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FIELD EFFICACY OF TWO BOTANICAL INSECTICIDE FORMULATIONS AGAINST CABBAGE INSECT PESTS, *CROCIDOLOMIA PAVONANA* (F.) (LEPIDOPTERA: PYRALIDAE) AND *PLUTELLA XYLOSTELLA* (L.) (LEPIDOPTERA: YPONOMEUTIDAE)

Dadang, Eva Dwi Fitriasari, and Djoko Prijono

Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University
Jl. Kamper, Kampus IPB Darmaga, Bogor, Indonesia 16680
Corresponding author: dadangtea@ipb.ac.id

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ABSTRACT

A field efficacy trial of two botanical insecticide formulations was conducted on cabbage crops in Bogor, West Java, Indonesia to assess the effectiveness of these formulations in reducing two major cabbage insect pest populations. The efficacy of two botanical insecticide formulations, mixtures of *Piper retrofractum* (Piperaceae) and *Annona squamosa* (Annonaceae) (RS) extracts and *Aglaia odorata* (Meliaceae) and *A. squamosa* (OS) extracts at 0.05% and 0.1% was compared to a synthetic pyrethroid, deltamethrin at 0.04% and a microbial insecticide, *Bacillus thuringiensis* at 0.15%. The application of both RS and OS formulations decreased the population of *Crocidolomia pavonana* (F.) (Lepidoptera: Pyralidae) and *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) and the treatments of RS and OS at 0.1% was more effective than deltamethrin, a synthetic insecticide. The application of RS and OS did not affect the performance of insect pest natural enemies. These botanical formulations can be used in the integrated pest management of cabbage.

Key words: *Annona squamosa, Piper retrofractum, Aglaia odorata,* safety

INTRODUCTION

The cabbage webworm, *Crocidolomia pavonana* (F.), (synonymous to *C. binotalis* Zeller) (Lepidoptera: Pyralidae) and the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) are two major insect pests of cabbage and other cruciferous crops which often cause heavy damage on cabbage crops particularly in the dry season. The common strategy adopted by farmers for insect pest management in cabbage crops and other vegetable crops such as tomato, potato, string bean, as well as broccoli is the use of synthetic insecticides which is still intensive due to its efficient, practical use, as well as effectiveness (Dadang et al., 2003a; Dadang et al., 2003b). Farmers spray synthetic insecticides 2-3 times a week and the total number of pesticide applications on cabbage cultivation in one season could reach 30-35 times (Rauf et al., 2005). Moreover, about 70% of cabbage farmers use about 25-30% of their total production input cost for pesticides.

The misuse and excessive use of synthetic insecticides may cause some undesirable effects not only to the agricultural ecosystem but also to human health due to insecticide residue in food. Insecticide residue in agricultural products particularly in vegetable and fruit products is a growing concern for producers, traders, and consumers in many parts of the world. Therefore, several efforts have been exerted to reduce the use of synthetic pesticides particularly synthetic insecticides. One of the efforts is the development of botanical insecticides as a novel and safer alternative strategy. Botanical insecticides, which contain plant extracts as active components, are safer as well as environmentally friendlier than synthetic insecticides. Therefore, this research was conducted to assess the effectiveness of two botanical insecticide formulations in reducing the major cabbage insect pests and their impact to natural enemies and cabbage crops in field trials.

MATERIALS AND METHODS

Plant Materials

The study was conducted from February to May 2008. Plant materials used in this experiment were seeds of *Annona squamosa* (Annonaceae), twigs of *Aglaia odorata* (Meliaceae), and inflorescences 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 yield a uniform size of powder which was soaked in methanol (1:10; w/v) for 48 hours. Each plant extract solution was filtered through filter paper (Whatman No. 1) and the methanol was evaporated using a rotary evaporator at 50 ºC under reduced pressure (400-450 mmHg) to produce a crude extract. Crude extracts were kept under low temperature (-4^{o}C) in the refrigerator until use.

Preparation of Extract Formulation

An appropriate amount of each crude extract was mixed to prepare extract mixtures of *P. retrofractum* and *A. squamosa* (RS*)* and *A. odorata* and *A. squamosa* (OS). The extract mixture was diluted with methanol, and then stirred until a homogeneous extract mixture was obtained. Each extract mixture solution was mixed with water, containing Agristik 400 L (a.i. alkylaryl polyglycol ether) as emulsifier and sticker, to produce the desired extract mixture concentration. The final concentrations of organic solvent (methanol) and emulsifier were 1% and 0.05%, respectively. Two extract concentrations of each formulation, 0.05% and 0.1% were applied. Water containing 1% organic solvent and 0.05% emulsifier served as a control. In order to compare the effectiveness of botanical insecticide formulations, a synthetic pyrethroid insecticide, Decis 2.5 EC (a.i. deltamethrin) and a microbial insecticide, Arial WP (a.i. *Bacillus thuringiensis*) were used and applied at recommended concentrations of 0.04% and 0.15%, respectively. All insecticides were applied using a lever operated knapsack sprayer. Applications of insecticides were done five times during the experiment with one-week interval between applications.

Cabbage Crop Management

The field application was conducted in a 1,500 m^2 area of a cabbage field in Bogor, West Java, Indonesia. Cabbage seedlings were transplanted to plots (8 m x 6 m) consisting of 12 rows of plants and each row having 12 plants, so that each plot contained 144 cabbage plants. The plots were fertilized with chicken manure applied two weeks before transplanting, at the rate of 10 ton ha $^{-1}$. Inorganic fertilizers (90 kg ha^{-1} urea, 30 kg ha^{-1} SP-36, and 30 kg ha^{-1} KCl) were applied twice, two and four weeks after transplanting. Weeds were removed mechanically, when necessary.

Field Observation

The field trials consisted of seven treatments and three replications. Ten cabbage plants in each plot were randomly selected as plant samples. The number of *C. pavonana* and *P. xylostella* larvae of each plant sample (larval density) were monitored weekly. The first and second observations were conducted before the first application of insecticides, while the third to seventh observations were conducted a week after each insecticide application. The effect of the application of insecticides on natural enemies, especially parasitoids, was evaluated by collecting 10 larvae of *P. xylostella* or *C. pavonana* from each plot at 7, 8, 9, and 10 WAT. All collected larvae were reared and fed with insecticide-free cabbage leaves in the laboratory until adult emergence. The percent parasitism was calculated by the following formula:

$$\text{Percent parasitism} = \frac{\text{Number of parasitized larvae}}{\text{Number of collected larvae}} \times 100\,\%$$

Other related data were collected such as cabbage yield and phytotoxic effect on cabbage crops. The experiment was arranged based on a complete randomized block design with seven treatments and three replications. The data obtained were analyzed by analysis of variance. The comparison of means of larval population and percent parasitism were done using Duncan Multiple Random Test at 0.05 level (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Plutella xylostella larval population.

Before the first application of insecticides, the population of *P. xylostella* larvae was monitored weekly starting 5 weeks after transplanting (WAT). The average *P. xylostella* larval populations were 0.06-0.37 and 0.06-0.33 larvae plant^{-1} at five and six WAT, respectively.

After the first application of insecticides, the population of *P. xylostella* increased slightly on RS 0.1% (0 to 0.05 larvae plant^{-1}) but decreased on *B. thuringiensis* treatment (0.33 to 0.11 larvae plant^{-1}). Other treatments, OS 0.05% and 0.1%, were able to maintain the larval population on the same level before and after insecticide application. The deltamethrin treatment showed highly increased larval population from 0.16 to 0.31 larvae plant^{-1} (Fig. 1).

After the second application, the larval population on three treatments, RS 0.1%, OS 0.1%, and *Bacillus thuringiensis* increased slightly, but treatments of RS 0.05%, OS 0.05%, deltamethrin, and control increased highly (Fig. 1). The increase of larval population might be caused by new infestation of *P. xylostella* from neighboring cabbage crop areas. In addition, the peak population of *P. xylostella* occurred on the eighth week after transplanting. This phenomenon might be correlated with the increasing larval population in all treatments.

The larval population decreased in all treatments starting on the ninth week but two treatments, deltamethrin and control, showed higher populations than the other treatments. These indicate that treatments with botanical insecticides (RS and OS particularly at 0.1%) were more effective than deltamethrin. In addition, the RS formulation showed better efficacy than the OS formulation.

***Crocidolomia pavonana* larval population**

The average larval populations of *C. pavonana* at 5 and 6 WAT were 0.17-0.83 and 0.05-2.23 larvae plant^{-1}, respectively. After the first application, all treatments decreased the larval population except deltamethrin treatment and control. The larval population increased from 0.05 to 0.78 larvae plant^{-1} and from 0.11 to 2.06 in the treatments of deltamethrin and control, respectively (Fig. 2).

The larval populations treated with RS 0.1%, OS 0.1% and *B. thuringiensis* at 8 to 11 WAT were consistently low and the larval populations treated with deltamethrin and control were consistently high. It indicates that the two botanical insecticides were more effective against *C. pavonana* than deltamethrin and was comparable to *B. thuringiensis*.

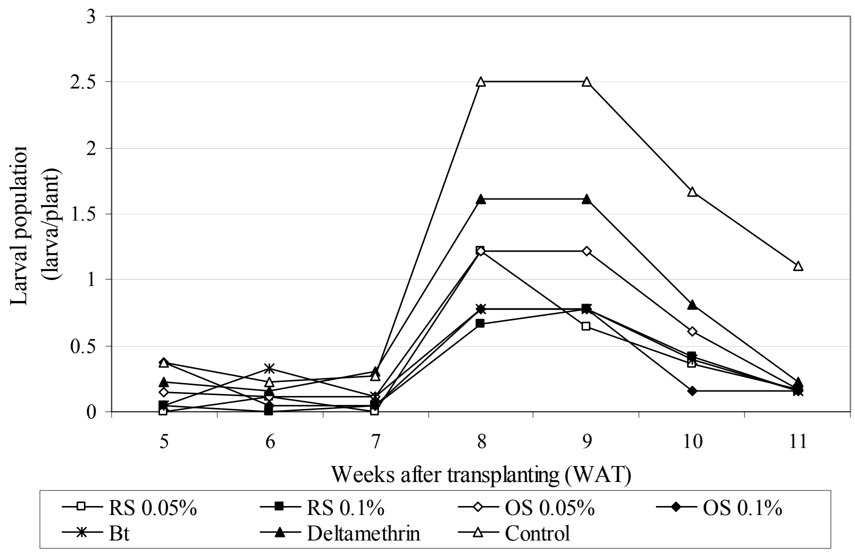

Fig. 1. Development of *P. xylostella* larval population on cabbage crops treated with botanical, synthetic, and microbial insecticides

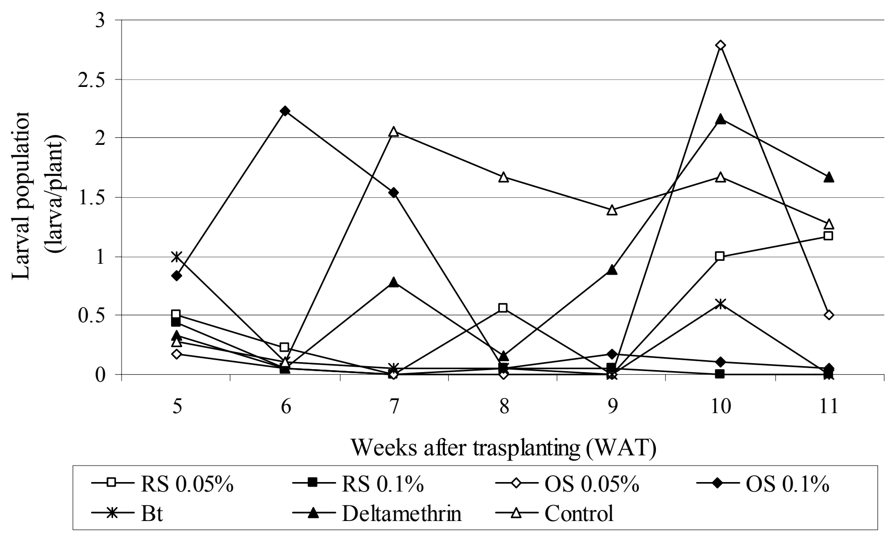

Fig. 2. Development of *C. pavonana* larval population on cabbage crops treated with botanical, synthetic, and microbial insecticides

The effect of two botanical insecticide formulations on *P. xylostella* parasitoid

The treatments of the two botanical insecticide formulations did not affect the performance of *Diadegma semiclausum* (Hymenoptera: Ichneumonidae), a parasitoid of *P. xylostella*. The parasitism level in the two botanical insecticide formulation treatments were higher than control and deltamethrin (Fig. 3). The percent parasitism on RS 0.1% and OS 0.1% at 7, 8, 9, and 10 WAT ranged from30% to 83%. Meanwhile, the percent parasitism on deltamethrin ranged from 0% to 50%. The application of RS and OS formulation are safe for the *D. semiclausum* parasitoid.

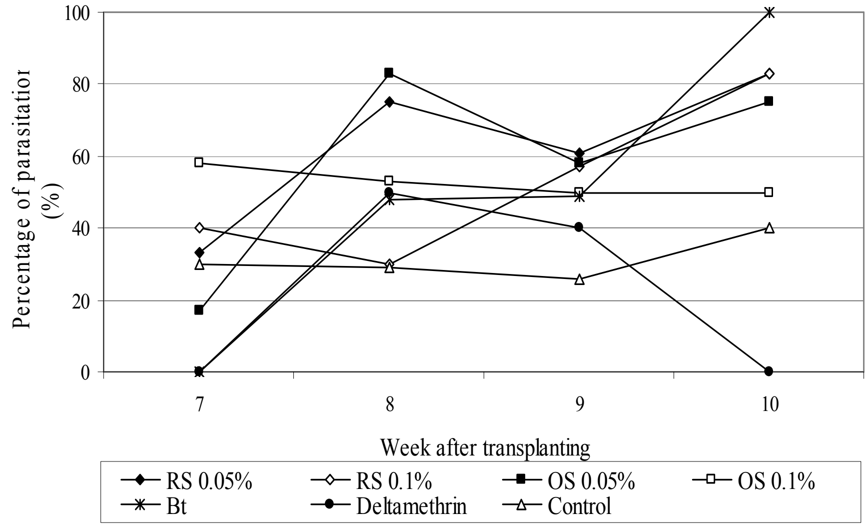

Fig. 3. Development of percentages of parasitism of *D. semiclausum* on cabbage crops treated with botanical, synthetic, and microbial insecticides

The effect of two botanical insecticide formulations on *C. pavonana* parasitoid

The RS 0.1% formulation showed higher percent parasitism than control and deltamethrin but the OS 0.1% formulation showed lower percent parasitism. The percent parasitism in RS 0.1% treatment at 7, 8, 9, and 10 WAT ranged from 25% to 75% while percentage parasitism of OS 0.1% treatment ranged from 0% to 44%. Meanwhile, the percent parasitism in the deltamethrin treatment ranged fro 0% to 75%. This result indicates that the application of the RS formulation is safe for *Eriborus argentiopilosus* (Hymenoptera: Ichneumonidae), a parasitoid of *C. pavonana*, however, the OS formulation affected slightly the performance of the parasitoid (Fig. 4).

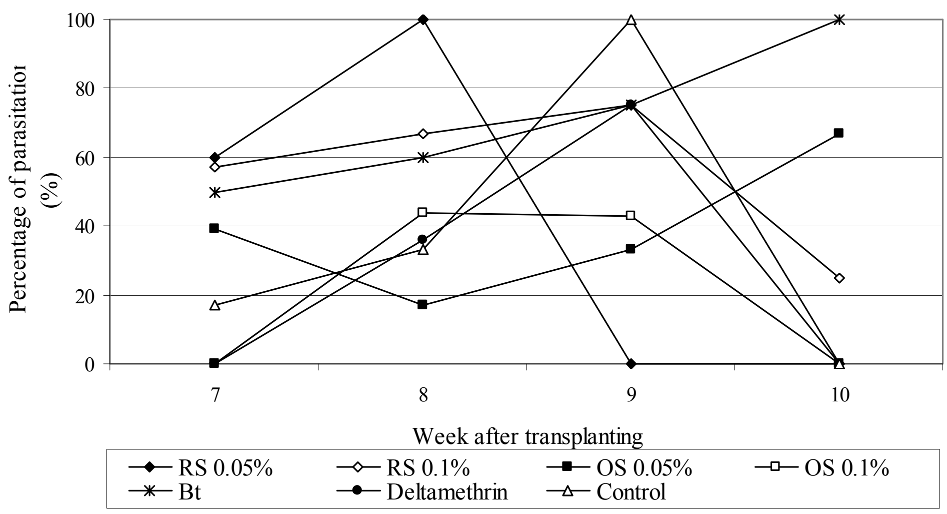

Fig. 4. Development of percentages of parasitism of *E. argentiopilosus* on cabbage crops treated with botanical, synthetic, and microbial insecticides

Cabbage damage and cabbage yield

The intensity of cabbage damage on all insecticide treatments except OS 0.05% treatment was lower than control (Table 1). The intensity of the cabbage damage on control compared to RS 0.05%, OS 0.05% and deltamethrin treatments showed non-significant difference and when compared to RS 0.1%, OS 0.1%, and *B. thuringiensis* treatments showed significant difference. RS 0.1%, OS 0.1%, and *B. thuringiensis* treatments reduced intensity of damage caused by *C. pavonana*. Among RS 0.1%, OS 0.1%, and *B. thuringiensis* treatments, intensity of cabbage damage on RS 0.1% and *B. thuringiensis* treatments were similar but was lower than OS 0.1% treatment.

The RS 0.1% treatment produced the highest average cabbage yield followed by deltamethrin and *B. thuringiensis* treatments, while RS 0.05% treatment produced lowest yield (Table 1). There was an inconsistent correlation between intensity of cabbage damage and yield. Intensity of cabbage damage on RS 0.05 treatment was lower than deltamethrin treatment but the yield in the deltamethrin treatment was higher than RS 0.05% treatment.

Table 1. Averages of intensity of cabbage damage and cabbage yield.

Treatment	Intensity of damage (%) ± sd	Yield (kg) ± sd[1]
RS 0.05%	10.4 ± 1.2^{ab}	$32.0 \pm 6.9b^{2)}$
RS 0.1%	6.3 ± 2.7^{b}	69.0 ± 4.8^{a}
OS 0.05%	12.5 ± 1.0^{ab}	55.5 ± 7.2^{a}
OS 0.1%	8.9 ± 3.2^{b}	43.2 ± 1.4^{b}
B. thuringiensis	6.3 ± 3.6^{b}	60.5 ± 2.9^{a}
Deltamethrin	11.0 ± 3.6^{a}	62.4 ± 5.2^{a}
Control	14.0 ± 2.0^{a}	48.0 ± 6.6^{b}

1 Standard deviation

2 Averages in the same column followed by the same letter are not significantly different by DMRT at α=0.05

Two botanical insecticide formulations containing extract mixtures of *P. retrofractum* and *A. squamosa* (RS) and *A. odorata* and *A. squamosa* (OS) have been tested on cabbage crops in order to evaluate the effectiveness of formulations in reducing *P. xylostella* and *C. pavonana* larval population. Overall, these two formulations at 0.1% were highly effective and more effective than deltamethrin, a synthetic pyrethroid insecticide. RS 0.1% was slightly more effective against *P. xylostella* than *C. pavonana*, while effectiveness of OS 0.1% was similar to both *P. xylostella* and *C. pavonana* larvae. These results indicate that these two botanical insecticide formulations have high potential for development as commercial products.

In single assays, the seed extract of *A. squamosa* exhibited high insecticidal activity against *C. pavonana* larva with LC_{50} was 0.208% (Basana and Prijono, 1994) and at the range of concentration 0.063-0.1% was effective to *P. xylostella* larvae (Istiaji, 1998). Acetogenin compounds such as annonin I and squamosin isolated from seed of *A. squamosa* were responsible for mortality activity of *A. squamosa*. These compounds together with asimisin were very toxic against *P. xylostella* (Ohsawa and Dadang, 1998). Acetogenin will inhibit conversion ADP to ATP in the ion transport system (Gu *et al*., 1975).

The *P. retrofractum* extract has been demonstrated to be insecticidal against *P. xylostella* and *C. pavonana* (Dadang, 1999; Dadang *et al*., 2007). The *P. retrofractum* extract at 0.5% gave 100% mortality against instar II of *C. pavonana* (Prijono *et al*., 2006). This extract also showed high knock down effect against several test insects including *P. xylostella* and *C. pavonana,* as well as *Forficula auricularia* (Dermaptera: Forficulidae), *Culex quinguefasciatus* and *Aedes aegypti* (Diptera: Culicidae), and *Coptotermes gestroi* (Isoptera: Rhinotermitidae) (Assabgui et al., 1995; Chansang et al., 2005; Alfian, 2007). Piperamide compounds which have isobutylamide and methylendioxyphenyl moieties have strong insecticidal activity by inhibiting impulse current on the axon of the nerve system (Miyakado et al., 1989).

The A. odorata extract was noted to be effective against several agricultural insect pests including *P. xylostella* and *C. pavonana* larvae. The extract of *A. odorata* at 1.0% produced 92% larval mortality of C. pavonana (Dadang et al., 2007). Treatment of the ethanol twig extract of A. odorata caused 100% mortality to *Spodoptera litura* (Lepidoptera: Noctuidae) (Koul et al., 1997). Nugroho (1999) isolated six rocaglamide derivate compounds from *A. odorata*, which were responsible for feeding inhibition, mortality, and growth regulatory activity against *Spodoptera littoralis*. Rocaglamide at 80 ppm caused 90% mortality of *C. pavonana* larvae (Sudarmo, 2001). In addition, rocaglamide has high toxicity activity against *S. litura.* indicated by LC_{50} and LC_{90} values of 4.8 ppm and 8.76 ppm, respectively (Janprasert et al., 1993).

Based on a single assay of extract and mode of action, it seems that each extract works on different target site. The combination of each extract in formulation will produce a good botanical insecticide formulation. Dadang et al. (2007) reported the strong efficacy of extract mixture of *P. retrofractum* and *A. squamosa* and *A. odorata* and *A. squamosa*. The extract mixture of *P. retrofractum* and *A. squamosa* and *A. odorata* and *A. squamosa* produced 100% and 94% mortality when *C. pavonana* larva were treated with 0.05% extract mixture at 48 hours after treatment. Extract mixture of *A. odorata* and *A. squamosa* gave a synergistic combination with multiple actions, as feeding inhibition and insecticidal activity (Dadang et al., 2007).

Generally, two effective botanical insecticide formulations did not affect the performance of both parasitoids *D. semiclausum* and *E. argentiopilosus* on cabbage crops. Istiaji (1998) mentioned that the increasing of *A. squamosa* extract concentration increased the sensitivity of *D. semiclausum* but did not affect the development and parasitism level of *D. semiclausum*. In the field test, treatment of *A. squamosa* extract did not affect the parasitism level of *D. semiclausum* (Nurmayanti, 1998). *A. odorata* is highly toxic against *C. pavonana* and *P. xylostella* but not against *E. argentiopilosus*. *A. odorata* extract works on insects as stomach poison and not as a contact poison due to the weak penetration ability of the active compound on the insect integument.

Moreover, *A. odorata* has no repellent effect to the parasitoid, *E. argentiopilosus* adults so application of this extract did not affect the parasitoid activity in terms of visiting cabbage crops (Sudarmo, 2001). It means that these botanical insecticide formulations is safe for field application and can be combined with the use of natural enemies in cabbage insect pest management program due to the selectivity in action. This result also proved that the application of deltamethrin affected the parasitoid population. Deltamethrin is a broad spectrum and unselective pyrethroid insecticide (Undiarto and Sastrosiswojo, 1997).

The RS 0.1% treatment gave the lowest intensity of cabbage damage and produced highest cabbage production. Treatment of OS 0.1% also gave low intensity of cabbage damage but produced lower cabbage production than deltamethrin treatment. The low cabbage production may be caused by *A. odorata* extract. Sometimes, the application of *A. odorata* causes phytotoxicity to the crops and inhibites plant growth. This phenomenon should be evaluated. It gives experience to us to evaluate the whole aspect before botanical insecticide formulations can be commercialized.

Based on the plant chemical natures in term of the concentration of active compounds that strongly influenced by geographic and climate conditions, the availability of botanical insecticide formulation in market is necessary. It will assure the quality of botanical insecticide formulation. Moreover, the formulations should contain two or more plant extracts to make the formulation more efficient in using plant materials, more economic in extract and formulation preparations, and more effective in toxicity and delay the development of insect resistance (Dadang et al., 2008). Another important matter to consider is the application of botanical insecticide formulation should be compatible to other integrated pest management strategies.

CONCLUSION

Two botanical insecticide formulations containing extrac mixtures of *Piper retrofractum* and *Annona squamosa* (RS) and *Aglaia odorata* and *A. squamosa* (OS) showed high effectiveness and more effective than synthetic pyrethroid. Between two formulations, RS 0.1% was more effective to *P. xylostella* than *C. pavonana*, while OS 0.1% gave the same effectiveness to both *P. xylostella* and *C. pavonana* larva. These formulations did not affect the performance of both parasitoids, *D. semiclausum* and *E. argentiopilosus* on cabbage crops. Treatments with RS 0.1% and OS 0.1% resulted in significant difference in reducing cabbage damage compared to the synthetic pyrethroid insecticide, deltamethrin treatment. In addition, treatment with RS 0.1% produced highest cabbage yield. The application of RS and OS did not affect the performance of insect pest natural enemies. These botanical formulations can be used in the integrated pest management of cabbage.

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