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Effectiveness of Biopesticides *Nicotiana tabacum* L and *Ageratum conyzoides* L As Controlling *Spodoptera exigua* in Red Onion (*Allium ascalonicum* L.)

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Abstract

Shallots (Allium ascalonicum) are a commodity in Indonesia that has high economic value. One of the pests that often attacks and reduces shallot yields is the onion caterpillar pest S. exigua. Tobacco plants and babadotan weeds are types of plants that can be used as botanical pesticides as a method of controlling and preventing pest attacks on plants. Tobacco and babadotan plants contain secondary metabolite compounds such as alkaloids, flavonoids, saponins, terpenoids and tannins which are able to kill insect pests on shallot plants. The aim of this research is to determine the effectiveness of tobacco extract (Nicotiana tabacum L.) and Babadotan (Ageratum conyzoides L.) as control of the onion caterpillar pest Spodoptera exigua on shallots. The highest combined mortality rate was treated with T6 at 96JSP with an average of 75%, the lowest larval mortality percentage test was found in T2 with an average mortality result of 22.50%. The results of biosafety testing show that the combination of tobacco extract and babadotan extract cannot be used together. From the conclusions that have been obtained, the extracts of (N. tabacum L.) and (A. conyzoides L.), both single extracts and combination extracts, are effective in controlling the pest S. exigua

Keywords: *Tobacco Extract, Babadotan, S. exigua*

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INTRODUCTION

Shallots (*Allium ascalonicum*) are a commodity in Indonesia that has high economic value. The increase in population causes an increase in consumer demand and consumer prices for shallots. Based on data from the Central Statistics Agency and the Directorate General of Horticulture (2016), shallot productivity in 2016 decreased to 9.67 tonnes/ha compared to 2015 of 10.06 tonnes/ha. The productivity of shallots in North Sumatra has decreased

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and is even less than optimal, this is due to pest and disease attacks (Sihole and Hutapea, 2023).

The problem that farmers often face in cultivating shallots is pest attacks which can result in decreased production yields. One of the main pests that attacks and reduces shallot yields is the onion caterpillar pest *S.exigua*. The *S.exigua* pest can damage shallot plants, resulting in loss of harvest (Paparang et al., 2016) and a population density of 3 to 5 onion caterpillar larvae in a cluster can cause production losses of 32% to 42%.

Farmers still often use synthetic pesticides to control plant pests (OPT). The use of synthetic insecticides can result in huge losses, such as resistance, human health and environmental pollution. Continuous use of synthetic pesticides can result in resistance to insect pests and their ability to grow rapidly. In addition, pest resurgence can occur which results in natural enemies becoming extinct (Susanti et al., 2016). One of the efforts that needs to be made to control and prevent *S.exigua* or Plant Pest Organisms (OPT) is to use botanical pesticides.Apart from that, one of the efforts to control nuisance pest organisms (OPT) includes using biological agents (parasitoids and predators) (Ginting et al., 2014).

Botanical pesticides are pesticides derived from plants that contain secondary metabolite compounds. Secondary metabolite compounds can control pests, especially insects, on plants. Botanical pesticides can be obtained from plant extracts from the leaves, stems, seeds and roots which contain secondary metabolite compounds (Krisna et al., 2022). One of the plants that can be used as a botanical pesticide is tobacco and babadotan weed.

Tobacco (*Nicotiana tabacum*) can be used as a botanical pesticide because it contains nicotine compounds (Avianto, 2021). The nicotine compound found in tobacco is also a secondary metabolite compound. The nicotine content in tobacco can inhibit the appetite of pests (Firma, 2019). Nicotine has rapidly toxic properties to insects (Andi, 2016). Apart from that, tobacco contains other secondary metabolite compounds such as polyphenols, saponins and flavonoids. Nicotine in tobacco can be used as a fumigant, which can kill insects (Aji and Amin, 2015).

Babadotan (Ageratum conyzoides) is a type of weed that belongs to the Ateraceae family (Krisna et al., 2022) *A.conyzoides* can be a vegetable pesticide, where the plant contains several secondary metabolite compounds such as alkaloids, flavonoids and polyphenols which provide repellent, anti-feeding effects on various types of pests, especially insects (Nurhudiman et al., 2018). Apart from that, *A.conyzoides* also contains monoterponoids, sesquiterpomoids, diterponoids, and other compounds such as cumin, benzofuran, chromenes, and sterol compounds which can provide toxic properties to various types of pests (Edwin et al., 2018). The compounds found in the *A.conycoides* plant play a very important role in controlling pests, which can interfere with the process of developing eggs into pupae and also make it difficult for female pests to produce. Based on the statement above, the aim of this research is to see the effectiveness of Nicotiana tabacum, Ageratum conyzoides extracts, and a combination of *Nicotiana tabacum* L and *Ageratum conyzoides* L extracts as control of the onion caterpillar pest *Spodoptera exigua* on shallots.

MATERIALS AND METHODS

This research was conductedin the Panca Budi Development University laboratory in Medan. This research will be carried out from November 2023 until completion. The tools used are plastic cups, tweezers, scissors, oven, beaker, Erlenmeyer, test tubes, filters, stirrers, blenders, black/brown glass bottles, plastic bottles, rubber bands, markers, cameras and writing utensils. The ingredients used are tobacco, babadotan leaves, distilled water, red onion leaves, *S. exigua* larvae, *Trichogramma* sp, (*Cheilomenes sexmaculata*), *Phenacoccus solenopsis*, label paper, honey, tape, cardboard, filter paper, gauze and cotton.

The research method used was a non-factorial completely randomized design (CRD). There were 4 replications and 7 levels of concentration treatment as follows: T0: No Application (Control), T1: Tobacco leaf extract 30%, T2: Tobacco leaf extract 60%, T3: Babadotan leaf extract 30%, T4: Babadotan leaf extract 60%, T5: combination of 15% Tobacco leaf extract and 15% Babadotan, M6: combination of 30% Tobacco leaf extract and 30% Babadotan. There are a total of 28 experimental units. Each experimental unit contained 10 3rd instar *S.exigua* larvae, where one larva was placed in one container.

Making Tobacco Extract and Babadotan Extract

Tobacco leaves and babadotan are cleaned from adhering dirt using running water. Then the leaves are dried by airing them without sunlight for ± 7 days Then it is powdered using a blender and filtered to extract flour. The flour powder is macerated with distilled water in a ratio of 1:10, for 3 x 24 hours. Then the filtering process is carried out using filter paper. The leaf powder waste is macerated again until the solution becomes clear. Next, evavoration was carried out to produce extract using an oven at a temperature of $50^{o}C$.

Phytochemical screening test

The results of the vegetable pesticide extract that have been macerated and evaporated are then carried out with a screening test to determine the secondary metabolite content. The screening test was carried out at the Biochemistry Laboratory, University of North Sumatra.

Rearing of *S.exigua* larvae

The larvae obtained from the onion plantings of UPT Horticulture Parent Seeds were kept in containers covered with gauze, the larvae were fed fresh spring onions. Larvae that have entered the prepupal stage are transferred to a container, the prepupal phase lasts 2-3 days. The larvae that have become pupae are transferred to a cotton-lined container. This phase takes 6-10 days. After 3-4 days the pupa is moved to a larger container before turning into an imago. After becoming imago, they are fed with a 10% honey solution impregnated with cotton and hung. After 2-8 days the imago lays eggs, and the eggs are transferred to a container covered with gauze. The eggs hatch in around 3-5 days, after

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that the hatched eggs are cared for until they enter instar 3 which takes around 9-14 days. 3rd instar larvae ready for testing.

Maintenance of Natural Enemies

The predator C.sexmaculata was obtained directly from the eggplant planting field, then kept in a plastic container covered with gauze and then fed with mealybugs (*P.solenopsis*). The larval life phase ranges from 6-10 days until they become pupae. Once the pupae have laid eggs, the eggs are transferred to another container. Egg phase 2-4 days. Eggs that have hatched are transferred to a plastic container to become larvae. The life phase of the pupa is around 2-4 days, after the pupa becomes an imago and changes color to orange it is ready to be tested, *Trichograma* sp eggs were obtained from the Bogor Agricultural Institute (IPB) which had been parasitized and propagated using C. pavonana eggs and placed on paper. After 10-14 days parasitoids will emerge from the eggs of the *C. pavonana* host. Parasitoids were transferred to the reaction tank as many as 10 individuals per replication. maintained in the laboratory of Panca Budi Development University as test insects.

Application to *S. exigua*

The application of pesticide extract is carried out using the dipping method. After the extract solution is mixed with water as a solvent, the leek feed is dipped for 15 minutes in each treatment. then food is given to 3rd instar *S.exigua* insects that have been hungry for 2-6 hours. The larvae used were 10 larvae per replication.

Natural Enemy Testing

Tests on natural enemies (predators and parasitoids) used extracts with the highest mortality and best results from *S.exigua* testing in the T6 treatment. Testing using the residue method, where the extract is sprayed into a test tube with a concentrated dosecombination of *N.tabacum* extract 30% and *A.conyzoides* 30% 4 repetitions, then air-dried for ± 15 minutes. After that, put natural enemies into each container, then observe their death for 24 hours.

Test Parameters

Phytochemical Screening Test

The evaporated leaf extracts of *N.tabacum* and *A.conyzoides* were then subjected to phytochemical screening at the Biochemistry Laboratory, University of North Sumatra.

S. exigua Mortality

S.exigua mortality was observed daily up to 96 JSA. The mortality percentage is calculated using the formula (Mawuntu, 2016).

$$P = \frac{X}{Y} X 100\%$$

Information:
P = Mortality percentage *S. exigua*
X = Amount *S. exigua* who died
Y = Amount *S. exigua* which was tested

Standardization and quality of pesticide extracts are said to be effective, which can be seen from mortality with 0-30% = not effective, 31-50% = less effective, 51-70% = effective, 71-100% = very effective (Aisyah, 2016).

Pest Attack Symptoms and Behavior

Symptoms of attack and larval behavior were observed for 4 days until the larvae died.

Pest Death Time

The duration of larval death was observed starting when the larvae were fed vegetable pesticides. The number of dead larvae was counted from 24 DAP to 96 DAP.

Biosafety Test

Application to predators and parasitoids was observed for 24 hours. Observation data is calculated using a formula.

$$P = \frac{X}{Y} X100\%$$

Information:
P = Percentage of predator and parasitoid deaths
X = Number of dead predators and parasitoids
Y = Number of predators and parasitoids tested

Data Analysis

Data on the percentage of deaths of *S.exigua* were analyzed using analysis of variance (ANOVA), then continued with the Duncan's Multiple Range Test (DMRT) at a significance level of 5%.

RESULTS AND DISCUSSION

Phytochemical Screening

Based on the results of phytochemical tests on tobacco extract and babadotan extract, tobacco contains alkaloid, terpenoid and steroid compounds. This is also appropriate inside Khalalia (2016) that tobacco extract contains secondary metabolite compounds in the form of nicotine and alkaloids. Meanwhile, babadotan extract contains flavonoids, terpenoids, steroids, saponins and tannins. In research (Mentari et al., 2020) that babadotan extract contains secondary metabolite compounds such as flavonoids and steroids. Purwanti, (2022) states that babadotan extract also contains flavonoids, steroids, saponins and tannins.

Table 1. Results of phytochemical screening of tobacco and babadotan

	Reactor	Tobacco	Babadotan	Change
alkaloids	Bouchardart	+	-	Brick Red Precipitate

	Maeyer	+	-	Yellowish White Precipitate
	FeCl3	-	+	Black Colloid
Flavonoids	Mg. HCl	-	-	Pink Solution
	H2SO4	-	-	Yellowish Orange Solution
Terpenoids	Liebermann-Bouchard	+	+	Bluish Green Solution
	Salkowsky	+	+	Red Solution
Steroids	Liebermann-Bouchard	+	+	Bluish Green Solution
	Salkowsky	+	+	Red Solution
Saponins	Aquades	-	+	Foamy
Tannin	FeCl3	-	+	Black Colloid

Description: there is a compound (+), there is no compound (-)

Mortality Rate of *S. exigua*

The results of the variance test showed similar results influential and effective on *S.exigua* mortality. Observation of 96 JSA in treatment T6 had an effect on *S.exigua* mortality by 75% compared to other treatments. Apart from that, T4 treatment also had an effect on *S.exigua* mortality of 72.50%. The T1 treatment had no significant effect on the mortality of *S.exigua* larvae, where the percentage produced was equal to 22.50%. This is due to the lower content of secondary metabolite compounds in tobacco. Apart from that, concentration is also a factor so that treatment T1 has no real effect on the mortality of *S.exigua* larvae.

Table 2. percentage of *S. exigua* mortality due to *N. tabacum* and *A. conyzoiden* extracts

Treatment	Average Mortality of *S. exigua*			
	24 JSA	**48 JSA**	**72 JSA**	**96 JSA**
T0 (Control)	0.00a	0.00a	0.00a	0.00a
T1 (Tobacco Leaf Extract 30%)	0.00a	0.00a	7.50ab	22.50cd
T2 (E. Tobacco Leaf 60%)	0.00a	5.00ab	10.00bc	30.00de
T3 (E. Babadotan Leaves 30%)	2.50a	10.00bc	22.50cd	47.50fg
T4 (E. Babadotan Leaves 60%)	7.50ab	22.50cd	42.50ef	72.50h
T5 (E. Tobacco Leaves 15% + E. Babadotan Leaves 15%)	5.00ab	12.50cd	27.50de	60.00gh
T6 (E. Tembakau leaves 30% + E. Babadotan leaves 30%)	15.00cd	30.00de	47.00fg	75.00h

Information: Numbers followed by the same letter indicate that they are not significantly different in the 5% Duncan test

The test results showed that the highest death rate for the test pests was found in T6 with a mortality percentage75% 96 JSA. The death of the test pest occurred due to the combination of N. tabacum extract which was more capable of causing the death of the test pest. N. tabacum extractif combined, the domain will release more nicotine compounds and alkaloid compounds which are toxic. This is in accordance with research (Ogbalu et al., 2014; Serdani et al., 2022) The combination of tobacco extract contains nicotine and alkaloid compounds which are toxic to insect pests. Apart from that, the death of the test pests was also caused by the smell of the A. conyzoides extract which was pungent and caused a high level of insect pest mortality. Kinasih et al., (2013) stated that the odor of A. conyzoides extract is very strong and high concentrations can cause increased death of insect pests.

The test results for the lowest larval mortality percentage were found in T1 with a mortality percentage of 22.50%. This is caused by the content of secondary metabolite compounds in tobacco being less than babadotan. Secondary metabolite compounds in tobacco are alkaloids, terpenoids and steroids. The secondary metabolite compounds in tobacco that are more efficient in killing larvae are alkaloids. This is in accordance with research by Khalalila (2016), stating that larval death is caused by active compounds in tobacco, namely alkaloids. Alkaloids Tobacco has a function as a contact poison, stomach poison and fumigant (Afifah et al., 2015). Terpenoids function as appetite inhibitors and cause dizziness in insect pests. Steroids have a function as inhibitors of the growth of insect pests (Purwatiningsih et al., 2019). In addition, low concentrations at T1 greatly influence the percentage of larval mortality. Wowor et al., (2022) stated that the higher the concentration used, the greater the percentage of pest mortality.

Mortality percentage of test pests in treatment T4 at 96 JSA, namely72.5%. Treatment T4 is very significantly different from treatments T1, T2, and T3. This is because babadotan has higher toxic properties than tobacco. Apart from that, babadotan has secondary metabolite compounds such as flavonoids, terpenoids, steroids, saponins and tannins which can influence pest growth and increase pest mortality. Flavonoids function as neurotoxins that enter through the insect's respiratory system (Khalalia, 2016). Tannin functions as defense for plants, as well as disrupting the digestive system of insect pests, resulting in death (Tahtameirosi et al., 2022). Saponin has a bitter taste and smell, so this compound functions as an inhibitor and reduces the appetite of insect pests (Ningsih, 2018). This is in accordance with the explanation (Suhardjadinata et al., 2019) stated that saponins greatly influence insect mortality rates by damaging nerve cells which can cause a decrease in appetite and weaken insects, resulting in the death of pests.

Pest Attack Symptoms and Behavior

The initial symptoms of death and behavior of *S.exigua* are changes in the color and body shape of the pest. The behavior of the pest *S.exigua* This is in accordance with Nuhurdiman et al., (2018) stating that the behavior that occurs in larvae slows down movement and the body becomes smaller. Symptoms of larval death include turning yellow, blackish brown. Rahmawati et al., (2023) that the symptoms of death are characterized by a gradual change in body color, namely from green, yellowish, brown or blackish and death. Another symptom is the lack of appetite of insect pests. This is caused by the terpenoid content found in babadotan extract (Ningsih, 2018) Terpenoid compounds inhibit appetite.

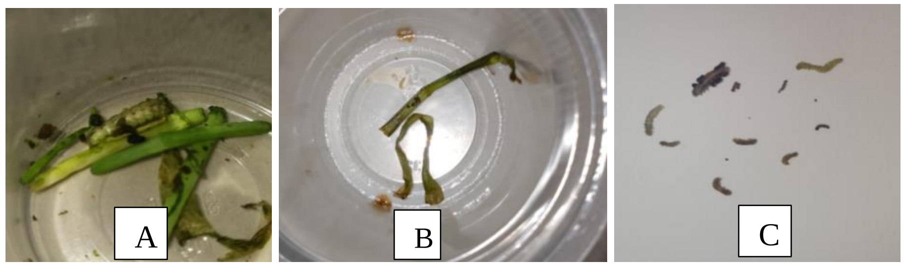

Figure 1. symptoms of *S. exigua* larvae. 1.) *S. exigua* larvae before application, 2.) larvae after application secrete fluid and are black, 3.) larvae dry out and turn black
Source: Live photo

Duration of Death of the Pest *S. exigua*

Based on the data obtained, the fastest larval death occurred in the T6 treatment at 24 JSA. The longest time to death was in treatment T1, which took 72 JSA. Based on the data above, the length of time for pests to die is influenced by the amount of concentration used. The higher the concentration dose used, the higher and faster the effect of pest death will be (Harris et al., 2023). (Nofrianti & Salbiah, 2020) stated that the higher the concentration, the more active compounds there are, which results in increased daily mortality.

Table 3. Time to death of *S.exigua* larvae

Treatment	Time of Death				Total	Average
	24 JSA	48 JSA	72 JSA	96 JSP		
T0	0	0	0	0	0	0
T1	0	0	3	6	9	2.25
T2	0	2	2	8	12	3
T3	1	3	5	10	19	4.75
T4	3	6	8	12	29	7.25
T5	2	3	6	13	24	6
T6	4	6	7	11	28	7

Biosafety Test

The research results were obtained from testing the highest mortality rate in *S.exigua* larvae, namely in the T6 treatment. Figure 2 shows that the combined extract of tobacco and babadotan botanical pesticides can cause mortality of the parasitoid Trhicograma spp and the predator *C.sexmaculata*. Where the test results for the parasitoid Trhicograma spp experienced very high mortality, with a result of 100%. In testing, *C.sexmaculata* experienced a mortality of 20%.

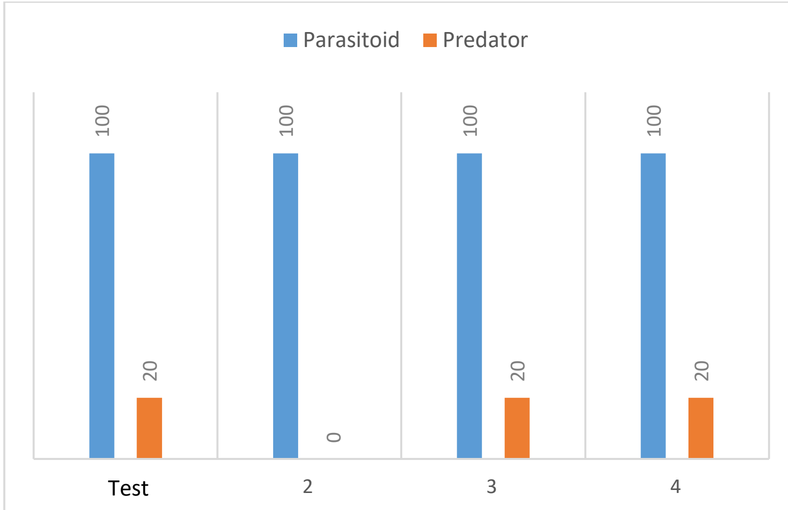

Figure 2. Mortality rate of natural enemies at the highest *S.exigua* mortality rate (T6)

Based on the results of the research above, the combination treatment of tobacco extract and babadotan extract has an effect on the mortality of natural enemies. This is thought to be because there is a high level of nicotine compounds when the two extracts are combined. Apart from that, this is also due to the presence of active compounds that resemble chemicals in babadotan extract which are able to suppress predatory insects. So babadotan extract cannot be combined with pesticides or even other pesticides (Susanti et al., 2016), because it will have a bad impact on natural enemies, but is very effective in controlling pests.

CONCLUSION

Based on the results and discussion, tobacco extract and babadotan extract are effective in controlling the pest *S.exigua*, both single extract and combination. The lowest mortality rate of each treatment is T0 with a result of 0% and T1, namely22.5% onmoment 96 JSA. Meanwhile, the highest mortality rate was in the T6 treatment, namely75% at time 96 JSA.

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