POTENTIAL OF PURE ETHYL ACETATE EXTRACT OF *SYZYGIUM AROMATICUM* LEAVES FOR CONTROLLING *SPODOPTERA EXIGUA* HUBNER A PEST OF GREEN ONION (*ALLIUM FISTULOSUM*)

Juniarny Silfana S. Waworuntu[1], Christina L. Salaki[2], Juliet M. E. Mamahit[3], Dantje Tarore[4]
[1]Universitas Klabat, Airmadidi, Indonesia
[2,3,4] Sam Ratulangi University, Manado, Indonesia

Abstract

Loss of harvest due to leaf damage from the pest *Spodoptera exigua* Hub. can lead to significant economic losses. Clove leaves (Syzygium aromaticum L.) are one of those plants that has potential as a vegetable insecticide to contorl pests. This research aimed to determine the potential of pure extract of clove leaves (S. aromaticum) in controlling *S. exigua* in green onion. The research was conducted at the Laboratory of BPPMTPH (Balai Perlindungan dan pengujian Mutu Tanaman Pangan dan Hortikultura or Food Plant Quality and Horticultural Testing and Protecting Centre) Kalase – Manado from July to October 2023. This research used a completely randomized design consisting of four treatment concentrations with three replications for each treatment. Four treatment concentrations of pure ethyl acetate extract; 0.15%, 0.25%, 0.35% dan 0.45% dan 0% (control) were administered. Each treatment used 15 instar 3 larvae of S. exigua. The testing was conducted using the feeding dip method. The data obtained: a. *S. exigua* mortality b. morphological changes in *S. exigua* larvae. The research results showed that the application of pure ethyl acetate extract from *S. aromaticum* leaves had an effect on controlling *S. exigua*. The potential of the 0.35% extract concentration was the best among all treatments for controlling *S. exigua*, with a total mortality of 86.87%. Morphological changes and death symptoms of *S. exigua* indicate slow larval movement, inactivity, decreased appetite, and incomplete development of pupae and imago.

Keywords: *Syzigium aromaticum*, *Spodoptera exigua* Hub; mortality; physical changes in *S. exigua*; ethyl acetate extract of *S. aromatiucm*

INTRODUCTION

Green onions are one of the leading horticultural commodities in Indonesia; this can be seen from the many regions in Indonesia that cultivate them. The average production growth of green onions in North Sulawesi in the last 3 years (2019 - 2021) has increased (BPS North Sulawesi Province 2021). North Sulawesi Province in 2021 produced 814,690 kw of green onions, an increase from the previous year's 733,996 kw. The increase in demand for green onions from households and food producers causes prices to rise. In supermarkets, green onions are currently 2-3 stalks/bundle IDR 5,000 – IDR 6,000, while retail traders or kiosks price 6-7 stalks/bundle of spring onions, IDR 5,000.

Several horticultural plant locations in North Sulawesi still use a mixture of several types of chemical pesticides with spraying intervals several times a week. Even for some vegetable crops during the vegetative period, farmers spray them almost every day. This is to prevent pest attacks and prevent losses incurred. One significant type of leaf-eating pest is *Spodoptera exigua* Hub. which are those that attack green onions usually attack plants quickly, simultaneously, and in fairly large areas. S. *exigua larvae* instar 3 – 5 bore the leaves and eat the leaf veins causing the plant to run out of leaves. Heavy armyworm attacks occur in the dry season, and cause very heavy leaf defoliation (Santosa & Sumarmi 2015). Therefore, it can be done by using the natural ingredient clove leaves (*Syzygium aromaticum*) as a botanical pesticide. One promising alternative contains bioactive chemicals (Vanichpakorn et al., 2010) and has the potential to be used in integrated pest control (Schmutterer, 1992). Nurhikma et al. (2017) explained that clove leaves (*Syzygium aromaticum L.*) have potential as a vegetable insecticide because they contain eugenol. Furthermore, Mu'nisa et al. (2012) reported that eugenol is the main content of clove leaves, which reaches 90%; eugenol is a respiratory poison that works as a neurotoxin. *S. aromaticum* is an insecticide made from plant materials which contains active compounds in the form of secondary metabolites which are capable of providing one or more biological activities, which can influence both the physiological and behavioral aspects of plant pests and fulfill the requirements for use in pest control (Safirah et al ., 2016). Alvionita Djau et al. (2022) applied pesticides made from clove leaves to control aphid pests (Aphid sp.) on red chili plants. This research aims to obtain a concentration of pure ethyl acetate extract from *S. aromaticum* leaves that effectively controls the armyworm *S. exigua*.

Materials and Methods

This work was carried out in the Laboratory of BPPMTPH (*Balai Perlindungan dan pengujian Mutu Tanaman Pangan* and Hortikultura or Food Plant Quality and Horticultural Testing and Protecting Centre) of North Sulawesi Province, from July to October 2023. The materials used for the experiment were *S. exigua* Hub. larvae, purple basil (*Ocimum sanctum).*

Sample collection

The test animals were collected directly from the green onion plantation in Passi Timur district, Bolaang Mongondow Regency, in the form of *S. exigua* eggs, and hatched in the Laboratory of BPPMTPH. The leaves of the basil (O. sanctum L) were collected from Touliang, Kakas district, Minahasa regency. As many as 20 green onions of the local variety were planted in BPPMTPH yard. The plants were reared without fertilizer treatments.

Method

The clove leaf-based extract was processed from 4 kg of fresh clove leaves cleaned and wind-dried for 3-4 days. The dry leaves were then blended into powder. As much as 500 gr of clove leaf powder was macerated in 1 L of technical methanol for one-night, then filtered to obtain the extract solution. The dregs were macerated again for another night and then filtered. The same procedure was repeated 2 times using the same dregs for a total of 4 macerations, and 3.5 L of extract solution was obtained. It was then evaporated to obtain 80 mL of viscous extract. The viscous extract was macerated again for one-night in methanol PA and x-hexane at the ratio of 1:1:1 (80 mL solid extract:80 mL of n-hexane:80 mL of methanol PA). The extract was then separated and evaporated, and 22 mL of n-hexane fraction was obtained. It was macerated for one-night in the distilled water and ethyl acetate at the ratio of 1:1:1 (25 mL of n-hexane extract:25 mL of distilled water:22 mL ethyl acetate), evaporated, and obtained 12 mL of ethyl acetate fraction extract. Each treatment used 15 larvae with 3 replications. The study used a Complete Randomized design with 4 treatments of *S. aromaticum* extract at the concentrations of 0.15% (0.15 ml/100 ml distilled water), 0.25% (0.25 ml/100 ml distilled water, 0.35% (0.35 ml/100 ml distilled water), and 0.45% (0.45 ml/100 ml distilled water), and a control treatment of 0%.

The 8 oz-plastic glasses layered with a sheet of tissue and a piece of fresh free pesticide green onion (3 cm x 3 cm) totally dipped into the extract solution until all leaf pieces submerged in the extract solution for 3 minutes and then wind-dried were prepared. The instar III larvae were placed into the plastic glass covered with a gauze to let the air in and out. The mortality of larvae was recorded daily, and the onion leaf fed was replaced with a new leaf piece on the new tissue. The data were analysed with ANOVA, then continued with Duncan test.

Results and Discussion

Daily Mortality (%)

Daily mortality of test insect *S. exigua* Hub. after application with several concentrations of pure extract of S. *aromaticum* leaves showed that there was death of *S. exigua* on the first day to the 12th day.

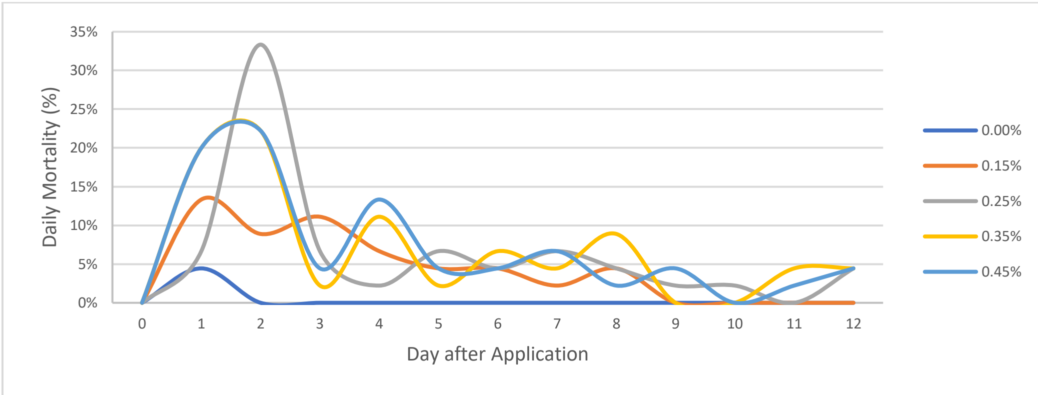

Figure 1. Daily Mortality of the Test Insect *Spodoptera exigua* after administration of pure ethyl acetate extract form *S. aromaticum* leaves

In Figure 1., mortality of *S. exigua* larvae on day 1 occurred at all treatment concentrations. The death of *S. exigua* larvae on day 1 at a concentration of 0.45% showed the highest mortality of 20%, then increased to 22% on day 2. However, the highest mortality was on day 2 at a concentration of 0.25% with a total mortality of 33%. The active ingredient eugenol contained in *S. aromaticum* leaves works as a stomach poison, damaging the digestive system of *S. exigua*, resulting in death. Rismayani and Laba (2015) explained that eugenol is effective in controlling *C. pavonana* with mortality of 72-86% at a concentration of 2-4 ml l^{-1}. The daily mortality of *S. exigua* continued to increase for each concentration of extract treatment until day 11 and day 12. Most of the mortality of *S. exigua* occurred in the 3rd and 4th instar larvae. The surviving larvae can reach the 5th instar, even up to the pupal and imago stages, but their development is not complete. The potential of pure ethyl acetate extract of *S. aromaticum* leaves given to *S. exigua* at the beginning of application has influenced the growth physiology of insects with the symptoms shown: less active, slow movement, reduced appetite, and eventually death. According to Lestari et al. (2014), the eugenol contained in clove leaves has neurotoxic properties which cause insects to become inactive. Neurotoxic works by suppressing the insect's nervous system. N. S. Aminah (2001) determined that the metabolites contained in S. aromaticum can interfere with insects in digesting food. This happens because tannins will bind to proteins in the digestive system which insects need for their growth so that the process of protein absorption in the digestive system is disrupted.

Total Mortality (%)

Treatment with a concentration of 0.45% showed the highest total mortality of 88.87% compared to other treatments. Eugenol is the main content of clove leaves which reaches 90%, the results of GC-MS analysis of active ingredient eugenol from pure extract of *S. aromaticum* leaves reached 97.74 %. Talahatu (2015) indicated that eugenol is a respiratory poison and can affect the nervous system, so it can cause death in insects.

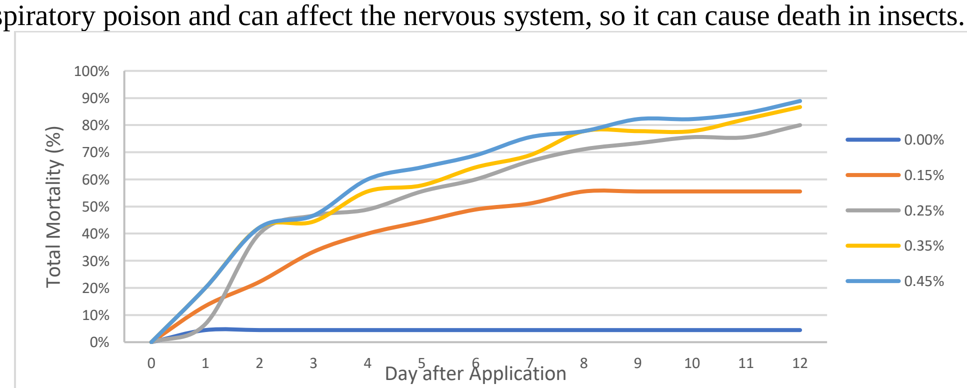

Figure 2. Total Mortality of *S. exigua* Hub. Larvae with Ethyl Acetate Fraction Extract Treatment from *S. aromaticum* leaves.

Figure 2. shows that the total mortality rate of *S. exigua* continues to increase until day 12. This proves that the pure ethyl acetate extract of *S. aromaticum* leaves, which has been applied to green onion as food for S. exigua, affects the *S. exigua* pest. The 0.45% concentration has a higher killing power compared to other concentrations. However, Duncan's analysis shows that a concentration of 0.35% is the best and most efficient for controlling the *S. exigua* pest. Duncan's test results showed that the application of *S. aromaticum* extract has an effect on the mortality of *S. exigua* Hub.

Table 3. Percentage of *S. exigua* larval mortality at various concentrations of *S. aromaticum* leaf extract

Treatment	Total Mortality (%)
Concentration without treatment (0%)	6.70 a
Concentration of extract 0.15%	71.13 b
Concentration of extract 0.25%	80.00 bc
Concentration of extract 0.35%	86.87 c
Concentration of extract 0.45%	88.87 c

Note: Average values in the column followed by the same letter in the same column indicate no significant difference

Table 3 shows that the percentage of total mortality of *S. exigua* larvae after administration of pure *S. aromaticum* leaf extract on day 1 to 12 of observation, in all concentration treatments shows that all concentration treatments are significantly different from the control. The lowest mortality of *S. exigua* was in the 0% concentration treatment with a mortality of 6.70%, while the highest mortality of S. *exigua* in the 0.45% concentration treatment was 88.87%, very significantly different from the 0.15% concentration but not significantly different from the 0.25% concentration and the 0.35% concentration. Meanwhile, the 0.25% concentration treatment was not significantly different from the 0.35% dan 0.45% concentrations. The concentration of 0.45% shows the concentration with the highest mortality of all treatments because it can kill 88.87% of insects. However, the concentration of 0.35% (3.5 $ml.l^{-1}$ water) is the best concentration which is not significantly different from the treatment with a concentration of 0.45% (4.5 $ml.l^{-1}$ water) showing that increasing the concentration of clove leaf extract from 0.35% menjadi 0.45% did not make a significant difference in *S. exigua* mortality. A concentration of 0.35% is a concentration that can kill 80% of the test insects and is an effective and more efficient concentration than a concentration of 0.45%. Dadang and Prijono (2008) stated that the extract concentration exceeds the determination as an effective vegetable insecticide if it causes the death of test insects equal to or greater than 80% for organice solvent extracts with a maximum concentration of 1%.

Symptoms of the death of the *S. exigua* insect are characterized by decreased appetite, slow larval movement and the insect's body color is blackish brown. This is in accordance with the opinion of Zulyusri (2000) that the symptoms of poisoning experienced by onion caterpillars are reduced activity, such as moving more slowly than before, staying in place, and decreasing appetite until finally experiencing death, which is marked by a change in body color. Djojosumarto (2008) reported that the eugenol compound inhibits the work of the acetylcholinesterase enzyme, resulting in a buildup of acetylcholine. As a result, it causes insects to experience tremors (shaking), convulsions and paralysis which ultimately causes death. In addition, the eugenol compound disrupts the insect's respiratory system by entering through the spiracles along with O2, making it difficult for the insect to breathe.

Morphological Changes in *Spodoptera exigua*.

The results of the research after the administration of pure extract from *S. aromaticum* leaves showed several morphological changes in *S. exigua*, including body color, body shape, and imperfections in metamorphosis. The caterpillars experiencing poisoning began with a fading body color, and after several hours, the surface of the caterpillar's body appeared darkening to a blackish hue. The indicators of death in *S. exigua* were marked by the darkening of the caterpillar's surface, which even turned black as if burnt. The change in the larval body to a brownish-black color is suspected to be due to the larvae undergoing melanization. According to Indriyanti et al. (2017), the formation of melanin is known as melanization, which is facilitated by the enzyme phenoloxidase, characterized by a brown or black color.

(a) (b) (c) (d) (e) (f)

Figure 3. Morphological changes in the test insect *Spodoptera exigua* Hub before and after the application of *S. aromaticum* (a) healthy larva without treatment (b) larva after application showing a brownish color, blackening, and becoming rigid like a mummy (c) pupa after application turning brownish-black, (d)pupa shrinking and failing to become an imago, (e) imago with imperfect wings and antennae, (e) healthy image without treatment.

The pupa turned brownish-black and its body shape shrank, with some test insects dying before completing pupation. Similarly, in the image stage, the development of wings and antennae was imperfect, ultimately resulting in death with deformities.

Conclusion

The mortality of *S. exigua* insects occurred on the first day for all treatment concentrations after applying pure ethyl acetate extract from S. *aromaticum* leaves. The concentration of 0.45% exhibited the highest mortality rate among all treatments at 88.87%. The concentration of 0.35% was found to be the most effective and efficient for controlling *S. exigua*, with a total mortality rate of 86.87%. After application, morphological changes and signs of death in S. exigua showed slow movement, inactivity, decreased appetite, and the larvae become rigid and mummified, with imperfect development of pupae and imagos.

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