Safety and effectiveness of mixed plant extracts formulation against cabbages pests under field conditions

Eka Candra Lina[1,*], Dadang[2], Syafrida Manuwoto[2] and Gustini Syahbirin[3]

ABSTRACT

This study was carried out to determine the persistency and safety of emulsifiable concentrate (EC) and wettable powder (WP) formulations of *Tephrosia vogelii* and *Piper aduncum* (1:5) against natural enemies and broccoli leaves (phytotoxic effects) as well as to assess their effectiveness against *Crocidolomia pavonana* in field. EC and WP formulations were safe to parasitoid *Eriborus argenteopilosus* and did not cause any phytotoxic effects on broccoli leaves. Persistency of EC and WP formulations were lower than bio-insecticide *Bacillus thuringiensis* (BT) and sythetic pesticide with active ingredient deltametrin. The mortality of *C. pavonana* treated with EC and WP formulations reached 100%, while BT and deltametrin only reached 73.33% and 88.33% respectively, at the first day treatment. The effectiveness of extracts were declined sharply at one day after treatment indicating low persistence of formulation. In field, EC and WP formulations suppressed the population of *C. pavonana* more than 80% compared to control. Both formulations showed better activity comp d to synthetic insecticide deltametrin.

Keywords: *Eriborus argenteopilosus*, field, parasitoids, persistency, phytotoxic.

MS History: 22.12.2016 (Received)-10.03.2017 (Revised)- 14.04.2017 (Accepted)

Citation: Eka Candra Lina, Dadang, Syafrida Manuwoto and Gustini Syahbirin. 2017. Safety and effectiveness of mixed plant extracts formulation against cabbages pests under field conditions. *Journal of Biopesticides*, **10**(1): 25-34.

INTRODUCTION

Crocidolomia pavonana (F.) (Lepidoptera: Crambidae) and *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) are main pests on cabbage plantations. *Plutella xylostella* has been reported effectively controlled by parasitoid *Diadegma semiclausum* (Bakri, 2015). Otherwise *C. pavonana* could not effectively controlled by it's parasitoid *Eriborus argenteopilosus*. Dono (2004) reported that *C. pavonana* has the ability to encapsulate parasitoid's egg or larvae. Furthermore, the use of synthetic pesticides to control *C. pavonana* may kill *D. semiclausum* because of pesticide residues. Both pests caused dilemna in field control effort.

Farmers still rely on synthetic pesticides to control *C. pavonana* and *P. xylostella*. Unfortunately, it many negative effects, including resistance and resurgence of target pests, mortality of natural enemies and non target organism, environmental pollution, and hazards of pesticide residues (Metcalf, 1982). Insecticides such as DDT and BHC can stimulate the development of human cancer cells, i.e. skin cancer, liver cancer, lung cancer, and cancer of the lymph (Matsumura, 1985; Kuroki, 1998). All those issues have been encouraging people to seek alternatives of pest control in order to meet the demands of consumers for free pesticide residue on vegetable products, either for domestic or export demands, and for local or foreign consumers. Botanical insecticides have a few demerits when compared to the synthetic insecticides such as easier as well as faster to decompose, and compatible with other integrated pest control techniques (Isman, 1995; Schmutterer, 1997).

Laboratory tests for mixture extracts formulations of *T. vogelii* and *P. aduncum* (1:5) showed that both formulations (EC/WP) have a good activity as insecticide against *C. pavonana* (Lina *et al.,* 2013, 2014). However, formulations effectiveness in field need to be assessed further. Prior to its application in field, persistency, safety to natural enemies, and phytotoxicity level of the formulations need to be evaluated and compared to other formulations.

This study was conducted to evaluate the persistance of EC and WP formulations of *T. vogelii* and *P. aduncum* (1:5) on broccoli plants and to find whether the formulations were safe against natural enemies' parasitoid *Eriborus argenteopilosus*, as well as to assess the effectiveness of mixed formulations against pests, *C. pavonana* and *P. xylostella* in field.

MATERIALS AND METHODS

The research was carried out in the laboratory of Physiology and Toxicology of Insects, at the Plant Protection Department, Faculty of Agriculture, Bogor Agricultural University and at the center of cabbage plantation in Mega Mendung Bogor from October 2013 to February 2014.

Extraction of Plant Materials

Plant materials used as the source of extract were leaves of *Tephrosia vogelii* with purple flower from Agropolitan area in Pacet sub district, Cianjur District, West Java Province and fruits of *Piper aduncum* from campus area of Bogor Agricultural University in Darmaga, Bogor. Leaves of *T. vogelii* and fruits of *Piper aduncum* were cut into small pieces then dried for week before milled into powder using a blender was sifted using a wire sieve (0.5 mm). The powder of *T. vogelii* (500 g) and *P. aduncum* (1000 g) were soaked in 5 and 10 L of ethyl acetate, respectively at least for 24 hrs. The liquid of marinade was filtered using glass funnel covered with filter paper Whatman No. 41 diameter 185 mm. The liquid was evaporated using a rotary evaporator at 50 °C and 240 mbar of pressure to become crude extract. Extract of *T. vogelii* showed a dark green liquid and *P. aduncum* showed brown-little greasy materials. Each extract was stored in the refrigerator (± 4 ° C) until used.

Production of the Formulations

Crude extract of *P. aduncum* (100 g) and *T. vogelii* (20 g) were prepared, then formulated as emulsifiable concentrate (EC) and wettable powder (WP) for field application (Lina *et al.,* 2017). Both formulations (EC: emulsifiable concentrate) and (WP: wettable powder), contain a 20% concentration of mixture extracts of *T. vogelii* and *P. aduncum* (1:5). Formulation of 20 EC was made separately by mixing the extract or active faction with best adhesive and solvent methanol by volume proportion of 20%, 10%, and 70%, repectively. Formulation of 20 WP was made by mixing the extract with the best adhesive and carrier materials kaolin by weight proportion of 20%, 10%, and 70%, respectively.

Safety test of formulations against natural enemy

Imago of *E. argenteopilosus* used in this test were produced in laboratory using a procedure from Dono (2004). Testing was performed by using a thin layer of residue on the glass surface against males and females of parasitoids imago. concentration of the formulation was 2 x LC_{95} (lethal concentration), based on result test against *C. pavonana*. testing conducted for the EC and WP formulations, EC and WP controls, and each treatment was done with ten replicates for parasitoid imago, males and females. materials preparation for the tests was conducted in the same way as in the toxicity test against larvae *C. pavonana* (Lina, 20014). Each lliquid formulation was put into a glass tube and leveled over the entire surface of the tube by turning it slowly. Parasitoid imago inserted into the tube after the formulation in the tube surface became dry. The observation was conducted in 24 hours after treatment and the number of dead parasitoids was recorded.

Residual effects of formulations on the plant leaves against the parasititation behavior of parasitoids was tested by cutting broccoli leaves in size of 6 cm x 6 cm and leaving the bottom leaves bones as a stalk. Leaves were placed in the bottle containing water to keep leaves fresh. The leaves were sprayed with EC and WP formulations, with equivalent concentration of 2 x LC_{95}. For control, leaves were sprayed with formulation without active ingredients of extract. Once dried, leaves were invested with 10 larvae of *C. pavonana*, then larvae were placed in a plastic cage with gauze window. The female parasitoids that had already mated with males for 24 hrs were released into the cage. After 10 minutes, parasitoid larvae were removed from the cage and were reared to see their parasititation behaviour. Each treatment was repeated three times.

Residual effects of formulation of residue on the host's body of parasitoids against the survival ability of parasitoid larvae was tested by dipping the broccoli leaves into extract solution equivalent with LC_{50}. The treated leaves were put in the petri dish containing of tissue paper and ten larvae of second instar *C. pavonana*. The larvae which had eaten the treated leaves during 24 hrs were later parasited to female of imago *E. argenteopilosus* one by one. After three days the larvae dissected to see parasitoid larvae inside. The treatment was repeated three times.

Persistency of Formulations Test

Experiment was arranged using random block design with five treatments and five replications. They were: (1) 20 EC formulation, (2) 20 WP formulation, (3) insecticide BT, *Bachillus thuringiensis* (Bactospeine WP), (4) control of EC, and (5) control of WP. Each formulation was tested at concentrations 2 x LC_{95} based on laboratory toxicity test result to consider the possibility of degradation by environmental factors.

Each treatment was prepared separately by diluted formulation with water as recomended concentration. Each treatment and control sprayed at broccoli plant using a hand sprayer. Broccoli leaves taken from each plant on 0, 1, and 2-days after spraying, then cuted into small parts and in a petri dish (9 cm diameter) with the same method as in laboratory toxicity tests. Fiftteen second instar larvae of *C. pavonana* were put in each petri dish. Observations of larval mortality was conducted at 72 hrs since the beginning of the treatment. Larval mortality data was processed with varians analysis using the SAS program (SAS Institute, 1990), followed by Duncan's multiple range test on the real level of 5% for knowing the difference in mean value between the treatments (Steel and Torrie, 1993).

Phytotoxicity Formulation Test

Phytotoxicity test was conducted following the procedure of Dono (2004). Broccoli plants were grown in polybags and used as plant experiment after were 1.5 months old. Treatments were using hand sprayer i.e. EC formulation, WP formulation (2 x LC_{95}), and control. Each treatment was repeated five times and all treatment plants were put on green house which was exposed to the sun but protected from rain. Observations were made on five days after treatment including the measurement of leaf necrosis surface area (phytotoxic). Leaf necrosis extent was converted square centimetres (cm^2). Phytotoxic data was analyzed using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) at α=5% (Steel and Torrie, 1993).

Efficacy Trials in the field

Field experiment was done in land comprising 30 plots, each plot having 4 beds (1 m x 4 m). Experimental plots were arranged in randomize block design with six replications. Processing and maintenance were done following the method of local farmers. Broccoli seeds (3 weeks of age) were planted in each bed with space 70 x 50 cm, and each plot experiment contains 60 broccoli plants.

Five field ttreatments are EC and wettable powder formulation of mixture extract of *P. aduncum* and *T. vogelii* (1:5), biopesticide *B. thuringiensis* (BT), a synthetic insecticide deltametrin, and control. A concentration of botanical insecticides for field aplication was determined based on the results of laboratory toxicity tests (equivalent to 2 x LC_{95}), whereas the comparison of insecticide according to dosage recommendations. Insecticide formulation was diluted with water to get the right concentration. The treatment for the first and the second week was approximately 2 L of liquid spray / treatment. The third week to the fifth requires three litres of spray liquids/treatment, and the sixth week to ninth week requires five litres of spray liquids / treatment.

Population of *C. pavonana* larvae was observed on six sample plants from each experimental plot except border plant. First spraying for all treatment was determined by action threshold level (≥ 3 cluster of egg of *C. pavonana* / 10 plants). Next treatments were done once a week. Parasitisation of *C. pavonana* was recorded by taking a 20 larvae from each plots. Larvae was brought to the laboratory and then dissected to observe the presence of parasitoid.

Observations were made on (1) populations of *C. pavonana* larvae, (2) populations of *P. xylostella* larvae, (3) population of *Helula undalis* larvae, and (4) the parasitisation of *C. pavonana* larvae by *E. argenteopilosus*. Data were analyzed with varians analysis followed by DMRT on the real level of 5% for knowing the difference value of mean between the treatments (Steel and Torrie, 1993). The effectiveness of the insecticide in field was calculated by Abbot formula if observations on the pest population (before application) did not differ markedly between the plot of treatment.

$$EI = \frac{Ca - Ta}{Ca} \, x \, 100$$

Where

EI = the effectiveness of insecticides tested (%)

Ca = target pest populations on control plots after insecticide application

Ta = target pests on a swath of treatment after the application of insecticide

RESULTS AND DISCUSSION

Formulation safety against natural enemy

Formulation of EC and WP of mixture extract *T. vogelii* and *P. aduncum* (1:5) did not cause mortality against *E. argenteopilosus* males and females (Table 1). An active ingredient of *T. vogelii* and *P. aduncum* have a specific characteristic and relatively safe against natural enemy such as *E. argenteopilosus*. Syahputra (2005) had stated that the plants secondary compounds were more toxic when applied through the food rather than applied by contact.

The mixture extract of *T. vogelii* and *P. aduncum* (1:5) tested on *C. pavonana* larvae with equivalent concentration of LC_{50} gave the result that 65.78% of treated larvae was parasited by parasitoid whereas control only parasited 55.56%. Visual observations against parasitisation behavior of *E. argenteopilosus* showed that parasitoids had no differences in behavior of parasitisation to the treated and untreated of *C. pavonana*. The number of parasitoid imago hatched at the treatment of WP formulations was 26.66%, EC formulations was 40%, and control 13%.

Table 1. Safety test of EC and WP formulations against natural enemy *Eriborus argenteopilosus*

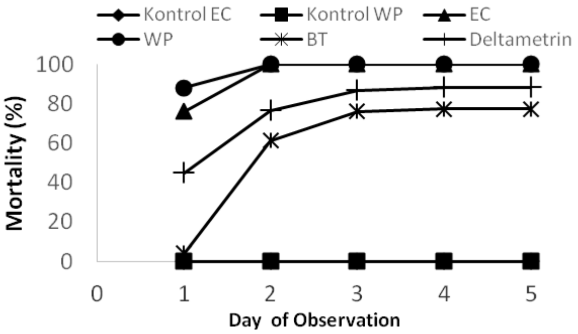
Treatment (N=10)	Time (hours)	Sex	Mortality (%)
Control	24	Male	0
		Female	0
	48	Male	0
		Female	0
EC	24	Male	0
		Female	0
	48	Male	0
		Female	0
WP	24	Male	0
		Female	0
	48	Male	0
		Female	0

The persistency of formulations

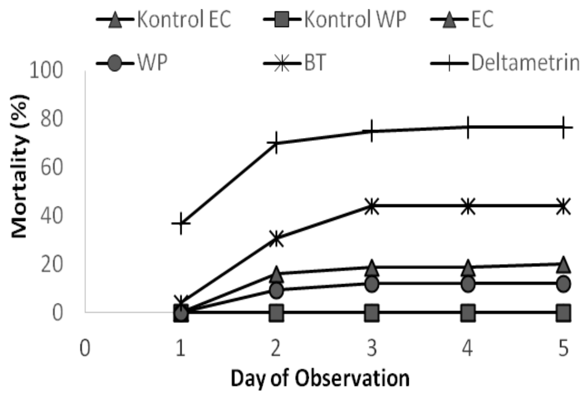
The persistencyy of EC, WP, commercial insecticide *Bacillus thuringiensis* (BT), and chemical pesticide Deltametrin (Decis 25 EC) presented in Fig.1. Observations at zero days after treatment showed mortality of larvae *C. pavonana* reached 100%, 100%, 88%, 33% and 77.33% because of the residue of formulations EC, WP, Deltametrin, and BT, respectively. Formulation of mixture extract of *T. vogelii* and *P. aduncum* (1:5) killed *C. pavonana* larvae more effectively than commercial insecticide BT and chemical pesticide (Deltametrin).

Residue of emulsifiable concentrate, WP, and BT formulations significantly decreased on the first day after treatment, but was not so with Deltametrin as shown in Figure 2. The activity of Deltametrin residual still killed 76.67% larvae of *C. pavonana* and the activity of BT residual killed 44% larvae of *C. pavonana* followed by EC formulation 20% and WP formulation 12%. Observation conducted on two days after treatment showed that the residual effect of emulsifiable concentrate and WP formulations decreased sharply in killing larvae of *C. pavonana*. Residue of EC and WP only killed 1.33% and 4% of larvae, respectively while Deltametrin and BT still killed 96.67% and 30.66% of larvae, respectively. Februlita (2013) had mentioned that the extract of *P. aduncum* from seven different locations had killed 100% larvae of *C. pavonana* in laboratory but the persistency of each extract was very low. They only killed less than 15% larvae of *C. pavonana* on the first day after treatment.

a



b



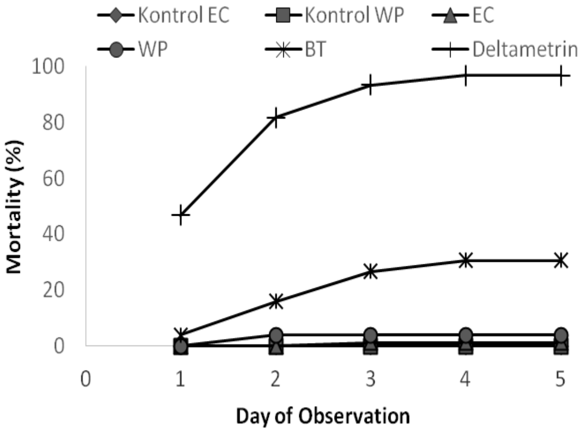


Fig. 1. The persistency of EC, WP, BT an Deltametrin formulations at 0 day (A), 1 day (B), and (C) 2 days after treatment

The ability of the formulation residues to persist in the environment after spraying is influenced by many factors, i.e microorganism activities, chemical reactions, and photodegradation, while residue spread is influenced by the flow of water and air. Sunlight as the main cause in the decomposition of active ingredients of the plants compounds (Matsumura, 1985) was a consideration in the selection and application of type of insecticides (Hassall, 1987). Speight *et al.* (1998) confirmed further that chemical components, concentration, as well as time and technology of the application of insecticide as considerations to increase the efficiency of the use of insecticides.

Time for the application of formulations plays an important role for the effectiveness of controlling target pests. Suitable times for emulsifiable concentrate and WP formulations application were found to be in the morning or in the afternoon when ultaviolet (UV) rays are at a low level. The suitable time aims is chosen so as to reduce decomposition of active ingredients by UV.

A research conducted by Scott *et al.* (2004) showed the piperamid decomposition under sunlight needs 6 hrs while decomposition under ultraviolet (UV) light needs 39 minutes only. This reveals that decomposition caused by the sunlight/photolysist. The use of piperamid is recommended for products under storage or for plants in the garden rather than in the open landscape.

Phytotoxic effect on broccoli leaves

Phytotoxic tests showed that emulsifiable concentrate and wettable powder formulations of mixed extracts of *T. vogelii* and *P. aduncum* (1:5) did not cause any necrotic symptoms on the broccoli leaves (Figure 2). This indicates that the formulations are safe to use as an alternative pest control of *C. pavonana* in the field.

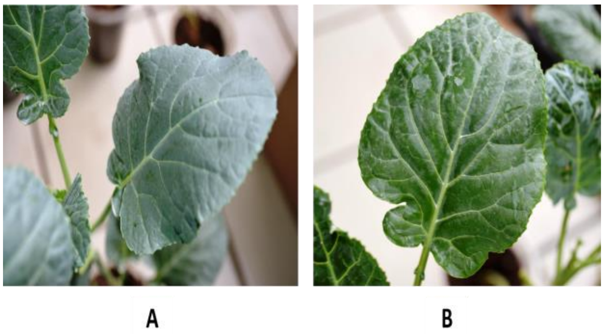


Fig. 2. Application of A) EC formulation, B) WP formulation on broccoli leaves did not show phytotoxic symptoms

Efficacy of formulations in field

Observations on the larvae population of *C. pavonana* at different treatments are shown in Fig. 3. In general, population of untreated pests was higher compared to treated pests. Larvae population started to increase at 2 weeks after planting (WAP) when the eggs laid in the first week hatched in the second week. The highest population found at 4 WAP then declined at 5 and 6 WAP. At 7 and 8 WAP, the population increased followed by a significant decreased at 9 WAP. At the highest population, the number of *C. pavonana* reached 8.5 larvae/plant.

The increasing of pest population at 28 days after planting (DAP) was due to climate changing factors, where there was low rainfall and interspersed heat at 7 until 21 days after planting (Fig. 3). Heavy rain over two days occured at 4 WAP causing declining of pest population at 35 and 42 days after planting. At 49 and 56 days after planting, the larvae population increased and started to decline entering harvest time at 63 days after planting. Population of larvae *C. pavonana* treated by EC and wettable powder formulations was below its control. WP formulation treatment showed an equal result with BT insecticide in repressing the population of larvae *C. pavonana*. Emulsifiable concentrate formulation showed a lower activity rather than wettable powder and BT formulation but it was better than the activity of chemical insecticides deltametrin (3).

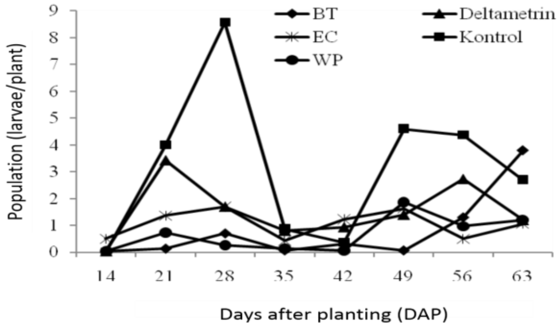


Fig. 3. Population of *C. pavonana* larvae on broccoli plant treated with insecticides

Data analysis using statistic showed that at 28 and 56 days after planting, all treatments of emulsifiable concentrate and wettable powder formulations, BT (Bactospein), and deltametrin gave a different result compared to control; however, there was no real difference between treatments (Table 2). This indicated that when pest population was high, emulsifiable concentrate and WP formulations of *T. vogelii* and P. *aduncum* (1:5) gave an equivalent activity with a commercial insecticide BT and deltametrin. At 28 days after planting, larvae population of *C. pavonana* were suppressed well by emulsifiable concentrate and wettable powder formulations with the effectiveness levels of formulation were 80.16% and 96.73% respectively compared to deltametrin which was only 70.25% (Table 3). At 56 days after planting, the effectiveness level of emulsifiable concentrate and wettable powder formulations were 88.56% and 85.43% respectively. It was higher in the case of BT and deltametrin which have the effectiveness level of formulations, 70.35% and 35.24% respectively (Table 3).

Table 2. Population of larvae *C. pavonana* on broccoli plant with several insecticide treatment

Treatment	Population of *C. pavonana* larvae on DAP (mean ± SE)*							
	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP	49 DAP	56 DAP	63 DAP
Control	0 ± 0 a	4.00 ± 12.0a	8.57 ± 24.7 a	0.87 ± 4.19 a	0.37 ± 0.81 a	4.60 ± 15.7 a	4.37 ± 9.66 a	2.70 ± 5.58 a
EC formulation	0.50 ± 2.74 a	1.37 ± 5.14a	1.70 ± 5.88 b	0.43 ± 0.97 a	1.30 ± 4.56 a	1.63 ± 6.38 a	0.50 ± 0.90 b	1.07 ± 4.02 a
WP formulation	0.03 ± 0.19 a	0.72 ± 2.81a	0.28 ± 0.65 b	0.14 ± 0.58 a	0.07 ± 0.26 a	1.93 ± 0.26 a	0.83 ± 2.16 b	0.89 ± 3.07 a
BT	0.03 ± 0.18 a	0.13 ± 0.43a	0.70 ± 1.70 b	0.07 ± 0.25 a	0.30 ± 0.95 a	0.07 ± 0.25 a	1.30 ± 4.18 b	3.80 ± 8.68 a
Deltametrin	0.03 ± 0.19 a	3.55 ± 10.5a	1.76 ± 5.44 b	0.83 ± 4.27 a	0.97 ± 3.91 a	1.45 ± 5.58 a	2.83 ± 5.69 b	0.75 ± 1.29 a

DAP: days after palnting, x: mean, SE: standar error, BT: *Bacillus thuringiensis;*
*Numbers with the same characters not significanly different (Duncan test, α=0.05)

Table 3. The effectiveness of several insecticides to control *C. pavonana* in the field

Treatment	Value of insecticide effectiveness (%)							
	14 DAP	21 DAP	28 DAP	35 DAP	42 DAP	49 DAP	56 DAP	63 DAP
Control								
EC formulation	-	65.75	80.16	50.57	-	64.56	88.55	60.37
WP formulation	-	82	96.73	83.90	81.08	58.04	81.00	67.04
BT	-	96.75	91.83	91.95	18.91	98.47	70.25	-
Deltametrin	-	11.25	79.46	4.59	-	68.48	35.24	72.22

DAP: days after planting, BT: *Bacillus thuringiensis*

Activity of deltametrin was lower than insecticide BT, EC and WP formulations, particularly at 21, 28, 35, and 56 DAP. This might perhaps be due to the effective resistance of *C. pavonana* to some commercial insecticides that commonly used by farmers, including insecticides with deltametrin as their active ingredient.

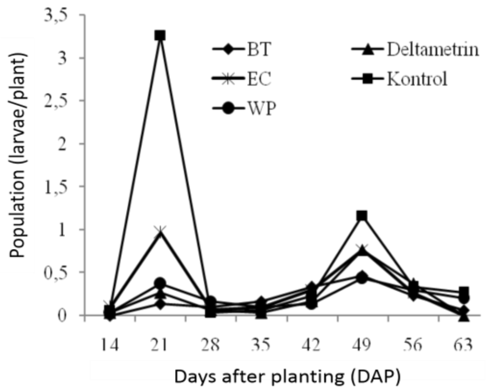


Figure 4. The population of larvae *H. undalis* on broccoli plants treated with insecticide

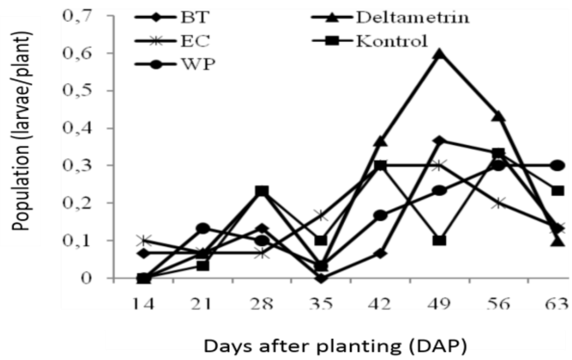


Figure 5. The population of larvae *P. xylostella* on broccoli plants treated with insecticide

Population of other pests of cabbage i.e *H. undalis* and *P. xylostella* was also observed during the observation of *C. pavonana* on the plant samples (Figs. 4-5). The population of *H. undalis* emerged earlier compared to *C. pavonana* (Fig. 3). The population of *H. undalis* increased at 21 DAP and decreased at 28 DAP, 35 DAP, and 42 DAP. The decline of population occurred due to the high rainfall which made many of the pests fail to survive. The larval population was re-increasing at 49 DAP and was re-decreasing on the next observation. Statistical analysis showed that there was no difference of the population of *H. undalis* compared to the control population.

In general, population of *P. xylostella* was found to be the lowest compared to *C. pavonana* and *H. undalis*. The population of *P. xylostella* was began to increase at 35 to 56 DAP. The population of *P. xylostella* found to be the highest in deltametrin treatment with an average of 0.6 larvae/plant (Figure 5) and it was still higher compared to control. This indicated that *P. xylostella* was resistant to chemical insecticides deltametrin. The statistical analysis against pests population showed no significant difference in treatment compared to control and the treatment itself.

The abundance of parasitoid of larvae *C. pavonana* was assessed on field efficacy. The result showed that the level of parasitisation of *E. argenteopilosus* against larvae of *C. pavonana* in the field was very low. None of the parasitoids emerged from the entire samples of larvae taken from the field to be maintained in the laboratory. This might due to the insecticides on cabbage plantation. Insecticide significantly supressed the population of *E. argenteopilosus*. Moreover, due to the scanty cultivation of vegetables in that area the availability host of parasitoids (*C. pavonana*) were not continuous. Visual observations on the field against parasitoids *E. argenteopilosus* imago was show the low abundance.

Formulations of EC and WP have low persistency and are relatively safe for the environment. The toxicity of mixtures extracts was very selective against parasitoid *E. argenteopilosus*. Formulation of EC and WP did not cause the death of the males and females of parasitoid in high concentration. Moreover, the formulation did not cause phytotoxic symptoms on the broccoli leaves. The effectiveness level of EC and WP formulations in field application equivalent to bioinsecticide contains *Bacillus thuringiensis* (BT) spora. The formulations were significantly success in suppressing pest populations by over 80% compared to control in high population.

REFERENCES

Bakri, M. S., Dien, M. F. and Kandowangko, D. S. 2015. Parasitisasi *Diadegma* *semiclausum* Hellen (Hymenoptera: Ichneumonidae) pada hama *Plutella xylostella* (Lepidoptera; Plutelidae) DI Tomohon). In *COCOS*, **6** (16): 1-4.

Dono, D., Prijono, D., Manuwoto, S. and Buchori, D. 1998. Pengaruh ekstrak biji *Aglaia harmsiana* Perkins terhadap interaksi antara larva *Crocidolomia binotalis* Zeller (Lepidoptera: Pyralidae) dan parasitoidnya, *Eriborus argenteopilosus* (Cameron) (Hymenoptera: Ichneumonidae). *Buletin Hama dan Penyakit Tumbuhan*, **10** (1): 38-46.

Dono, D. 2004. Aktivitas insektisida rokaglamida dan penghambatan respons imunitas larva *Crocidolomia pavonana* (F.) (Lepidoptera: Pyralidae) terhadap parasitoid *Eriborus argenteopilosus* (Cameron) (Hymenoptera: Ichneumonidae) [disertasi]. Bogor (ID): Institut Pertanian Bogor, Program Pascasarjana, Program Studi Entomologi.

Februlita, Y.M. 2013. Aktivitas insektisida ekstrak *Piper aduncum* asal Riau terhadap larva *Crocidolomia pavonana* [tesis]. Bogor (ID): Institut Pertanian Bogor, Program Pascasarjana, Program Studi Entomologi.

Hassall KA. 1990. *The Chemistry of Pesticides*: *Their Metabolism, Mode of Action and Uses in Crop Protection.* London (GB): Macmillan.

Isman, M.B. 1995. Leads and prospects for the development of new botanical insecticides. *Reviews in Pesticide Toxicology*, **3**:1-20.

Komisi Pestisida. 2000. Syarat peredaran dan perdagangan pestisida nabati. Di dalam: Soetopo, D., Supriadi, Djazuli, M., Hadipoentyati, Yuliani, S., Prijono, D. (Edts.). Prosiding Forum Komunikasi Ilmiah Pemanfaatan Pestisida Nabati. Bogor, 9-10 November 1999. Bogor (ID): Pusat penelitian dan Pengembangan Tanaman Perkebunan. Hlm 21-30.

Kuroki, T. 1998. Cancers as a disease of genes and a disease due to environmental factors. Di dalam: Kuhr, R.J. and Motoyama, N. (Eds.) *Pesticides and the Future: Minimizing Chronic Exposure of Humans and the Environment*. Washington (AS): IOS Press. hlm 113-118.

Lina, E.C., Dadang, Manuwoto, S., Syahbirin, G. and Prijono, D. 2013. Synergistic action of mixed extracts of *Brucea javanica* (Simaroubaceae) *Piper aduncum* (Piperaceae), and *Tephrosia vogelii* (Leguminosae) against cabbage head caterpillar *Crocidolomia pavonana*. *Journal of Biopesticides*, **6**(1):77-83.

Lina, E.C., Dadang, Manuwoto, S., Syahbirin, G. and Prijono, D. 2014. Ekstraksi dan Bioaktivitas *Brucea javanica*, *Tephrosia vogelii*, dan *Piper aduncum.* Seminar Nasional dan Lokakarya Membangkitkan Patriotisme Pertanian. Forum Komunikasi Perguruan Tinggi Pertanian Se Indonesia. Universitas Andalas, Padang 8-10 September, 2014.

Lina, E.C., Dadang, Manuwoto, S., Syahbirin, G. and Prijono, D. 2017. Formulation of Mixed extracts of *Tephrosia vogelii* and *Piper aduncum.* Asia-Pacific Conference on Life Sciences and Biological Engineering. Higher Education Forum (HEF), Nagoya-Japan March 29 – April 31, 2017.

Matsumura, F. 1985. *Toxicology of Insecticides*. 2nd Edition. New York (AS): Plenum Press.

Metcalf, R.L. 1982. *Insecticides in pest management*. *In:* Metcalf, R.L. and Luckman, W.H. (Eds.). *Introduction to Insect Pest Management*. 2nd Edition. New York: J Wiley. *Pp* 217-253.

SAS Institute. 1990. *SAS/STAT User's Guide*, Version 6, Vol 2. 4th Edition. Cary (North Carolina) (US): SAS Institute.

Sastrosiswojo, S. and Setiawati, W. 1993. *Hama-hama kubis dan pengendaliannya*. Di dalam: Permadi AH, Sastrosiswojo S, editor. Bandung (ID): Balithor Lembang. hlm 39-50.

Schmutterer, H. 1995. *The Neem Tree, Azadirachta indica A. Juss, and Other Meliaceous Plants: Sources of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes*. Weinheim (DE): VCH.

Scott, I.M., Jensen, H.R., Nicol, R., Lesage, L., Bradbury, R., Sachez-Vindas, P., Poveda, L., Arnason, J.T. and Philogene, B.J.R. 2004. Efficacy of piper (Piperaceae) extracts for control of common home and garden insect pests. *Journal of Economic Entomology*, **97**(4): 1390-1403.

Speight, M.R., Hunter, M.D. and Watt, A.D. 1999. Ecology *of Insects: Concepts and Applications*. Blackwell Science Ltd.

Steel, R.G.D., Torrie, J.H. and Dickey, D.A. 1997. *Principles and Procedures of Statistics: A Biometrical Approach*, 3rd Edition. Boston (AS): McGraw-Hill.

Sudarmo, Prijono D, Manuwoto, S. and Buchori, D. 2001. Selektivitas ekstrak ranting *Aglaia odorata* Lour. (Meliaceae) terhadap *Crocidolomia binotalis* Zeller dan *Eriborus argenteopilosus* (Cameron). *Hayati*, **8**: 112-116.

Syahputra, E., Prijono, D., Dadang, Manuwoto S. and Kadarusman, L.K. 2005. Bioaktivitas insektisida botani *Calophyllum soulattri* Burm.F. (Clusiaceae) sebagai pengendali hama alternatif [disertasi]. Bogor (ID): Institut Pertanian Bogor.

Eka Candra Lina[1,*], Dadang[2], Syafrida Manuwoto[2], Gustini Syahbirin[3]

[1]Andalas University, Plant Protection Department, Agriculture Faculty, Kampus Unand Limau Manis-Padang

[2]Bogor Agriculture Institute, Plant Protection Department, Agriculture Faculty, Jl. Kamper Kampus IPB Darmaga, 16680

[3]Bogor Agriculture Institute, Chemistry Department, Faculty of Mathematics and Natural Science, Kampus IPB Darmaga 16680

*Corresponding author
Phone +62 813-82568905
Email: eka_candra@faperta.unand.ac.id