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Insecticidal activity of *Piper aduncum* fruit and *Tephrosia vogelii* leaf mixed formulations against *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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ABSTRACT

The emulsifiable concentrate (EC) and wettable powder (WP) of Piper aduncum and *Tephrosia vogelii* mixed formulations were tested for their activities in the laboratory and their effectiveness in the field against cabbage pest *Plutella xyostella*. Cabbage leaves soaked in six different mixed formulation concentrations were tested against second instar larvae of *P. xylostella* and observed until the larvae reached fourth instar stage. From the survived larvae, growth and development, antifeedant effects were recorded. Field efficacy test was based on LC_{95} value of formulations from laboratory test result. The experiment used a randomized block design with 5 treatments and 3 replications to determine the insecticide effectivity against populations of *P. xylostella* larvae. The results showed that the EC and WP mixed formulations have insecticidal activity against *P. xylostella* larvae, with LC_{95} value of 0.35% and 0.37%, respectively. The highest antifeedant effect on EC mixed formulation was 85.01% and WP mixed formulation was 86.23%. Both mixed formulations also slowed the development of larvae when compared with control. Field effication result showed that applications of EC mixed formulation were able to restrain the population of *P. xylostella,* with effectivity value of 71.06%. Insecticide effectivity value of EC mixed formulation was higher than WP mixed formulation and *Bacillus thuringiensis* formulation.

Keywords: Botanical insecticide, efficacy, emulsifiable concentrate (EC), *Plutella xylostella,* wettable powder (WP).

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INTRODUCTION

Leaf caterpillar (*Plutella xylostella* L., Lepidoptera: Plutellidae) is one of the main pests in Brassicaceae plants, especially broccoli species in Indonesia. *P. xylostella* starts to attack plants from the first instar larvae stage. The newly hatched larvae slit the broccoli leaf and get to in tissue until they reach second instar larvae. The second instar larvae come out from leaf tissue and cause thin transparent layer symptoms eventually form torns and holes. In high populations, almost all leaves are eaten and the leaf bone alone is left. Heavy attacks usually occur in the dry season or in 5-8 weeks after they are planted (Dirlintura, 2013). Nowadays, farmers rely on synthetic insecticides to control *P. xylostella* because it is practically easy and gives fast response. The use of synthetic insecticides continuously can cause some negative impact on non-target organisms and the environment as well as pest resistance and resurgence. *P. xylostella* from some vegetable cultivation area in Indonesia such as lembang, pengalengan, kejajar/dieng and batu are resistant to deltametrin and profenofos (Moekasan *et al.,* 2004). Furthermore, *P. xylostella* resistance against pyrethroid and organophosphates insecticide has also reported in other countries such as Australia, China, India, Nicaragua, Pakistan, Philippines, South Africa, and South Korea (Furlong *et al.*, 2013). One of the prospective alternative control methods is the use of plant active secondary metabolites for insecticides (botanical insecticides). Botanical insecticide has advantages. It is biodegradable, safer for non-target organisms (natural enemies), synergistic extract components, delays resistance, goes well with other integrated pest control techniques, and some botanical insecticides can be served at farmer level (Dadang dan Prijono, 2008) . Some species of plants such as Kacang Babi (*Tephrosia vogelii*) and Sirih Hutan (*Piper aduncum*) have a potential to be used as botanical insecticide. The mixture of both materials have a potential to control pest. LC_{95} from the *T. vogelii* and *P. aduncum* mixture extract is less than 0.5% so that the extract has potential to be alternative control against *P. xylostella*, related to pesticide resistance management. Former research found that the mixture extract of *T. vogelii* and *P. aduncum* (1:5) have a synergistic action to be used as alternative insecticide against another main pest of *Croccidolomia pavonana.* The mixture extract work as toxic and secondary antifeedant effects (Prijono, 1999; Furlong *et al.*, 2013; Lina, 2014).

MATERIALS AND METHODS

Eexperiment was conducted in Insects Bioecologycal Laboratory and Wire house, Andalas University from January to March 2017. Field test was conducted in Tanah Datar District from June to September 2017. The tools used in these experiments were rotary evaporator, analytical scales, micropipette, blender, sieve screen 0,5 mm, volumetric flask (25 mL, 50 mL, and 100 mL), measuring cup 100 mL, petri dish, pipette (1 mL, 5 mL, and 10 mL), bolt, film bottle, bottles for extract, bottles for formulation, spatula, erlenmeyer flask, glass funnel (diameter of 5 cm and 9 cm), boiling flask, small brushes, plastic box (30 cm x 20 cm x 10 cm), measuring cup, insect cage (50 cm x 50 cm), plastic container (34 cm x 26 cm x 7 cm), polybags and sprayers. The materials used in these experiments fruits of sirih hutan (*Piper aduncum*) obtained from Bukit Lampu, Padang and leaf of kacang babi (*Tephrosia vogelii*) obtained from an agriculture area in Cianjur. Seeds of the broccoli comes from SAKATA varieties (as food for *P. xylostella* larvae), ethyl acetate, methanol, tween 80, acetone, filter paper of Whattman No. 41, filter paper, aluminum foil, cotton, honey, observation book, and fertilizer. Laboratory toxicity experiment of mixed formulation (EC and WP) were conducted using Completed Randomized Design with six treatments and five replications, there were 60 Petri dishes of experiment unit. Each Petri dish consists of 10 larvae of *P. xylostella.* Efficacy experiment was conducted in field, consisting of 30 trial plots. Each plot has four beds (1 m x 4 m). The experimental plots were arranged in Randomized Block Design with five treatments and six replications. The three week old broccoli seedling were planted in 70 cm x 50 spacing. Each plot had 60 plants of broccoli. The treatments were *P. aduncum* and *T. vogelii* mixed formulation in EC and WP forms, *Bacillus thuringiensis*, Deltametrin, and control. The concentration level was determined based on laboratory toxicity test results (equivalent to 2 x LC_{95}), whereas comparative insecticides were used according to recommendation dose. The observed data was analyzed using POLO PC software program (Furlong *et al.*, 2013) to determine LC_{50} and LC_{95} levels based on larvae mortality. Mortality data also analyzed using Statistic 8 and continued by Least Significant Different (LSD) test at confidence level 5%.

RESULTS AND DISCUSSION

Mortality of *P.xylostella* larvae

Variant analysis of *P. xylostella* mortality data showed significantly different results (Table 1).

Table 1. *P. xylostella* larva mortality in some concentrations of mixed *P. aduncum* and *T. voglii*

Treatments	Mortality (%)
0.30% EC	98.0 a
0.30% WP	96.0 ab
0.19% WP	82.0 bc
0.19% EC	80.0 c
0.12% WP	64.0 d
0.12% EC	64.0 d
0.08% WP	58.0 d
0.08% EC	56.0 de
0.05% EC	42.0 ef
0.05% WP	44.0 f
0.00% EC	0 g
0.00% WP	0 g
CV = 8,79%	

Increasing of concentration was followed by mortality level rates of insect test. Mortality of *P. xylostella* larvae treated with different concentration of EC and WP formulations are presented in Fig. 1 and Fig. 2.

Figure 1. Mortality of *P. xylostella* larva due to treatment of EC formulation mixture of *P.aduncum* fruit extract and *T. vogelii* leaf extract (5:1)

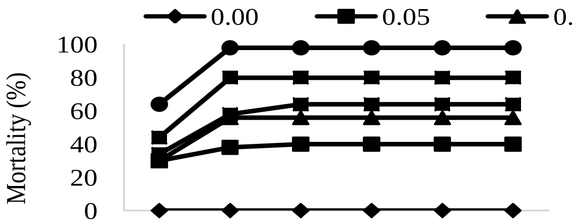
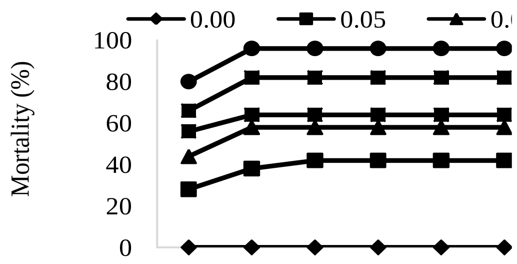


Fig. 2. Mortality of *P. xylostella* larvae due to treatment of WP formulation mixture of *P.aduncum* fruit extract and *T.vogelii* leaf extract (5:1)



P. xylostella larval mortality increased from first to second day, then on the third day the mortality did not increase significantly because the treated leaves were replaced with leaves without treatment. It makes the survival larvae recover. It shows that the formulation tends to be a toxic than growth and development regulator (Lina *et al.*, 2008; Lina, 2014). The mortality of *P. xylostella* treated with WP was formulation higher than *P. xylostella* treated with EC formulation at the first day treatment. This is because leaves eaten by *P. xylostella* in WP formulation treatment is much more than EC formulation treatment. But, in the second day *P. xylostella* treated with EC formulation ate the treated leaves as much as the leaves treated with WP formulation. The quantity of leaves consumed related to toxic limit of active compound that work on target site.

Feeding activity

EC and WP formulation mixed extract of *P. aduncum* and *T. vogelii* has a toxic effect as well antifeedant effect. The antifeedant effect as contributed to mortality of *P. xylostella* larvae. Observation result of antifeedant activity in *P. xylostella* treated with EC and WP formulation mixture extract of *P. aduncum* and *T.vogelii* (5:1) showed significant different result in different concentrations (Table 2). Toxic compounds that enter the insect body require enormous energy of insect to detoxify these toxins. Toxic compounds derived from plant can inhibit growth and development of insect tested due to energy allocation. The energy of insect is used for growth and development, in this case the energy is used to detoxify toxic compounds (Parkinson and Ogilvie, 2008).

Table 2. The antifeedant activity of *p. xylostella* larvae on some concentration of extract *p. aduncum* and *t. vogelii* mixed formulation

Treatments	Average of eaten leaf area (cm²)	Antifeedant activity (%)
0.30% WP	4.67	88.09 a
0.30% EC	3.39	82.23 a
0,19% WP	7.99	79.08 ab
0,19% EC	6.00	71.70 abc
0,12% WP	11.33	71.37 abc
0,08% WP	15.44	61.13 bcd
0,12% EC	10.69	51.03 cde
0,08% EC	11.22	48.73 de
0,05% WP	23.56	40.67 ef
0,05% EC	15.28	31.23 fg
0,00% EC	24.51	0.00 g
0,00% WP	39.78	0.00 g
CV = 18,49%		

The mean values in the same column and followed by unequal small letters are significantly different from the LSD advanced test at a real 5% level.

Researchers have found the impact in food assimilation of the insect when treated with *T. vogelii* and *P. aduncum* mixed extracts. Impact of food assimilation caused interference of a relative growth due to toxins that enter the insects body (Lina *et al.*, 2014). There is a correlation between concentration and antifeedant activity. The highest concentration 0.30% of WP and EC formulations that inhibited feeding activity of *P. xylostella* larvae were 88.09% and 82.23% respectively.

At the lowest concentration 0.05% of WP and EC formulations inhibited feeding activity of *P. xylostella* larvae by 40.67% and 31.23% respectively. The concentration will increase in followed by increasing toxic compound and also increase in the antifeedant effect. The toxic properties of plant compounds to insects can be a hindrances to the development of insects directly (intrinsically) or indirectly (extrinsic), whereas the antifeedant effects contained in plants can be detected by insects through the sensory system (primary antifeedant effect) or the central nervous insects that govern the eating process (secondary antifeedant effect) (Miller and Stricler, 1984; Prijono 1999). Mixture extract of *P. aduncum* and *T. vogelii* (5:1) has secondary antifeedant effects on *C. pavonana* larvae (Lina, 2014). This feeding inhibition is derived from single extract of *P. aduncum* and *T. vogelii*, in accordance with another researcher who found that *T. vogelii* leaf extract has insecticidal, antifeedant, and repellent against *P. xylostella* larvae (Morallo-Rejesus, 1986). Secondary compounds derived from Piperaceae species are also antifeedant to insects (Scott *et al.*, 2004).

Antifeedant effect contributed to mortality of *P. xylostella* larvae, inhibition of larvae feeding activity causes sickness and weakness. The purpose of feeding activity on insects is to achieve a nutritional balance. The nutrients are vitamins, casein, amino acids, sucrose, salt and others to reach 100%. If the content of food is less than the supposed amount, the insects will consume more in quantity but not gain weight or restrict consumption (Dethier, 1970). The suitability of insects to the host plant will affect the fitness of insects and offspring. When there are no choice of qualified food the insects would be forced to eat unsuitable food to survive or decide not to eat at all. This decision will lead to consequences of inhibition of growth and development and even death (Schoonhoven *et al.*, 2005).

Growth and development of *P. xylostella*

Based on *P. xylostella* larvae growth and development observations, the difference in concentrations of EC and WP formulation of *P. aduncum* and *T. vogelii* (5:1) show a significantly different result.Generally, the development of survival larvae will be inhibited from 2^{nd}-3^{rd} instar and form 2^{nd}-4^{th} (Table 3 and Table 4).

Table 3. Growth and development of *P.xylostella* 2^{ND} - 3^{RD} instar larvae at several concentrations of mixed *P.aduncum* and *T.vogelii*

Treatments	Prolonged development of *P. xylostella* 2^{nd}-3^{rd} instar larvae (days) X ± SD
0.30% WP	3.50 ± 0.70 a
0.30% EC	3.00 ± 0.00 b
0.19% WP	2.90 ± 0.33 bc
0.12% WP	2.85 ± 0.98 bcd
0.19% EC	2.55 ± 0.81 bcde
0.12% EC	2.36 ± 0.78 cdef
0.08% WP	2.19 ± 0.96 cdef
0.08% EC	2.01 ± 0.81 def
0.05% WP	1.75 ± 0.92 ef
0.05% EC	1.74 ± 0.77 ef
0.00% WP	1.74 ± 0.75 ef
0.00% EC	1.66 ± 0.65 f
CV = 11,58%	

The mean values in the same column followed by unequal small letters are significantly different from the LSD advanced test at a real 5% level. In the highest concentration of EC and WP formulation prolonged from second to third instar stage about 1.34 and 1.76 days longer than control respectively (Table 3).

Table 4. Growth and development of *P. xylostella* second and fourth instar larvae at several concentrations of mixed *P. aduncum* and *T.vogelii*. X ± SD

Treatments	Prolonged development of *P. xylostella* 2^{nd}-4^{th} instar larvae (days)
0.30% WP	6.00 ± 0.00 a
0.30% EC	5.50 ± 0.70 b
0.19% WP	4.80 ± 0.44 c
0.12% WP	4.70 ± 0.57 c
0.19% EC	4.68 ± 0.48 cd
0.12% EC	4.58 ± 0.52 cd
0.08% WP	4.34 ± 0.82 de
0.08% EC	4.15 ± 0.71 ef
0.05% WP	4.10 ± 0.99 ef
0.05% EC	3.82 ± 0.66 fg
0.00% WP	3.70 ± 0.64 g
0.00% EC	3.68 ± 0.65 g
CV = 2,96%	

Further more for second to fourth instar stage about 2.30 and 1.82 days longer than control respectively (Table 4).

The mean values in the same column followed by unequal small letters are significantly different from the LSD advanced test at a real 5% level.

Insects consumed treated leaves with mixed extract formulations though very little, especially at high concentrations. This provides an opportunity for active ingredients to enter and cause the death or physiological disturbance of test insects. The active compound enters *C. pavonana* larvae body at feeding activity on treated leaves. Active compound will reach toxic accumulation points and cause larva mortality or disrupting the growth of survival *C. pavonana* larvae (Lina, 2014).

Table 5. Regression proportion parameters of *P. aduncum* and *T. vogelii* (5:1) mixed extract relation between EC and WP Formulation to mortality of *P. xylostella* larvae

Treatments	b ± ES	LC_{50} (%)	LC_{95} (%)
EC Formulation	2.34 ± 0.35	0.07	0.35
WP Formulation	2.19 ± 0.35	0.06	0.37

Notes: b = slope of regression; ES=error standard

Correlation of mortality *P. xylostella* and concentration of formulations was analyzed using PoLo PC program (Table 6). Analysis result showed that the concentration of EC formulations for LC_{50} and LC_{95} were 0.07% and 0.35% respectively, with a regression slope 2.34. Forever, concentration for LC_{50} and $LC_{95}$of WP formulations were 0.06% and 0.37% respectively, with a regression slope 2,19. Generally, LC_{50} value of EC and WP formulation for *P. xylostella* is lower than LC_{50} of *Croccidolomia pavonana* (Lina, 2014). LC_{95} value of EC formulation for *P. xylostella* is the same LC_{95} on *C. pavonana* (0.35%). But, LC_{95} value of WP formulation for *C. pavonana* (0.31%) was lower than LC_{95} for *P. xylostella*. The result showed that the pest management activity for both main pests in Brassicae can be applied using the same concentration in field, that is 2 x LC_{95} (0.74%).

Percentage of affected plant in field

Observation of affected plants in field at various treatments can be seen in Table VI. Percentage of attacked plant by *P.xylostella* in trial plot which is treated with Deltametrin showed a significanly different result compared with control.The effectivity value of Deltametrin, *Bacillus thuringiensis*, EC Formulation and WP formulation are 61,30%, 35,49%, 12,90% and 25,81% respectively. Syntethic pesticide Deltametrin suppressed *P. xylostella* population better than EC, WP and *Bacillus thuringiensis* formulation.

Table 6. Percentage of plants attacked by *P.xylostlla* in field

Treatments	Attacked Plants (%)		Effectivity (%)
Control	68.89± 6.78	a	0
EC Formulation	60.00±24.03	a	12.90
WP Formulation	51.11 ± 3.84	ab	25.81
Bacillus Thuringiensis	44.44 ± 7.69	ab	35.49
Deltametrin	26.66± 1.54	b	61.30

Amount of *P. xylostella* larvae population at various treatments can be seen in Table 7. In general, larvae populations in control were higher than the number of larvae in the treatment. The number of larvae begins to increase and peaks on the 28th day after planting (DAP), then decreases in the 35th DAP to 42nd DAP. This happened because of heavy rain over two weeks that occured at 42 DAP.In other research with the EC and WP formulation against *Croccidolomia pavonana* had decrease in population on 35 DAP due to a heavy rain. The occurence of climate changes would affect the population dynamics of insect pest (Karuppaiah and G.K Sujayanad, 2012).The mixed formuation of *P. aduncum*fruit extract and *T. vogelii* leaf extract (5:1) in EC and WP forms showed insecticidal activity against *P. xylostella*.

Table 3. Population of *P. xylostella* larvae on broccoli plants with treatments In Field

Treatments	*P. xylostella* larvae population on DAP (x ± SD)*						
	21 DAP	28 DAP	35 DAP	42 DAP	49 DAP	56 DAP	63 DAP
Control	0.17±0.30 a	0.53±0.40 a	0.30±0.13 a	0.02±0.03 b	0.04±0.03 a	0.04±0.07 a	0.11±0.10 a
EC Formulation	0.06±0.11 a	0.44±0.37 ab	0.28±0.21 ab	0.13±0 a	0.04±0.07 a	0.08±0.10 a	0.11±0.10 a
WP formulation	0.06±0.06 a	0.15±0.13 ab	0.17±0.16 ab	0.13±0 a	0.08±0.04 a	0.13±0.13 a	0.04±0.07 a
Ba.thuringiensis	0.06±0.06 a	0.26±0.17 ab	0.2±0 ab	0.02±0.03 b	0.04±0.03 a	0.11±0.10 a	0.02±0.03 a
Deltametrin	0.11±0.10 a	0.11±0.10 b	0.02±0.03 b	0±0 b	0.02±0.03 a	0.02±0.03 a	0.04±0.07 a

DAP : Days after planting, x: mean, SD: Standart deviation

These formulations caused mortality, antifeedant effect and slack the growth of *P. xylostella* larvae. On the whole this formulation showed the same activity. Application on field showed that EC and WP formulation can reduce the larvae population of *P. xylostella*. In high pest population, WP forms showed an insecticidal activity that was almost equivalent to deltametrin. The use of EC and WP forms still allowed the population of some natural enemies in fields.

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