The efficacy of seed extract of *Tephrosia vogelii* and *Annona squmosa* on larvae of *Helicoverpa armigera*

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Abstract. *Nenotek PS, Ludji R. 2020. The efficacy of seed extract of* Tephrosia vogelii *and* Annona squmosa *on larvae of* Helicoverpa armigera. *Trop Drylands 4: 5-9.* Corncob borer (*Helicoverpa armigera*) is important pest of corn, urging the use of insecticide for its eradication. While using chemical insecticides is not sustainable, the use of plant-based insecticides is thus recommended. This study aimed to determine the efficacy of *Annona squamosa* and *Tephrosia vogelii* seed extract mixture on the mortality of 3rd instar larvae of *Helicoverpa armigera*. A range of concentration, as treatments, were evaluated, i.e., 0.05%, 0.11%, 0.28%, 0.65%, 1.5% and a control (water only) applied with three replicates in each treatment. Ten larvae of *H. armigera* at each treatment were infested in baby corn, using the residue method. Mortality was observed at 24, 48, 72, 96, and 120 hours after treatment (HAT). Percent mortality of the 3rd instar of *H. armigera* larvae was analyzed using Probit Polo PC, then proceed with mixed activity analysis. The results showed that the mixture of *A.squamosa* + *T. vogelii* seeds extracts killed *H. amigera* larvae with the value of LC_{50} and LC_{95} of 0.07% and 2.07%, respectively. A mixture of *A. squamosa* seeds + *T. vogelii* seeds extract was synergistic to *H. armigera* larvae with combined index values at LC_{50} and LC_{95} were 0.53 and 0.58, respectively. Thus, the use of a mixture of *A. squamosa* seed extract and *T. vogelii* seed is more efficient because the raw materials used were less at low concentrations to control *H. armigera* larvae than the extract applied separately.

Keywords: *Helicoverpa armigera*, *Annona squamosa*, *Tephrosia vogelii*, mortality

INTRODUCTION

Corncob borer (*Helicoverpa armigera*) is one of the main pests of some crops in Indonesia, attacking corn, soybeans, tomatoes, and cotton (Setiawati et al. 2002; Tenriwawe 2011; Indrayani 2013; Bedjo 2015). In corn, yield losses caused by this pest can reach 60% (Luther et al. 2007). The larvae bor the cob and eat the kernels, decreasing the quality and quantity of corn yields. In some corn plantations around the City of Kupang and Kupang Regency, East Nusa Tenggara Province, symptoms are often found due to corn cob borer. To suppress its development and damage, farmers often mix up three types of synthetic pesticides (i.e. carbofuran, BMPC, and diazinon) which are applied on a weekly basis.

The extensive uses of synthetic pesticides in controlling pests can cause various negative impacts such as pest resistance, environmental pollution, residues, and various types of diseases in humans (Gill and Grag 2004). For example, there are 260 species that are resistant to pesticides from the organophosphate group, 48 species are resistant to pyrethroids, and 85 species are resistant to carbamates (Dhaliwal et al. 2006). *H. armigera* is one of the pests that has been resistant to these three groups of pesticides (Ahmad 1997; Ahmad 2001; Ahmad 2007; Torres-Villa et al. 2002; Chaturverdi 2007). Corncob borer in India has been resistant to insecticides from the organophosphate, pyrethroid, and carbamate groups (Armes et al. 1996).

One of the pest control technologies that can minimize the negative effects of synthetic pesticides is plant-based pesticides. Each type of plant produces a variety of secondary metabolites that are sometime toxic to pests and diseases, act as repellent or attractant, can reduce appetite, and fertility (Vickery and Vickery1981; Schmutter 1990). In Indonesia, there are many plants that are potential as plant-based pesticides. It is estimated that 2400 species are potential as plant-based pesticides including *Annona squamosa* and *Tephrosia vogelii* (Kardinan 1999; Hasyim et al. 2015).

Botanical insecticide has several advantages, one of which is two or more species of plants can be mixed as a single pesticide. Through this mixture, insects are not easily resistant to the extracted plant compounds, and it can also increase synergism and reduce the use of raw materials. The compatibility of the mixture can be inferred from the synergistic nature of the mixture with higher toxicity rate compared to non-mixing plant extract. For example, the mixture of extracts of *Piper retrofractum*, *A. squamosa,* and *Aglaila odorata* was effective in controlling major pests in the cabbage, *Crocidolomia pavonana* and *Plutella xylostella* (Dadang et al. (2011). The mixture of *T. vogelii* seed extract and *Quassia amara* leaf extract had insecticidal properties against *C. pavonana* larvae, which was stronger than separately extract treatment (Nenotek 2010). In a previous study, separate testing of *A. squamosa* and *T. vogelii* seeds effectively killed the larval instar III *H. armigera*, with LC_{50} values of 0.89 and 0.15 (Nenotek and Ludji 2016). Information about the synergy of a mixture of *A. squamosa* and *T. vogelii* seed extracts has not been reported. Therefore, this study aims to determine the

insecticidal nature of the mixture of *A. squamosa* and *T. vogelii* seeds extract against mortality of 3rd instar *H. armigera* larvae, and to obtain LC_{50} and LC_{95} values smaller than separately testing.

MATERIALS AND METHODS

Research design

This research used a Completely Randomized Design. A mixture of *A. squamosa* seeds and *T. vogelii* seeds at a ratio of 1: 1 (w/w) was tested at a concentration of 0.05%; 0.11%; 0.28%; 0.65%; 1.5%; and control. Each treatment of concentration had three replicates and each replicate consisted of 10 larvae instar 3rd *H. armigera*.

Test insects rearing

Helicoverpa armigera larvae were obtained from farmers corn crops around Baumata Village, Tarus, and University Nusa Cendana Dryland Laboratory, Kupang, Indonesia. Larvae *H. armigera* instar 1st and 2nd were kept in 30 cm x 10 cm plastic boxes (the lid was made of rectangular holes and fixed with gauze as air circulation) and fed with baby corn. Up to 3rd instar larvae to pupae were kept in a pudding cup, each cup contained one larva. Sawdust was given as a media for the end instar larvae to pupae, then the pupae were transferred into plastic cages until they became imago. Imago was kept in a 30 x 30 x 30 cm^3 wooden frame, fed with 10% liquid honey, which was absorbed on a lump of cotton. Cotton was replaced every day because imago lay their eggs on it. Cotton containing *H. armigera* eggs was put in a plastic box. Before the eggs hatch, baby corn or corn was given to the container as food for newly hatched larvae.

Extraction of plant material as a botanical insecticide

The extraction process was carried out at BioScience Laboratory Universitas Nusa Cendana. *A. squamosa* and *T. vogelii* seeds were dried at room temperature that was not exposed to direct sunlight. *A. squamosa* seeds were separated from the seed coat, whereas *T. vogelii* seeds were not separated from the seed coat. Each seed of the plant was blended with a blender and then sieved using a 0.5 mm edged sieve until it became powder. Soursop seed powder was soaked with methanol at a ratio 1: 10 for at least 24 hours, filtered using a glass funnel (9 cm in diameter) lined on no.12 Waltman paper. The extract was collected in a vaporizer flask, then evaporated with a rotary evaporator at 45°C and 337 mbar pressure. The remaining methanol obtained from evaporation was used to soak the pulp of extract and then evaporated. This activity was carried out until it was colorless. The extract obtained was stored in the refrigerator at $\pm 40^{o}$C until the time of testing. Whereas *T. vogelii* seed flour was soaked with ethyl acetate at the same ratio.

Toxicity assay of mixed extracts on *H. armigera* larvae

Mixed form of *A. squamosa* seed extract and *T. vogelii* seeds were tested at a ratio of 1: 1 (w/w) at a concentration of 0.05%, 0.11%, 0.28%, 0.65%, 1.5%, and control. Each active component was mixed with methanol solvent and Agristick adhesive (final concentrations of methanol and agristick in the test preparation were 1% and 0.2%, respectively) and then diluted with aquadest to the specified concentration.

Pesticide-free baby corn was dipped into the mixed extract at each concentration until it was evenly wet, then dried for a few months into a 5 cm diameter custard cup with hole in the cover and covered with gauze for air circulation. In each pudding cup containing one 3rd instar, *H. armigera* larvae were given feed treatment for 48 hours, after which it was replaced with feed without treatment. The dead larvae were counted and removed from the pudding cup while the living ones were reared with baby corn without treatment until the larvae became pupae. Mortality was observed at 24, 48, 72, 96, and 120 hours after treatment (HAT). The percentage of *H. armigera* larvae mortality was analyzed using Probit Polo PC analysis (LeOra Software 1987).

Analysis of the mixture activity nature

The mixed *A. squamosa* and *T. vogelii* seeds extracts were analyzed with different working models to calculate the combination index (IK) at the LC_{50} and LC_{95} levels (Chou and Talalay 1984):

$$IK = \frac{LCx^{1(cm)}}{LCx1} + \frac{LCx^{2(cm)}}{LCx2} + \frac{LCx^{1(cm)}}{LCx1} \times \frac{LCx^{2(cm)}}{LCx2}$$

The two active component extracts LCx in separate tests, $LCx^{1\ (cm)}$ and $LCx^{2\ (cm)}$ respectively are extract components in the mixture resulting x mortality (50% and 95% of sample concentration), LCx values in the mixture are the result of LCx multiplication with a proportion of the component concentration in the additives mixture. The nature of the interaction of the mixture was divided into four categories namely (i). IK <0.5, the mixed composition is strongly synergistic; (ii). IK = 0.5-0.77, the mixed component is weakly synergistic; (iii). IK = 0.77-1.43, the mixture component is additive; (iv). IK> 1.43, the mixture component is antagonistic (Kosman and Cohen 1996).

RESULTS AND DISCUSSION

The results of the study showed that *A. squamosa* seed extract, *T. vogelii* seed and a mixture of *A. squamosa* + *T. vogelii* seed extract had the ability to kill larvae 3rd instar *H. armigera*. However, the toxicity of the mixture (*T. vogelii* + *A. squamosa* seed extract) killed the test insect more strongly than the separate method (Table 1). At a concentration of 0.11%, a mixture of *A. squamosa* + *T. vogelii* extract killed the test insects up to 70%, while mortality of *H. armigera* larvae that were given separate extracts of *A. squamosa* and *T. vogelii* was 26.26% and 50%, respectively. At a concentration of 1.5%, *A. squamosa* seed extract can only kill 56.67% of test insects, while the toxicity of mixed *T. vogelii* extract and *A. squamosa* + *T. vogelii* against test insects were 86.67% and 96.67%, respectively.

Table 1. Percent mortality of *H. armigera* larvae treated with *A. squamosa*, *T. vogelii* seed extract, and mixture of *A. squamosa* + *T. vogelii*

Conc. (%)	Cumulative mortality (%)		
	A. squamosa	*T. vogelii*	Mix *A. squamosa & T. vogelii*
Control (0)	0.00	0.00	0.00
0.05	20.00	26.67	36.67
0.11	26.67	50.00	70.00
0.28	40.00	56.67	80.00
0.65	43.33	80.00	80.00
1.50	56.67	86.67	96.67

The number of killed test insects at concentration of 0.05% for all treatments was categorized as low (Table 1). This shows that the toxin contained in each treatment is still low so it can be tolerated or neutralized by 3rd instar *H. armigera* larvae.

The mortality of 3rd instar *H. armigera* larvae treated with mixed extracts is presented in Table 2. At a concentration of 0.05% the mortality of test insects has not reached 25%, whereas at other concentrations the mortality percentage has reached 45-60%. Mortality rates continued to increase at 48 HAT and 72 HAT (hours after treatment) for all concentrations except controls. At the concentrations of 0.05% and 0.11% still found another test insects died at 96 HAT and 120 HAT.

In the mixed extract, mortality had begun to appear at 24 HAT at all concentrations. This shows that during 24 HAT there was a reaction of secondary metabolite compounds from the extracts of *T. vogelii* and *A. squamosa* seeds that poisoned the body of *H. armigera* larvae that worked as antifeedants, stomach poisons, and contact poisons. As an antifeedant, larvae recognized that the feed given was foreign object and not for consuming so that its body did not get energy, getting weak, and eventually dies. As a stomach poison, larvae consume food that already contains toxins and was carried into the digestive system thereby damaging cells in the digestive system that cause the death of test insects. When larvae activity directly contacts the feed, poison enters the insect's body through the pores of the cuticle. Furthermore, the poison was carried to the target site by the blood of the insect, thus damaging the cells and causing the death of the test insect. Poisoned larvae showed dislike to eat, lack of activity or movement, body-color changed to light brown and black when it died which began to appear on the first day after treatment.

The results of probit analysis (Table 3) show that a mixture of *A. squamosa* + *T. vogelii* seeds was more toxic to *H. armigera* larvae compared to separately extracts. LC_{50} value of *A. squamosa* seed extract + *T. vogelii* seeds was more toxic 2.14 times to *T. vogelii* seed extract and 12.71 times more toxic to *A. squamosa*. At LC_{95}, mixed extracts were more toxic 1.91 times than extracts of *T. vogelii* alone and 132.18 times more toxic than *A. squamosa* seeds alone. Thus a mixture of *A. squamosa* seeds + *T. vogelii* seeds was more effective against 3rd instar *H. armigera* larvae compared to extracts separately.

The combination index value (CI) of *A. squamosa* seed extract + *T. vogelii* extract has synergistic activity properties and stronger efficacy on the mortality of *H. armigera* larvae than the two extracts applied separately. The LC_{50} and LC_{95} combination index values are 0.53 and 0.58, respectively. The synergistic nature is probably caused by the activity of the active compound on different targets and at the same time resulting in a greater effect than the activity of the active compound separately. Another possibility is the presence of compounds from *A. squamosa* seeds and *T. vogelii* seeds can increase the workability of other compounds. Interaction of compounds in a synergistic mixture is a result of the activity of active compounds at different targets simultaneously providing a stronger effect than those compounds alone (Nenotek 2012). Mixture of several active compounds is synergistic if these compounds can increase the effectiveness of control of a species that is tested and the characteristics are called compatible (Clody 2010). Conversely, mixing two or more types of incompatible compounds is called an antagonist. Metclaf (1967) states that the mechanism of synergism occurs in the body of insects because toxic compounds can inhibit enzymes that function to decompose poisonous compounds in the insect's body.

Table 2. Percentage of mortality of *H. armigera* larvae treated with a mixture of *T. vogelii* + *A. squamosa* seed extracts

Concentration (%)	Percentage of mortality				
	Observational time (JSP)				
	24	48	72	96	120
Control (0)	0.00	0.00	0.00	0.00	0.00
0.05	13.33	20.00	26.67	30.00	36.67
0.11	50.00	60.00	63.33	66.67	70.00
0.28	46.67	70.00	80.00	80.00	80.00
0.65	53.33	76.67	80.00	80.00	80.00
1.50	66.67	83.33	96.67	96.67	96.67

Table 3. Estimating the toxicity parameters of *A. squamosa* seeds + *T. vogelii* seed extracts against *H. armigera* larvae

Type extract	a ± GBb	b ± GBb	LC$_{50}$ (CI 95%) (%)	LC$_{95}$ (CI 95%) (%)
Seeds of *A. squamosa* + *T. vogelii*	0.72 ± 0.11	0.51 ± 0.15	0.07 (0.02-0.13)	2.06 (0.08-32.07)
Seeds of *A.* [*] *squamosa*	0.31 ± 0.15	0.66 ± 0.20	0.89 (0.42-6.92)	272.31 (19.42-2124600.5)
Seeds of *T. vogelii*[*]	0.95 ± 0.17	0.17±0.22	0.15 (0.08-0.23)	3.95 (1.67-23.37)

Note: CI: Confidence Interval (Nenotek and Ludji 2016)

Anonna squamosa seeds contain acetogenin (Pomper et al. 2009). Some of the acetogenin compounds in *A. squamosa* are annonin I, squamosin, and asimine (Isman 2006). In general, acetogenin works to cut the energy supply by inhibiting the production of ATP energy in the mitochondria so that the test insects infested by these compounds will become weak and eventually die (Alali et al. 1999). These compounds are thought could kill various types of pest insects, including the *Sitophilus zeamais* (Nenotek et al. 2018), *Croccidolomia pavonana* larvae (Nenotek and Ludji 2014), and *Thrips* sp. (Nahak et al. 2018).

The active compounds in *T. vogelii* seeds are rotenone, rotenolene, and rotenoids (containing tephrosin and deugelin) and all are water-soluble (Enyiukwu et al. 2016; Chukwu 2018; Cabizza et al. 2004). Rotenon works as a contact, systemic, and selective poison to slowly kill test insects (Perry et al. 1988; Wirawan 2006). Tefrosin and deugelin in rotenone not only directly kill insects but also work to inhibit their feeding activity and growth development (Morris 1999).

Rotenone works in the body of insects by interfering with the function of respiration enzymes that work between NAD and coenzyme Q which causes failure in respiratory function. Rotenone also works in the mitochondria which inhibits the transfer of electrons in the NADH-coenzyme ubiquinone reductase so it can inhibit cellular respiration and decrease the energy source of ATP. This causes the test insects to become weak, paralyzed, and eventually die (Lu et al. 2006; Hollingwort 2001; Matsamura 1985; Wirawan 2006). A mixture of *A. squamosa* seed extracts + *T. vogelii* seeds is recommended to control *H. armigera* larvae because they are compatible, synergistic, require fewer raw materials, and increase application efficiency in the field at lower concentrations. Using a mixture of plant-based insecticides in different ways can delay the possibility of pest resistance to the components of the mixture (Georghiou 1983), reduce the negative impact on the environment, phytotoxicity, and non-target organisms.

In conclusion, a mixture of *A. squamosa* seed extracts + *T. vogelii* seeds has synergistic activity properties against *H. armigera* larvae. The LC_{50} and LC_{95} values of a mixture of *A. squamosa* seeds + *T. vogelii* seeds were 0.07% and 2.07%, respectively. Thus, the use of a mixture of *A. squamosa* seed extract and *T. vogelii* seed is more efficient because the raw materials used were less at low concentrations to control *H. armigera* larvae than two extracts applied separately.

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