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

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Manuscript received: 5 February 2020. Revision accepted: 5 May 2020.

Abstract. Nenote PS, Ludji R. 2019. The efficacy of seed extract of Tephrosia vogelii and Annona squamosa on larvae of Helicoverpa armigera. Trop Drylands 4: 5-9. 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 in 3 replications., Ten larvae of H. armigera at each test 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. armigera larvae with the value of LC50 and LC95 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 LC50 and LC95 were 0.53 and 0.58, respectively.

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

INTRODUCTION

In Indonesia, Helicoverpa armigera is one of the main pests in some plants including corn, soybeans, tomatoes, and cotton (Setiawati et al. 2002; Tenriwawe 2011; Indrayani 2013; Bedjo 2015). In corn, yield losses caused by this pest reach 60% (Luther et al. 2007). The larvae bor the cob and eat the kernels that are being filled so that the quality and quantity decreases.

In some corn plantations around the City of Kupang and Kupang Regency, East Nusa Tenggara, symptoms are often found due to corn cob borer. To suppress its development and damage, farmers mix up to three types of synthetic pesticides (including carbofuran, BMPC, and diazinon) and are applied on corn weekly.

Farmers who are always dependent on synthetic pesticides in controlling pests and their unwise use can cause various negative impacts such as pest resistance, resistance, environmental pollution, residues, and various types of diseases in humans (Gill and Grag 2004). The case of pest resistance 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 species 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 botanical pesticides. Each type of plant produces a variety of secondary metabolites that are 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 including Annona squamosa and Tephrosia vogelii are potential as plant-based pesticides (Kardian 1999; Hasyim et al. 2015).

Botanical insecticide has several advantages, one of which is two or more types of plants can be mixed as a single pesticide. Through this mixture, insects are not easily resistant to some plant extract compounds, but can increase synergism and reduce the availability of raw materials. The compatibility of the mixture can be seen from the synergistic nature with higher toxicity compared to non-mixing plant extract. Mixture of extracts of piper retrofractum + A. squamosa and Aglaila odorata + A. squamosa was effective in controlling major pests in the cabbage, Crocidolomia pavonana and Platella xylostella (Pondag et al. 2011). The mixture of T. vogelii seed extract + Quassia amara leaf extract had insecticidal properties against C. pavonana larvae, which was stronger than separately extract treatment (Nenote 2010). In a previous study, separate testing of A. squamosa and T. vogelii seeds effectively killed the larval instar III H. armigera, with LC50 values of 0.89 and 0.15 (Nenote 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 mixed activity of A. squamosa + T. vogelii seeds extract against mortality of 3rd instar H. armigera larvae and obtain LC50 and LC95 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 in a ratio of 1: 1 (w/w), tested at a concentration of 0.05%; 0.11%; 0.28%; 0.65%; 1.5%; and control. The concentration was repeated 3 times and each repetition consisted of 10 larvae instar 3rd H. armigera.

Test insects rearing

Helicoverpa armigera larvae were obtained from farmers corn crops around Baumata Village, Tarsu, and Undana Dryland Laboratory. 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 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 candidate

The extraction process was carried out at Undana's BioScience Laboratory. 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 evaporate. This activity was carried out until it was colorless. The extract obtained was stored in the refrigerator at ± 40°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 aguades to the specified concentration.

Pesticide free baby corn was dipped in mixed extract preparations 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 and the 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 larva became pupae. Each treatment and control was repeated 3 times with 10 larvae each repetition. 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 LC50 and LC95 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, LCx1 (cm) and LCx2 (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 1) IK <0.5, the mixed composition is strongly synergistic; 2) if IK 0.5-0.77, the mixed component is weakly synergistic; 3) if IK 0.77-1.43, the mixture component is additive; 4) if 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 mixed method (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 respectively, was 26.26% and 50%. 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.
The number of killed test insects at concentration of 0.05% for all treatments was still in the low category (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 development of 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 test, 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 not getting energy, and eventually dies. As a stomach poison, larvae consumed 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. LC50 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 LC50, 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 LC50 and LC95 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. Metcalf (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 1. Percent mortality of H. armigera larvae treated with A. squamosa, T. vogelii seed extract, and mixture of A. squamosa + T. vogelii.

<table>
<thead>
<tr>
<th>Conc. (%)</th>
<th>Cumulative mortality (%)</th>
<th>A. squamosa</th>
<th>T. vogelii</th>
<th>Mix A. squamosa &amp; T. vogelii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>20.00</td>
<td>26.67</td>
<td>36.67</td>
<td></td>
</tr>
<tr>
<td>0.11</td>
<td>26.67</td>
<td>50.00</td>
<td>70.00</td>
<td></td>
</tr>
<tr>
<td>0.28</td>
<td>40.00</td>
<td>56.67</td>
<td>80.00</td>
<td></td>
</tr>
<tr>
<td>0.65</td>
<td>43.33</td>
<td>80.00</td>
<td>80.00</td>
<td></td>
</tr>
<tr>
<td>1.50</td>
<td>56.67</td>
<td>86.67</td>
<td>96.67</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Percentage of mortality</th>
<th>Observation time (JSP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>Control (0)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>0.05</td>
<td>13.33</td>
<td>20.00</td>
</tr>
<tr>
<td>0.11</td>
<td>50.00</td>
<td>60.00</td>
</tr>
<tr>
<td>0.28</td>
<td>46.67</td>
<td>70.00</td>
</tr>
<tr>
<td>0.65</td>
<td>53.33</td>
<td>76.67</td>
</tr>
<tr>
<td>1.50</td>
<td>66.67</td>
<td>83.33</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Type extract</th>
<th>a ± GBb</th>
<th>b ± GBb</th>
<th>LC50 (CI 95%) (%)</th>
<th>LC95 (CI 95%) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeds of A. squamosa + T. vogelii</td>
<td>0.72 ± 0.11</td>
<td>0.51 ± 0.15</td>
<td>0.07 (0.02-0.13)</td>
<td>2.06 (0.08-32.07)</td>
</tr>
<tr>
<td>Seeds of A. squamosa</td>
<td>0.31 ± 0.15</td>
<td>0.66 ± 0.20</td>
<td>0.89 (0.42-6.92)</td>
<td>272.31 (19.42-2124600.5)</td>
</tr>
<tr>
<td>Seeds of T. vogelii</td>
<td>0.95 ± 0.17</td>
<td>0.17±0.22</td>
<td>0.15 (0.08-0.23)</td>
<td>3.95 (1.67-23.37)</td>
</tr>
</tbody>
</table>

Note: CI: Confidence Interval (Nenotek and Ludi 2016)
Annona squamosa seeds contain acetogenin (Pomer et al. 2009). Some of the acetogenin compounds in A. squamosa are annonin I, squamosin, and asimine (Isman et al. 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), Crocidoloma pavanana (Nenotek and Ludji 2014), and Thrips sp. (Nahak et al. 2018).

The active compounds in T. vogelii seeds are rotenone, rotenolene, and rotenoids (containing teephrosin 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). Tefrosis and deugelin in rotenin not only directly kill test insects but also work to inhibit the feeding activity and development of test insects (Morris 1999).

Rotenon works in the body of insects test 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; Matsamur 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. Another advantage is the use of plant-based insecticides in mixed form can reduce the need for raw materials. Using a mixture of plant-based insecticides in different ways can delay the possibility of pest resistance to the components of the mixture (Georghiu 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 LC50 and LC95 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 are less at low concentrations to control H. armigera larvae than two extracts are applied separately

ACKNOWLEDGEMENTS

The researcher is grateful for the Director-General of Higher Education who has funded this research. Thank you also to Yosep Tang and Damianus Nahak helping to extract A. squamosa and T. vogelii seeds as well as rearing of test rearing.

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