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Biocontrol potential of *Bacillus thuringiensis* var. *aizawai* and *Metarhizium anisopliae* SPW isolate against insect pests of pechay and lettuce grown under protected and open field cultivation

Reny G. Gerona[1*] and Bernardita I. Lauro[1]

ABSTRACT

Insect pests are one of the constraints in lettuce and pechay production. Although synthetic chemical insecticides are widely used, organic growers are interested in using alternative options which have no toxic residues. This study aimed to evaluate the biocontrol potential of *Bacillus thuringiensis* var. *aizawai* (*Bta*) and *Metarhizium anisopliae* (*Ma*) SPW isolate against insect pests of pechay and lettuce grown under protected and open field cultivation. Weekly spraying of each treatment was done using the recommended rates (RR) of application: *Ma* at 3L spore suspension per 13L water ($1x10^8$ spore concentration); *Bta* at 20g per 16L water; cypermethrin 5EC at 30mL per 16L water. Insect infestation was monitored weekly based on insect count and damage ratings. Yields were recorded at harvest. Results showed that application of either *Bta, Ma* or cypermethrin significantly reduced populations and damage of *Spodoptera litura* Fabr. and *Plutella xylostella* L. Pechay plants applied with *Bta* and *Ma* showed higher yields than cypermethrin in both types of cultivation. These findings suggested that *Bta* and *Ma* SPW isolate were effective against *S. litura* and *P. xylostella* and can be used as an alternative non-chemical option for their management.

Keywords: entomopathogens, alternative insect management, pechay, lettuce

INTRODUCTION

Pechay (*Brassica rapa,* Family: Brassicaceae) and lettuce (*Lactuca sativa,* Family: Asteraceae) are two of the leafy vegetables commonly grown by farmers. Just like other crops, insect pests are one of the constraints in lettuce and pechay production. In a study conducted in La Tranidad, Benguet, Tibao (2012) observed the following insect pests associated with pechay: *Phyllotreta spp.(*Chrysomelidae), *Pieris rapae* (Pieridae), *Trichoplusia ni* and *Spodotera litura*

[1] Visayas State University, Baybay City, Leyte, 6521-A, Philippines

* **Corresponding Author**. Address: Visayas State University, Baybay City, Leyte, 6521-A, Philippines; Email: rggerona@yahoo.com
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(Noctuidae), *Liriomyza huidobrensis* (Agromyzidae), *Plutella xylostella* (Plutellidae) and *Myzus persicae* (Aphididae). On the other hand, in the study of Oatman and Platner (1972) in Southern California, they found that *Spodoptera exigua*, *Trichoplusia ni*, *Agrotis ipsilon* and *Feltia subterranea* (Noctuidae) were the principal lepidopterous pests of lettuce. The lepidopterous species are the most damaging group. The adults lay eggs on the leaves and hatching caterpillars feed voraciously on leaves and heads of the plant. Reduction in yield and the downgrade of marketability of the crop is due to feeding damage, contamination by their presence or excreta on the leaves.

The use of synthetic insecticides is the common option that growers employ against vegetable insect pests. Two options that organic growers can also use are the biological pesticide, *Bacillus thuringiensis* (*Bt*) and entomopathogenic fungus, *Metarhizium anisopliae (Ma)* (Dutta 2015, Loc et al 2007). *Bt* is a soil bacterium that produces spores which contain endotoxins that have insecticidal activity. Their primary action is to lyse midgut epithelial cells by inserting into the target membrane and forming spores (Sanahuja et al 2011). Different strains of *Bt* have different insecticidal activity spectrums and were first used as an agricultural insecticide in the 1930ies. *Bt kurstaki (Btk*) and *Bt aizawai* (*Bta*), are commercial formulations that kill caterpillars. The endotoxin acts as a stomach poison and is dose dependent hence are more effective against early instars.

Bacillus thuringiensis var. kurstaki (Btk) and *Bt aizawai* (*Bta*) are used against lepidopterous larvae while other *Bt* strains are effective against species of beetles and dipterans. A commercial preparation of *Btk* was effective in reducing caterpillar populations and insect feeding damage in cabbage and lettuce which was comparable to the standard chemical insecticide (*lambda-cyhalothrin*) (Zehnder et al 1997) and eggplant fruit and shoot borer (Cruda 2012).

Metarhizium anisopliae (*Ma)* is a fungal pathogen that attacks a wide range of arthropods, more than 200 species from over 50 families (Ujjan & Shahzad 2012). *Ma* was first recorded as an agricultural insecticide in the 1880's in Russia (Vega et al 2009). The general mode of infection of *Metarhizium spp*. comprises six stages in the following order: adhesion, germination, appressorium formation, penetration, colonization of haemolymph and extrusion and sporulation which can also be found in other entomopathogenic fungi (Moon San Aw & Hue 2017). *Ma* also has strains with specific host ranges and various commercial formulations have been developed and registered for use. It was effective against the mealybug of tomato (Panyasiri et al 2007) and diamondback moth under laboratory and greenhouse conditions (Lo & Chi 2007). Laboratory and field evaluations of *Ma* SPW isolate against the fruit borer of jackfruit proved to be effective thus recommended as one of the best options for its management (dela Cruz et al 2013) and against the eggplant fruit and shoot borer under greenhouse conditions (Cruda 2012).

For leafy vegetables like lettuce and pechay which are either eaten raw as green salads or with brief cooking, the use of alternative insect pest control like entomopathogens is a better option since they leave no toxic residues. However, local farmers still rely on the use of synthetic chemical insecticides as the only solution against insect pests of these vegetable crops. This study hypothesizes that entomopathogens like *Bt* and *Ma* are effective against lepidopterous and other insect defoliators of pechay and lettuce. Specifically, this study was conducted to evaluate the pest control potential of *Bacillus thuringiensis* var. *aizawai* and

Metarhizium anisopliae SPW isolate against insect pests of pechay (*Brassica rapa*) and lettuce (*Lactuca sativa*) grown under protected and open field cultivation.

MATERIALS AND METHODS

Experimental Sites (Protected and Open Field)

This study was conducted under protective structure (ie, igloo type) and an open field at the experimental area of Visayas State University, Baybay City, Leyte, Philippines. The protective structure measuring 5mx40m and stands 4m high was made up of coconut palm lumber covered with 0.002mm thick UV-treated plastic film. An open field of similar dimension as the structure was used for the open field set-up. Both experimental sites were divided into two parts wherein the first half was planted with pechay while the second was planted with lettuce. The two experiments were simultaneously conducted from January-February, 2015.

Land Preparation

In both experimental set-ups, the soil was ploughed twice at one week interval followed by two harrowing. Three raised beds (1mx40m) were prepared at one meter apart in each set-up.

Preparation of Seedlings

Seeds of pechay (var. Pavo) and lettuce (var. Grande) were sown in seedling trays with sterilized soil mixture of garden soil, compost and carbonized rice hull (2:1:1) and were placed in the nursery. One week before transplanting, seedlings were hardened.

Transplanting and Fertilizer Application

At two to three leaf stage (about two weeks old) seedlings were transplanted into the prepared beds at the rate of one seedling per hill at 30cm distance between rows and 25cm between hills. Basal application of complete fertilizer followed by weekly drenching of calcium nitrate were done following the recommended rate of 90-60-60kg N, P2O5, K2O/ha.

Water and Weed Management

The plants were watered daily, usually in the morning, through drip irrigation. Weeds were removed manually two-three times monthly in the open field but usually only spot weeding under the protective structure due to scanty weed growth.

Preparation of Ma SPW Isolate Spore Suspension

Cultures of bagged *Ma* SPW isolate fungus were secured from the DA-RIARC, Balinsasayao, Abuyog, Leyte, Philippines. Spore suspension was prepared by adding 300mL water with a pinch of neutral soap per bag and squeezed to dislodge the spores. Additional 800mL of water was poured into the bag, shaken, squeezed and strained to extract a net of 1L spore suspension per bag of cultured *Ma.*

Application of Treatments

Treatment application was done weekly by spraying the test plants (60 for pechay and 52 for lettuce per replicate) at wetting capacity with a spray volume of 200-650mL depending on the age/size of plants either early morning or late afternoon using the recommended rates (RR) of application for each treatment.

The treatments were:

T_0 = tap water
T_1 = *Metarhizium anisopliae (Ma)* SPW Isolate at 3L spore suspension per 16L water (RR)
T_2 = *Bacillus thuringiensis* var. *aizawai (Bta)* at 20g per 16L water (RR)
T_3 = *Bta* and *Ma* at RR applied at 3 day interval between sprays
T_4 = cypermethrin 5 EC at 30mL per 16L water (RR)

Data Gathered

Actual insect counts and damage ratings (adopted from Navasero & Manipol 2005) were gathered weekly from 20 and 15 inner plants per replicate for pechay and lettuce, respectively. Yields (marketable & non-marketable) were recorded at harvest.

Damage Rating Scale for Chewing Insects

Scale	Description
0	No damage
1	Leaf feeding on 1-5% of leaves
3	Leaf feeding on 6-15% of leaves
5	Leaf feeding on 16-25% of leaves
7	Leaf feeding on 26-50% of leaves
9	Leaf feeding on more than 50% of leaves

Harvesting

Harvesting was done early in the morning or late in the afternoon at 18-25 (pechay) and 30-35 (lettuce) days after transplanting. The harvested crops were sorted into marketable and non-marketable yield.

Experimental Design and Data Analysis

The experiment was laid-out and arranged in Randomized Complete Black Design (RCBD) replicated three times. Data were analyzed using ANOVA following the Statistical Tool for Agricultural Research (STAR) version 2.0.1 and means were compared using Least Significant Difference at 5% level of significance.

RESULTS AND DISCUSSION

Effects of *Metarhizium anisopliae (Ma)* SPW Isolate and *Bacillus thuringiensis var. aizawai (Bta)* on insect pests of pechay

Low numbers of cluster caterpillar (*Spodoptera litura* Fabr.) and the diamondback moth (*Plutella xylostella* L.) were observed throughout the cropping period. Figures 1-4 show that under protective structure, generally all treatments got significantly lower damage ratings and population counts than the control except for *S. litura* in cypermethrin treated plots, particularly during weeks 2 and 3. During this period, lower damage ratings and population counts of *S. litura* were recorded in three biological treatments compared to the cypermethrin treated plots. In the open field, higher damage ratings and population counts were also obtained in the water sprayed control compared to the rest of the treatments especially with *P. xylostella*. However, cypermethrin usually had lower damage ratings and population counts than the biological treatments, particularly in the weeks 3 and 4 observations. Weekly rainfall ranged from 13-32mm (Figure 5) and may have washed the field applied *Bta* and *Ma* off more easily than the cypermethrin.

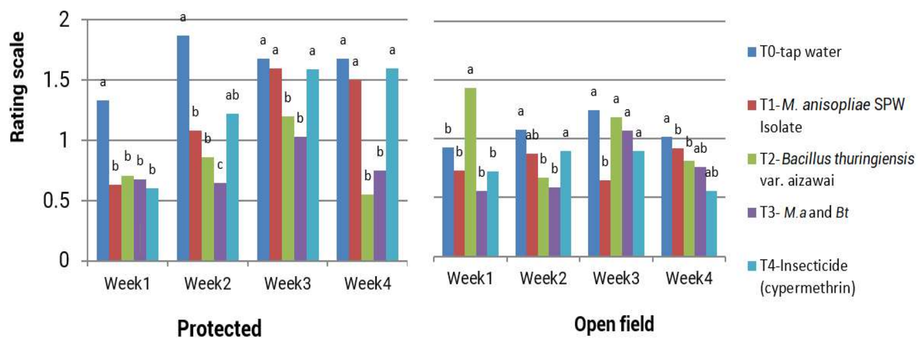

Figure 1. Mean damage ratings of cluster caterpillar (*Spodoptera litura* Fabr.) on pechay under protected and open field cultivation

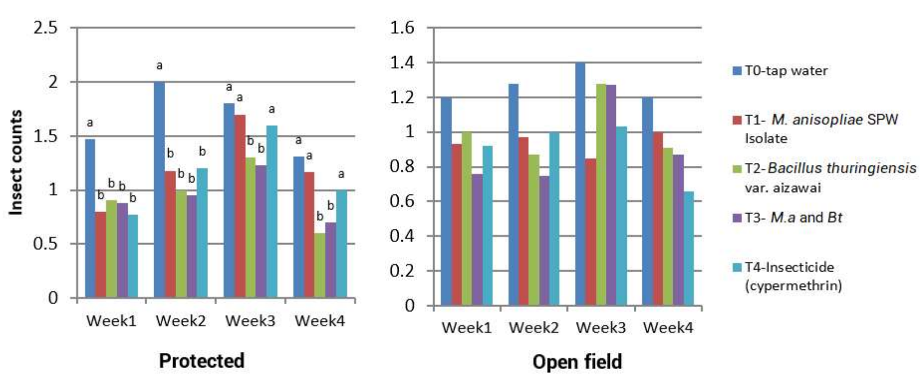

Figure 2. Mean population counts of cluster caterpillar (*Spodoptera* litura) on pechay under protected and open field cultivation

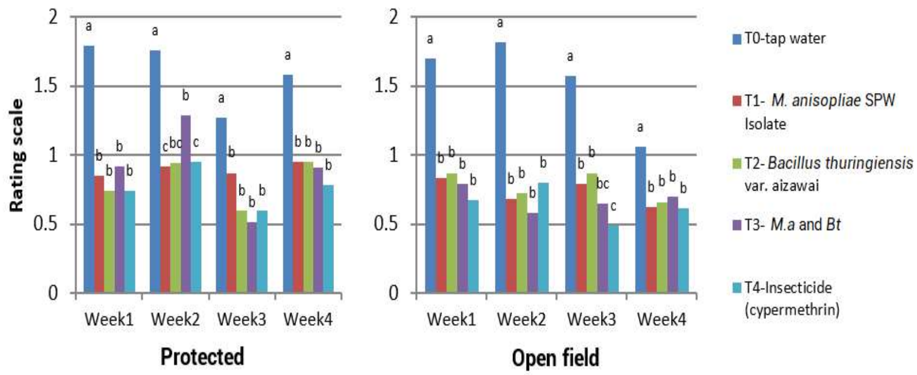

Figure 3. Mean damage rating of diamondback moth on pechay under protected and open field cultivation

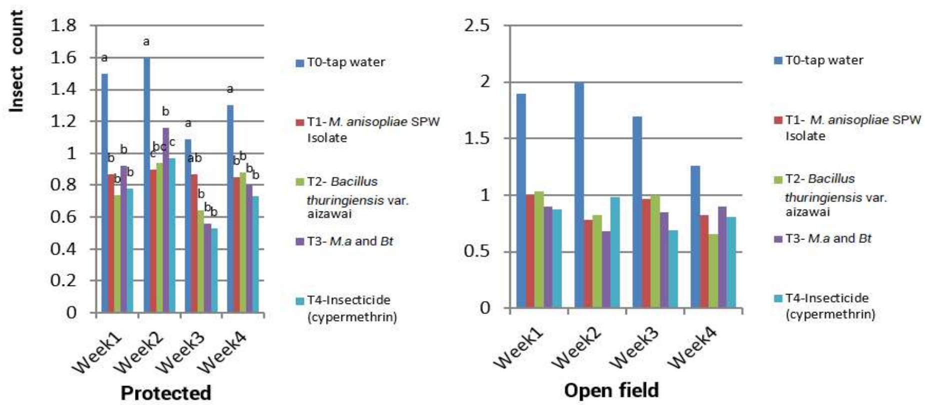

Figure 4. Mean population counts of diamondback moth on pechay under protected and open field cultivation

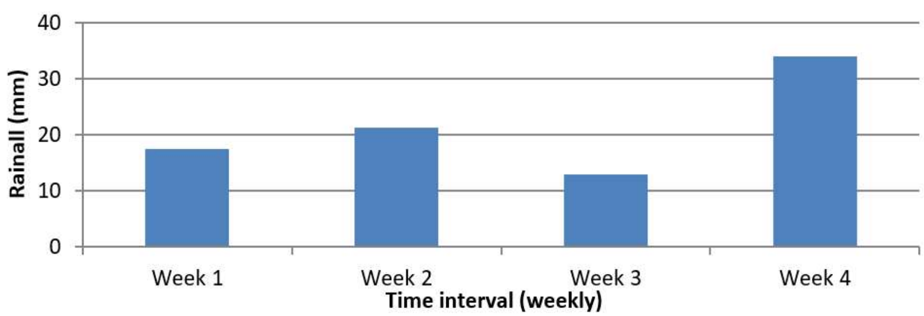

Figure 5. Weekly rainfall (mm) during the cropping period

Effects of *Metarhizium anisopliae SPW Isolate* and *Bacillus thuringiensis var. aizawai* on insect pests of lettuce

The insect infestation of lettuce was minimal compared to pechay during the cropping period. *Spodoptera litura* was the only species observed damaging lettuce. Figure 6 shows that damage ratings in all treatments were significantly lower than those in the control (T0) in both types of cultivation. This result indicated that *Ma* SPW and *Bta* were as effective as the insecticide (cypermethrin) against *Spodoptera litura*. Infected larvae were observed wherein late instars were not able to complete pupation (Figure 7).

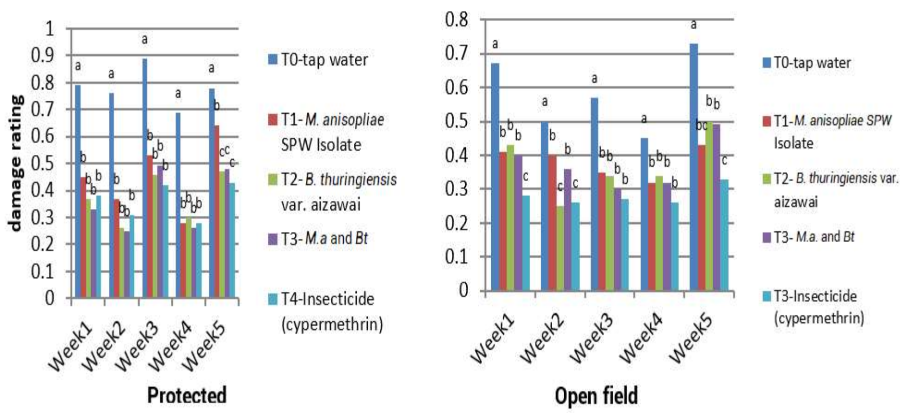

Figure 6. Mean damage ratings of cluster caterpillar (*S. litura*) on lettuce under protected and open field cultivation

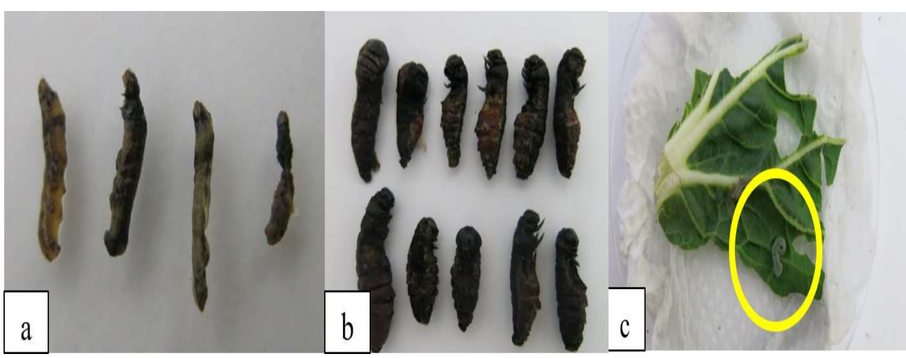

Figure 7. Infected early instar (a) and late instar (b) larvae of *S. litura* with Bta; (c) infected larva of *S. litura* with Ma SPW isolate

The effectiveness of *Bta* and *Ma* shown in this study was in consonance with some reported studies. Similar results were reported by Mau et al (2016) who investigated the effect of *Bta* against *P. xylostella* on cabbage. They found that *Bta* provided moderate levels of control which was better than the conventional insecticides used. In a related study, Wanna et al (2012) reported moderate toxicity of *Bta* against *Helicoverpa armigera* under laboratory and potted experiments.

Furthermore, Freed et al (2012) mentioned that entomopathogenic fungi are efficient tools in the biocontrol of a wide range of insect pests particularly in agricultural crops. In their investigation, *Ma* was effective against *Spodoptera exigua*.

Yield of Pechay and Lettuce as Affected by the Different Treatments Under Protected and Open Field Cultivation

Pechay grown under protected cropping had up to three times greater yield than those grown in the open field (Figure 8). The only significant treatment differences in yield were observed in plants grown under protective structures (Table 1). Plant survival ranged from 40% in the water sprayed treatments to 72% in the *Bta sprayed* treatment. Highest marketable yields by both number and weight were obtained with weekly *Bta* sprays (46, 10.3kg) or the weekly *Bta* and *Ma* sprays (43, 9.4kg) followed by *Ma* sprays alone (34, 7.3kg) and weekly cypermethrin sprays (29, 6.5kg). The water sprayed control yielded 4.3kg, less than half the yield of the *Bta* plots with lower plant survival. Lowest yield was observed in water sprayed control. However, it was noted that fewer plants in control survived as shown in Table 1 due to bacterial wilt incidence. The non-marketable plants recorded were mainly damaged by insects wherein the *Ma* (T2) and *Bta* (T3) treated plots had the lowest number and weight obtained. Results indicated that differences in yield were not only due to the effects of treatments on insect pests and uneven number of surviving plants but also on the cropping systems. The highest yields were obtained under structure (Figure 8). Plants were growing well and with greener leaves under protected cultivation than in the open field (Figure 9). This observation supported the findings of Gonzaga et al (2012) that generally vegetable crops have better performance under protective structure than in the open field particularly during the rainy season. However, in lettuce, yields were just comparable between treatments and types of cultivation which could be due to the low level of insect infestation observed. In addition, unlike pechay, lettuce plants were growing well in both types of cultivation (Figure 9) which was probably favoured by the cooler climate brought by the scattered rainfall throughout the season (Figure 5).

Table 1. Mean number and weight of marketable and non-marketable pechay plants under protected cultivation[1]

Treatment	Marketable plants			Non-marketable plants
	Number	Total Weight (kg)	Head wt/ (g)	Weight (kg)
T0-Tap water	24[b]	4.3[c]	179	4.7[a]
T1-*M. anisopliae* SPW Isolate	34[ab]	7.3[ab]	215	3.3[ab]
T2-*B. thuringiensis var. aizawai*	46[a]	10.3[a]	224	1.2[c]
T3-*M.a.* and *Bt*	43[a]	9.4[a]	219	1.7[bc]
T4-Insecticide (Cypermethrin)	29[b]	6.5[b]	224	4.8[a]
CV%	19.5	29.1		32.9

[1]based on 60 plants/replicate

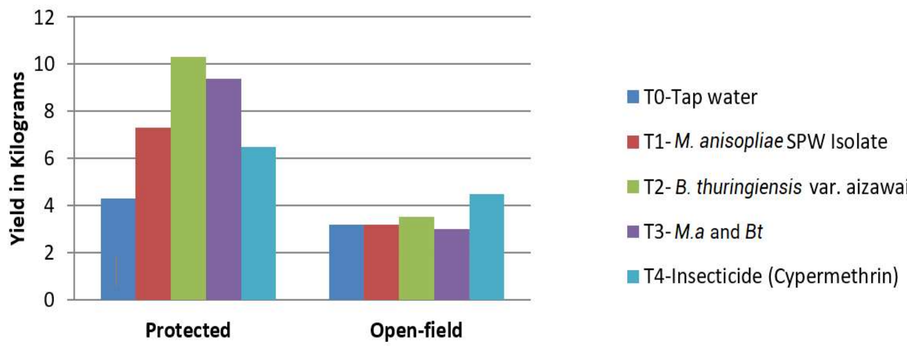

Figure 8. Mean weight of marketable pechay plants harvested under protected and open-field and protective cultivation

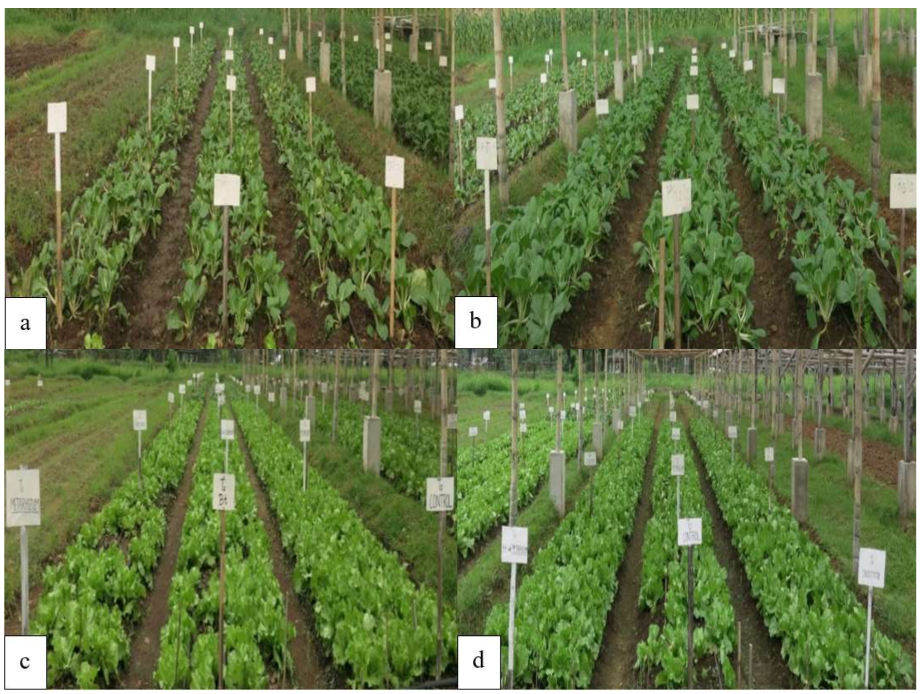

Figure 9. Experimental set-up at three weeks after transplanting: pechay open field (a) and protected (b); lettuce open field (c) and protected (d)

CONCLUSION

This study has shown that *Bta* and *Ma* SPW isolate were effective against the *S. litura* and *P. xylostella* of pechay and *S. litura* of lettuce grown under protected and open field cultivation. Applications of *Bta, Ma* and cypermethrin significantly reduced insect pest populations and damage compared to control water sprayed plants. Pechay plants applied with *Bta* and *Ma* showed higher yields than cypermethrin in both types of cultivation. These findings suggest that *Bta* and *Ma* can be used as an alternative non-chemical option for the management of *S. litura* and *P. xylostella* in pechay and lettuce. Lepidopterous pests are a problem for many vegetables in the Southern Philippines and further research to test the efficacy of *Bta* could see it as a viable alternative to broadspectrum synthetic pyrethroid insecticides. *Ma* has potentially a wider host range and efficacy research on how broad the *Ma* SPW isolate host range, including its impact on beneficial, would be helpful to determine where it fits within an integrated pest management strategy for vegetable production in the Southern Philippines. Flea beetles can be a significant pest of pechay and testing one of the *Bt* strains with coleopteran activity could see the removal of the need for any broadspectrum insecticides in the production system. In the Southern Philippines the only significant pests of lettuce are lepidopterous hence it is possible to grow lettuce without any need for broadspectrum insecticides.

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