** Implemented insurance outcome

| Indicators | Unit | 1981 | 1982 |
|---|---|---|---|
| No. of cooperatives | unit | 83 | 85 |
| No. of cooperatives involved in insurance | unit | 74 | 37 |
| Rate of cooperative | % | 89.2 | 43.5 |
| Turnover | million vnd | 556.7 | 790 |
| Compensation | million vnd | 464.1 | 300 |
| Compensation rate | % | 83.2 | 38 |

Source: Bao Viet, 2001 - Price 1982/1983

This insurance earned profits, however, it stopped operations after two working years because of unsuitable and unattractive insurance guidelines and shortage of scientific researches before putting insurance in practice.

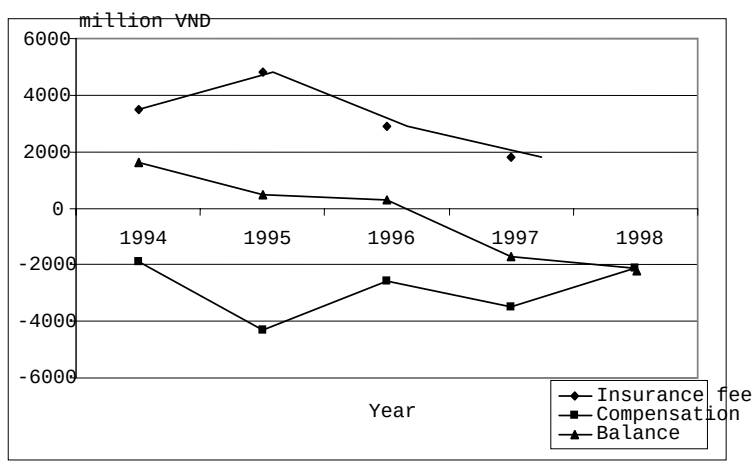
**From 1991 to 1998:** In December 1991, the Vietnamese Government assigned Bao Viet to conduct research on crop insurance. After its researches in 1993, Bao Viet drew the following conclusions: (i) compulsory commercial insurance was needed to put the seasonal insurance into mass production and (ii) experiment time for this insurance was around 5 - 7 years. Based on Bao Viet's conclusions, the Ministry of Finance permitted Bao Viet to provide insurance for rice paddy crop in 16 provinces with 90,000 farm households involved. In addition, Bao Viet also provided several livestock insurance (Table 2).

**Table 2.** Outcome of insurance

| **Indicators** | **Unit** | **1994** | **1995** | **1996** | **1997** | **1998** | **Total** |
|---|---|---|---|---|---|---|---|
| Insured paddy area | ha | 64,500 | 78,000 | 38,000 | 19,500 | | 200,000 |
| Buffalo and Cow | head | 38,500 | 36,700 | 66,700 | 12,000 | 7,900 | 161,000 |
| Pig | head | 33,700 | 60,000 | 66,000 | 18,000 | 12,000 | 189,700 |
| Poultry | head | 24,000 | 40,500 | 16,000 | 10,000 | - | 90,500 |
| Dairy Cow | head | 1,500 | 3,700 | 4,700 | 950 | 300 | 11,150 |
| Insurance Fee | million vnd | 3,500 | 4,800 | 2,900 | 1,800 | | 13,000 |
| Compensation | million vnd | 1,900 | 4,300 | 2,600 | 3,500 | 2,100 | 14,400 |
| Balance | million vnd | 1,600 | 500 | 300 | -1,700 | -2,100 | -1,400 |

Source: Report of Bao Viet, 2001

The agricultural insurance is smaller than its potential because the paddy planted area was 7.4 million ha, buffalo and cow were 7 million, pigs were 18 million and more than 166 million poultry (Statistical Year Book 2000). In 1998, the agricultural insurance of Vietnam VND 1,400 million in losses for the following reasons: (i) farmers did not understand production insurance. (ii) unsuitable insurance modality. (iii) Bao Viet implemented insurance alone (iv) insurance payment fees were not enough to cover for expenses and(v) the Government did not have suitable insurance policies for agriculture. As result, the agricultural insurance sector was merged into the vehicle insurance department.




Source: Bao Viet, 2001

**Fig. 2.** Fee, compensation and balance in agro-insurance operation of Bao Viet Company

**From 1999 to the present**

Agricultural insurance does not exist except for the France' Insurance Corporation called "Grouppama" which began operations in South Vietnam in 2001. It gives certain agricultural insurance products such as pork and shrimp insurance but on a very limited scale. Although agricultural insurance does not exist, the researches on this insurance are still ongoing to determine solutions for agricultural insurance in Vietnam (Table 3).

**Table 3.** Researches on agricultural insurance in Vietnam

| Organization and year | Objectives | Results |
|---|---|---|
| 1. FAO, 1999 | Consultation for Agricultural Insurance Program of Bao Viet | Giving recommendations on ability, conditions and needs to promote agricultural insurance operations of Bao Viet |
| 2.Vietnamese Farmer Association (VFA), 2000-2001 | Research on insurance for paddy rice crop | Concluded that mutual insurance is suitable to this crop and foreign insurance companies should be encouraged to invest in the agricultural insurance sector in Vietnam |
| 3. Ministry of Finance, 2001 | To propose agricultural insurance policies in Vietnam | Some financial policies for agricultural insurance development in Vietnam were proposed. |
| 4. Tam Duong Extension Station, 2001 | Improvement of livestock production through veterinary maintenance | Testing and conclusion of pig insurance capacity on the basis of self – management by groups. |
| 5. F2.1 and F2.2 Subprojects under the Uplands Program, 2002- 2004 | Finding suitable insurance types to help farmers for hunger and poverty reduction | Conclusions: (i) potential for voluntary agricultural insurance development.<br>(ii) farms want and are able to participate in suitable insurance schemes<br>(iii) analysis of factors impacting insurance demand. |

Source: Dung 2002, Dufhues.T et al 2004, FAO 1999, Loi 2001, Thanh 2002, VFA 2001

The biggest success of this stage include: the state institutions and scientific organizations pay more attention on insurance and conducting research to avoid failures in the past. In summary, the agricultural insurance of Vietnam has not played a role in agricultural production yet.

## Suggestions for Development of the Agricultural Insurance in Vietnam

1. The government should enforce policies to support agricultural insurance through

   a) issuance of regulations, legal writings and guidelines for the agricultural insurance;
   b) appropriate policies for insurance business;
   c) special policies for high - risk agricultural insurance to help agricultural companies buy reinsurance;
   d) establishment of some agricultural insurance programs for heavy risk areas; and
   e) offer some advantages in income tax and advertisement for agricultural insurance companies.

2. Combine communication with policies to stimulate farmers to buy insurance

   a) to help farmers to know insurance and the agricultural insurance by medias;
   b) to offer preferential credit, especially in credit for buying insurance;
   c) to support a part of the insurance fee for farmers;
   d) to increase role of state offices in solving contract relationship between insurance companies and farmers in risk compensations; and

3. Diversity of agricultural insurance

   a) to develop more kinds of insurance business such as state, foreign, joint venture which must be equity in operation;
   b) to develop many agricultural insurance products such as crop, livestock insurance, asset insurance, price, voluntary & compulsory and business & mutual insurances;
   c) to combine insurance services with other services such as financial services, material supplies, advisement services, extension services.

On one hand the Government must request big state insurance companies to have the agricultural insurance. On the other hand the Government needs to have special policies to encourage the investments of foreign organizations in the agricultural insurance sector.

4. Conduct more researches and tests on agricultural insurance
   a) to lead in choosing appropriate research topics;
   b) to validate the researches and tests on commercial insurance models in big agricultural companies, farms, enterprises;
   c) to validate the researches and tests on mutual insurance models for small farms, self-subsistence households;
   d) to survey the potential of agricultural insurance in order to design suitable insurance products; and
   e) to seek more funds to test some demand driven - insurance schemes in rural and in agriculture.

5. Development of overall insurance network in the rural areas

   The commercial insurance network focuses mostly on big cities and is marginalized in the rural areas especially in the mountainous regions. Therefore, the development of an insurance network in rural and remote areas will help farmers know more about insurance benefits. On the other hand, insurance companies will have opportunities to determine suitable contacts for agricultural production. In addition, a linkage between scientific institutions and farmers be set up to facilitate the re-establishment and development of agricultural insurance in Vietnam

## CONCLUSIONS

Agricultural insurance is the commercial insurance linked with socio - economic development tasks in rural areas. Presently, agricultural insurance is the weakest sector in insurance in Vietnam due to many different reasons. The development of agricultural insurance is difficult, but this is a useful work to help farms reduce risks. For the success of insurance, it is necessary to have some careful researches and tests on pilot samples before implementation. The development of agricultural insurance is the responsibility not only of the Government, but also by the insurance companies and agricultural producers. Besides the Insurance Law, the government needs to give priority to organizations involved in agricultural insurance.

There is a need to institutionalize agricultural insurance in Vietnam in order to:

(i) enforce more compulsory and mutual insurances in agriculture.
(ii) identify what types of risk will be covered by the state fund, what types of risk is covered of premium from insurance companies.
(iii) enforce some priority policies for companies and organizations which relate to agricultural insurance and,
(iv) promote the roles of the Government and intermediate institutions in payment for agricultural insurance buyers.

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