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Bioactivity effect of *Elephantopus scaber* Linn. extracts against *Spodoptera litura* and the soil microbial community

Efeito da bioatividade de extratos de Elephantopus Scaber Linn. em Spodoptera litura e na comunidade microbiana do solo

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ABSTRACT

This study investigates the biopesticidal effects of *Elephantopus scaber* Linn. extract on mortality of *Spodoptera litura, Plutella xylostella*, and non-target organisms and investigate the impact on *S. litura* protein levels and soil microbial community structure. The experiment was performed using a completely randomized design. Methanol extracts from *E. scaber* leaves, at concentrations of 2%, 4%, 6%, 8%, 10%, and 12%, were tested for bioactivity against the 2nd-instar larva of *S. litura, P. xylostella*, and earthworms. Mortality rates of the larvae and worms were observed. The collected data were analyzed using analysis of variance (ANOVA), followed by probit and descriptive analysis. The results showed that methanol extracts of *E. scaber* (12%) influenced the highest mortality rates for both *S. litura* (93.35%) and *P. xylostella* (96.65%) with LC50 and LC80 of *S. litura* was 1.867 and 4.763; for *P. xylostella* were 4.488 and 7.92, respectively. However, the application of *E. scaber* biopesticide also influences earthworms' mortality rate. The 6% *E. scaber* extract resulted in 60% death of earthworms during a 20-days period. In addition, higher concentrations of *E. scaber* extracts resulted in lower molecular weights and levels of *S. litura* proteins. The diversity and density of the soil microbial community also decreased by 6% concentration.

KEYWORDS: biopesticidal; community structure; mortality; protein level.

RESUMO

Este estudo tem como objetivo investigar os efeitos biopesticidas de *Elephantopus scaber* Linn. extrair na mortalidade de *Spodoptera litura, Plutella xylostella* e organismos não-alvo e investigar o impacto nos níveis de proteína de *S. litura* e na estrutura da comunidade microbiana do solo. Experimento realizado em delineamento inteiramente casualizado. Extratos de metanol de folhas de *E. scaber*, nas concentrações de 2%, 4%, 6%, 8%, 10% e 12%, foram testados para bioatividade contra a larva de 2º ínstar de *S. litura, P. xylostella* e minhocas. Taxas de mortalidade de larvas e vermes foram observadas. Os dados coletados foram analisados por meio de análise de variância (ANOVA), seguida de probit e análise descritiva. Os resultados mostraram que os extratos metanólicos de *E. scaber* (12%) influenciaram as maiores taxas de mortalidade para *S. litura* (93,35%) e *P. xylostella* (96,65%) com CL50 e CL80 de *S. litura* foi de 1,867 e 4,763; para *P. xylostella* foram 4,488 e 7,92, respectivamente. No entanto, a aplicação de biopesticida de *E. scaber* também influencia a taxa de mortalidade de minhocas. O extrato de 6% de *E. scaber* resultou em 60% da morte de minhocas durante o período de 20 dias. Para além disso, maiores concentrações de extratos de *E. scaber* resultaram em menores pesos moleculares e níveis de proteínas de *S. litura*. A diversidade e a densidade da comunidade microbiana do solo também diminuíram na concentração de 6%.

PALAVRAS-CHAVE: biopesticidas; estrutura da comunidade; mortalidade; nível de proteína.

INTRODUCTION

The application of synthetic pesticides has increased and has recently been considered an effective and efficient way to control pests and diseases. However, synthetic pesticides may cause environmental pollution through residual active compounds that are toxic to soil and affect non-target organisms, microorganisms, and enzymatic activity (TEHRI & SINGH 2015, OLESZCZUK et al. 2014). Therefore, in line with agroecosystem management strategies, pest and disease control strategies have begun to lean towards biological control measures.

Biopesticides represent a promising alternative to synthetic pesticides. Biopesticides or bioactive compounds derived from plants are considered to be safer and more friendly for the environment, and less likely to affect non-target organisms. Plant compounds are generally easy to degrade. Therefore, residues are less likely to have negative impacts on the environment. Bioactive compounds can be used to replace at least some hazardous chemical pesticides when incorporated into an integrated crop management strategy (KUMAR 2012).

In addition to producing primary compounds during plant metabolism, plants also produce secondary metabolites, such as phenolic compounds, alkaloids, terpenoids, and sulfur compounds (SENGOTTAYAN 2015). Various parts of plants can be used as biopesticides, including leaves, stems, flowers, seeds, and fruit (WINK 2010). Secondary metabolites, such as tannins, alkaloids, flavonoids, saponins, and phenols, are produced by plants and used during defense mechanisms against insects and many of these compounds can inhibit insect metabolism (LARRY 2002, CRUZ et al. 2018).

The use of plant secondary metabolites as insecticides can cause death at an early age, declining growth rates, shrinking body sizes, relatively short life spans, abnormal morphologies, and anxiety or other abnormal behaviors in insects (LARRY 2002). Active compounds found in the leaves, roots, tubers, seeds, and fruits, which are secondary metabolites, may interfere with the physiology and growth of insects (BIDLACK et al. 2000, KUSNADI 2003, MOSSA 2016), through various mechanisms. Terpenoids and diterpenoids act as antibacterial and antifungal agents, reducing cell membrane permeability. Terpenoid compounds can bind to protein and lipid molecules, affecting the physiological functions of cell membrane proteins and protein enzymes. In contrast, phenol compounds can form complexes with proteins through hydrogen bonds, inhibiting the formation of proteins and nucleic acids. In addition, phenol can dissolve lipids from cell walls due to the presence of an -OH group, which can interfere with and affect the integrity of the cytoplasmic membrane, causing cell lysis and inhibiting ATPase binding to the cell membrane (ROBINSON 1975, ABDUL et al. 2018).

Elephantopus scaber Linn. contains several secondary metabolites, such as flavonoids (methanol leaf extracts), alkaloids, tannins (methanol and chloroform leaf extracts), phenols (methanol leaf-rhizome extracts), proteins, glycosides, saponins (methanol rhizome extracts), terpenoids (methanol and chloroform leaf-rhizome extracts), steroids (methanol and chloroform leaf-rhizome extracts), triterpenoid, and elephantopin (YULIANI et al. 2018). *E. scaber* extracts also contain epifriedelinol, lupeol, stigmasterol, lupeol acetate, deoxyelephantopin, isodeoxyelephantopin, sesquiterpene, and luteolin-7-glucoside, which demonstrate various pharmacological activities, such as anti-tumor, anti-cancer, anti-inflammatory, antibacterial, and antifungal activities (FARHA & REMANI 2014). Ethanol and/or methanol extracts from the leaves and roots of *E. scaber* demonstrate antibacterial properties and have been shown to inhibit the growth of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (MOHAN et al. 2010). Alcohol and chloroform *E. scaber* extracts contain toxic germacranolide (sesquiterpene lactone), acting as an analgesic, diuretic, and anti-inflammatory in water extracts (LI et al. 2004).

Recently, biopesticide research has become relatively well-developed. BALFAS & WILLIS (2009) used *E. scaber* leaf extracts as insecticides. They found that a concentration as low as 0.5% could cause more than 90% mortality in *Spodoptera litura* that lived in the taro plant, Bogor City, Indonesia. Methanol extracts from the *Pluchea* genus demonstrated insecticidal effects, whereas chloroform extracts may act as anti-nematode and anti-bacterial treatments (TRAITHIP 2005). YULIANI & RAHAYU (2017) demonstrated that *Pluchea indica* leaf extracts, at a concentration of 12%, may cause optimal mortality among *S. litura* (81.9%). A study conducted by BRISCA & KITHERIAN (2009), who investigated the effects of *Ageratum conyzoides* and *Ageratum vulgaris* collected from the Western Ghats and around Coonoor, India, concluded that ethanol extracts, at a concentration of 0.01 µL/insect, can significantly decrease the total protein levels of *S. litura*.

Environmental changes caused by active biopesticide compounds might also affect other organisms living in these ecosystems, including both target and non-target organisms, which will respond to environmental changes by synthesizing certain proteins. Therefore, the presence of certain proteins may be used as an indicator that an applied biopesticide might cause environmental changes that induce stress on other organisms within the same ecosystem.

Biopesticides refer to any pesticide derived from natural materials. However, the use of biopesticides may affect flora and fauna, in addition to the physicochemical factors of the soil (CHANDLER et al. 2011). Moreover, the active compounds found in biopesticides may influence non-target organisms that live in the same ecosystem as pests, such as soil fauna (earthworms) and microorganisms. Soil microorganisms play important roles in the decomposition of organic matter, the maintenance of crop productivity, and nutrient cycling (SETHI & SAKSHAM 2013). The soil microbial structure can be influenced by several factors,

including environmental conditions, the local microorganisms, and the ecological niche (JURG et al. 2015).

Therefore, biopesticide development should include the use of scientific studies to determine the pests that can be eradicated and controlled without affecting non-target organisms and determine the proper dosages required to avoid unnecessary effects on non-target organisms and soil microbes. Moreover, understanding the adaptability of non-target organisms during the response to biopesticides is essential to avoid unintended consequences on the density and diversity of the soil microbial community, which are beneficial for the agroecosystem.

Therefore, the objective of this study was to investigate the effects of *E. scaber* Linn extracts on pest mortality, especially for *S. litura* and *Plutella xylostella*, and the impacts of these extracts on non-target organisms (earthworms), the protein levels of *S. litura,* and the soil microbial community structure.

MATERIAL AND METHODS

Manufacturing plant extract

Elephantopus scaber Linn. leaves were obtained from middle lands habitat altitude in Mojokerto, East Java, Indonesia. *E. scaber* leaves were dried for ten days (at room temperature), then grounded into powder, and sieved with a 40-mesh. The extraction process followed used a modified version of the procedure described by DAMIEN & RAIMO (2004). *E. scaber* powder was macerated with petroleum ether (1:4 w/v), at room temperature for 24 hours. The dried residue was then extracted with methanol (1:15 w/v), using a Soxhlet extractor, at 65 °C, for three hours. Finally, the solvent was evaporated under low pressure, using a rotary evaporator to obtain the methanol extract.

Analysis of bioactive compounds

Total phenolic contents were measured using the Folin-Ciocalteu method (SINGLETON et al. 1999, STANOJEVIĆ et al. 2009) using UV Spectrophotometry 1800. The analysis used gallic acid as the standard (concentrations from 0.125 to 0.625 mg/mL). First, 1 ml of *E. scaber* extract was added to 4 mL sodium carbonate (75 g L^{-1}) and mixed until homogeneous. After that, the mixture was added to 0.2 mL of Folin-ciocalteus phenol reagent. Finally, the mixture was diluted with distilled water. After 90 minutes, the mixture was measured at a wavelength of 760 nm to determine the total phenolic content equivalent to gallic acid (GAE).

Total flavonoid contents were measured based on the aluminum chloride colorimetric method, described by KUMAR et al. (2008) and WIJAYA et al. (2011). 1 mL of *E. scaber* extract was added with 4 mL 0f distilled water and 0.3 mL of 5% (w/v) $NaNO_2$ solution. After 5 minutes, the mixture was added with 0.3 mL of 10% (w/v) AlCl3 and 2 mL of 1 mol L^{-1} NaOH. The mixture was diluted with distilled water. The absorbance was measured at a wavelength of 352 nm to determine flavonoid content equivalent to quercetin (QE).

Biopesticide bioactivity against target organisms

The target organisms used in this experiment were *S. litura* and *P. xylostella* 2nd-instar larvae. The experimental design included various concentrations of plant extract (0%, 2%, 4%, 6%, 8%, 10%, and 12%) and two different larvae (*S. litura* and *P. xylostella*) as experimental variables, whereas the response variable was the mortality rate. The experiment was performed using a completely randomized design (CRD), with the extract concentration and the larvae species as the two treatment factors. Each treatment was repeated three times, using ten larvae each. A total of 42 different treatments were tested. The research outline included the manufacturing of the extract, the preparation of leaves for examination, larval preparation, and bioactivity testing.

Furthermore, a bioactivity test was conducted with *E.scaber* extract exposed to *S. litura* and *P. xylostella* 2nd-instar larvae. Methanol extract from *E. scaber* was diluted with 10% dimethylsulfoxide (DMSO) to obtain the proper concentrations. Mustard leaves were used as larval food. Cabbage leaves were cut into a circular shape, weighed, and placed into a container, followed by adding 0.2 mL of methanol extract. The container was closed and observed every day for seven days. The measured parameter was larval mortality (the number of dead larvae each day). Larval mortality was determined based on Equation 1 (BASKAR et al. 2011):

$$\text{Larval mortality} = \frac{\%\ \text{mortality of treatment sample} - \%\ \text{mortality of control sample}}{100 - \%\ \text{mortality of control sample}} \times 100 \quad (1)$$

Biopesticide bioactivity against non-target organisms

Earthworms, *Lumbricus* sp., weighing approximately 0.6 g, were used as non-target organisms. Earthworms were placed into a container containing a growth medium enriched with methanol extract and

observed for 20 days. Methanol extracts were administrated once every ten days. The experimental design used different concentrations of *E. scaber* methanolic extracts (0%, 2%, 4%, and 6%) as the only treatment factor. The experiment was performed using a completely randomized design (CRD), with three replications, using 15 earthworms each. 500 g of regosol soil was used as a medium for growing earthworms and placed in a pot. Earthworms were acclimatized for three days in planting media, with each pot consisting of three earthworms. 5 mL of methanol extract from *E. scaber* leaves was added to the pot on the first and tenth days. Earthworm mortality was observed every 48 hours for 20 days.

Quantification of *S. litura* protein content

Target organisms (*S. litura*) were cultured on a laboratory scale and exposed to *E. scaber* extracts, at concentrations of 4% and 6%, for three days (concentrations were determined according to the 80% lethal concentration/LC80 values of the extracts). After treatment, proteins were isolated from *S. litura* without exposed with *E. scaber* extracts as control and *S. litura* that were exposed to *E. scaber* extracts. The concentration of the protein was determined with UV spectrophotometry at a wavelength of 550 nm. The isolated proteins were also visualized using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (FREDERICK et al. 2003). The parameter observed during SDS-PAGE analysis was the presence of different protein bands for target organisms exposed to biopesticides, including the molecular weights (MWs).

The impacts of biopesticide applications on soil microbial communities

Testing of methanol extract from *E. scaber* leaves was carried out on bacteria present in soil samples from organic rice paddy soil. Each of the 4% and 6% *E. scaber* extracts was added to 250 mL of nutrient agar (NA) growth medium (NA medium at a temperature of ± 50-60 ^{o}C). Then, the extract and the NA medium were homogenized and added to a petri dish containing 1 mL of the soil sample suspension. The soil suspension used was seven soil suspensions with different soil content made through graded dilutions. Testing of each suspension was carried out two times. Then, the isolates were incubated for 24 hours at 30 °C. The impacts of biopesticides on bacterial isolates were recorded, including the total microbial growth, diversity, and community structure (soil microbial community structure before and after treatment).

Statistical analysis

Bioactivity data were analyzed by two-way analysis of variance (ANOVA) to determine the effects of different biopesticide concentrations and larval species on the percentage of larval mortality. The effects of biopesticide concentrations on earthworm mortality were analyzed by one-way ANOVA, followed by Duncan's post hoc test, to measure differences between pairs of means. The 50% and 80% lethal concentrations (LC50 and LC80, respectively) were determined by probit analysis using Minitab 17.

RESULTS

Levels of phenolic and flavonoid compounds from *E. scaber* methanolic extracts

The phytochemical analysis of phenolic and flavonoid contents, assessed by ultraviolet-visible (UV-Vis) spectrophotometry, can be observed in Table 1. The results showed that *E. scaber* contains higher phenol contents (0.208 mg/mL) than flavonoid contents (0.010 mg/mL). The plant extracts were then administered to both target organisms, *S. litura* and *P. xylostella*, and non-target organisms.

Table 1. Phenol and flavonoid contents of *E. scaber* methanolic extracts.

Replication	Flavonoid contents (mg/mL)	Phenolic contents (mg/mL)
1	0.009	0.214
2	0.010	0.200
3	0.011	0.211
Average	0.010	0.208

Biopesticide bioactivity against target organisms

The results indicated no significant differences in larval mortality between the two tested species when treated with different concentrations of *E. scaber* extracts (two-way ANOVA; Sig.<0.05 where 0.001<0.05 and the calculated F-value>F critical value where F critical value is $F_{(6.14)}$=2.8484; 7.345>2.848). Therefore, the larval mortality rates of *S. litura* did not significantly differ from those of *P. xylostella*. In addition, no significant interaction was observed between the concentration of *E. scaber* extract used and the larval species on larval mortality rates (Sig.<0.05 where 0.995<0.05 and the calculated F-value<F critical value where F critical value is $F_{(6.14)}$=2.8484; 0.104<2.848). The larval mortality percentages for each extract concentration and each species are presented in Table 2.

Table 2. Mean larval mortality rates for *S. litura* and *P. xylostella* treated with *E. scaber* extracts.

Concentration of *E. scaber* extract	Mean percentage of larval mortality rate	
	Spodoptera litura	*Plutella xylostella*
0% (Control)	10.00 ± 1.414 a	13.350 ± 1.887a
2%	36.65 ± 2.354 b	60.00 ± 4.242 b
4%	70.00 ± 2.361 bc	78.35 ± 2.595 bc
6%	73.30 ± 2.361 bc	86.65 ± 1.887 bc
8%	76.65 ± 2.354 bc	90.00 ± 1.414 bc
10%	81.70 ± 2.121 c	93.35 ± 0.940 c
12%	93.35 ± 0.940 c	96.65 ± 0.473 c

Note: The same letters in the same row and column represent statistically insignificant according to BNT 5%.

Duncan's post hoc test was performed to investigate further significant differences among the different concentrations of extracts used. The lowest mortality rate was found for *P. xylostella* larvae treated with the control (0%) dose of *E. scaber* extract, which was significantly different from other treatments. Treatment using the 2% concentration of *E. scaber* extract showed significantly different mortality rates compared with the 10% and 12% treatments, whereas no significant differences were observed when compared with the 4%, 6%, and 8% treatments. The highest mortality rate was found for the highest *E. scaber* concentration (12%), although no significant difference was observed compared with those for 4%, 6%, 8%, and 10% concentrations.

LC50 and LC80 values for *S. litura* and *P. xylostella*

Biopesticide treatments containing various concentrations of *E. scaber* extracts significantly influenced the larval mortality of both *S. litura* and *P. xylostella* (probit analysis, p=0.000). Furthermore, the regression coefficient indicated a positive correlation between *E. scaber* concentration and larval mortality rate, indicating that higher concentrations of *E. scaber* resulted in higher larval mortality rates.

The LC50 value for *P. xylostella,* which was approximately 4.48806%, was higher than that for *S. litura*, indicating that an *E. scaber* extract concentration of 4.48806% can kill 50% of the total *P. xylostella* larval population. The LC80 value was approximately 7.92188%, implying that an *E. scaber* concentration of 7.92188% can kill 80% of the *P. xylostella* population. These results showed that the toxicity of *E. scaber* extract against *P. xylostella* is quite high, although the extracts appear to be more fatal for *S. litura* (Table 3).

Table 3. LC50 and LC80 values for *S. litura* and *P. xylostella* treated with *E. scaber* extract.

Larvae species	Concentration of *E. scaber* methanolic extract (%)	
	LC50	LC80
Spodoptera litura	1.86773 ± 0.466	4.76338 ± 0.447
Plutella xylostella	4.48806 ± 0.432	7.92188 ± 0.524

Bioactivity test against non-target organism

Biopesticide bioactivity was also evaluated against non-target organisms, earthworms, to determine the impacts of the biopesticide on other organisms that live on organic rice farms. The extract concentration used to test non-target organisms were 0%, 2%, 4%, and 6%, which were determined based on the LC50 and LC80 values. The results of Duncan's test showed that the lowest mortality rate was detected for earthworm treated with the *E. scaber* control treatment, which was significantly different from those for the other concentrations. The mortality rate for the 2% treatment was also significantly different from those for the other concentrations of *E. scaber* extracts. The highest mortality rate was detected for earthworms treated with the highest concentration of *E. scaber* extract (6%), which was not significantly different from that for the 4% treatment (Table 4).

Table 4. Mean percentage of earthworm mortality rate.

The concentration of *E. scaber* extract	Percentage mortality of earthworm
Control (0%)	22.22 ± 10.183 a
2%	44.44 ± 7.696 b
4%	66.66 ± 6.665 c
6%	80.00 ± 17.637 c

Earthworm mortality began to be observed on day six for extract concentrations of 2%, 4%, and 6%. The earthworm mortality rate, recorded every 48 hours for 20 days, is presented in Figure. 1. The results showed no recorded mortality on days two, four, 14, or 20. However, oin day six, the highest mortality was recorded among earthworms treated with the 4% and 6% concentrations of *E. scaber* extract.

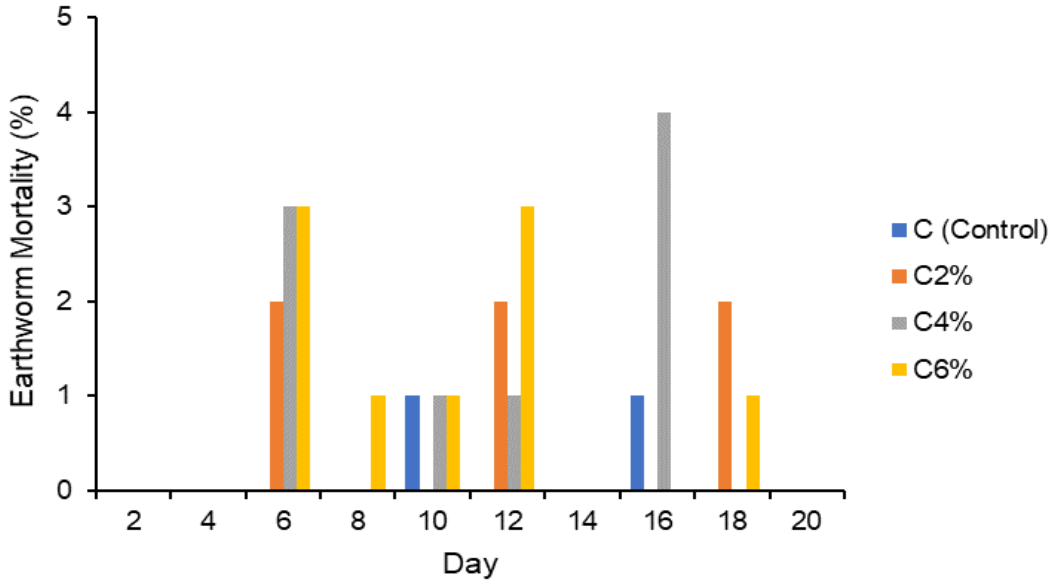


Figure 1. Earthworm mortality rate observation after treatments of various concentration *E. scaber* extract.

Protein profile of *S. litura* treated with *E. scaber* extract

The protein profile of the target organism *S. litura* was investigated following treatment with *E. scaber* methanolic extract. Mortality began to occur on the third day of treatment, when changes on protein profiles began to be recorded. Meanwhile, *P. xylostella* was already undergone lysis and dead on the 2nd day of treatment. Therefore, the total protein level and MW were selected as the parameters for protein profiling. The protein levels and MWs of *S. litura* proteins were reduced after treatment with *E. scaber* methanolic extract. The highest concentration of *E. scaber* extract resulted in the lowest protein level, approximately 0.000254 mg/µl, and a molecular weight of 13.83 kDa. (Table 5).

Table 5. Protein levels of *S. litura* larvae treated with *E. scaber* methanolic extract.

Treatment	Absorbance range (A)	Average protein level (mg/µl)	Average molecular weight (kDa)
Control	0.069-0.084	0.000332 ± 0.000042	73.04 ± 10.58
4%	0.071-0.077	0.000269 ± 0.000051	36.46 ± 7.11
6%	0.071-0.078	0.000254 ± 0.000057	13.83 ± 2.71

Soil microbial community

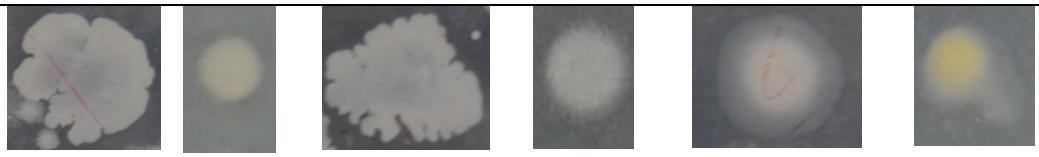
A total of 12 bacterial isolates were identified in the organic agriculture soil, which is considered to be a high level of microbial diversity, resulting from the availability of organic materials to support microbial life. As predicted, the microbial community was altered following treatment with *E. scaber* methanolic extracts, as shown in Table 6. The control treatment showed no changes in the soil microbial community, as all 12 of the originally identified isolates were found. However, treatment with 4% or 6% *E. scaber* extract decreased the microbial diversity. Only seven isolates were identified in the soil treated with 4% *E. scaber* extract, whereas treatment with 6% extract decreased the soil microbial diversity to six isolates. Furthermore, as shown in Table 7, the application of biopesticides containing either 4% or 6% *E. scaber* methanolic extracts have reduced the number of soil microbes.

DISCUSSION

Plants use various mechanisms to defend themselves against insect attacks, such as producing secondary metabolites that act as biopesticides. These chemicals may penetrate the body of an organism through the digestive tract, lungs, and skin (LU & KACEW 2002, KORTBEEK et al. 2019). As a contact poison, active compounds in plants may enter insect bodies through the body wall, the surface of the skin, and the nervous system on the surface of the skin. Insecticides enter the body when the insect makes

contact with or walks on the surfaces of plants that produce insecticides. Contact poisons can rapidly kill insects immediately following contact with the insect body. The penetration of chemicals into the insect body occurs through the epicuticle, causing damage to the waxy substance of the cuticle layer, resulting in water loss and death (COTTRELL 1987). Active compounds can also penetrate the tissues beneath the integument, towards the target neurosecretory regions of cells, and enter the corpora cardiaca via axonal transport. Once in the corpora cardiaca, active compounds can inhibit the excretion of α-ecdysone, a molting hormone, from the prothorax into the hemolymph. When the secretion of α-ecdysone is disrupted, β-ecdysone becomes disrupted, resulting in the inhibition of the new cuticle formation. As a result, the old cuticle remains attached to the body, preventing the insect from thriving and growing into an adult insect (KATHIRVELU et al. 2010).

Table 6. Microbial diversity in organic farming soils, after treatment with biopesticides containing *E. scaber* methanolic extracts.

Treatment						
	1	2	3	4	5	6
Control	+	+	+	+	+	+
4%	-	+	-	+	-	+
6%	-	+	-	+	+	-
						
	7	8	9	10	11	12
Control	+	+	+	+	+	+
4%	-	+	+	+	+	-
6%	-	+	+	+	–	-

Note: + indicates the microbe is present; – indicates the microbe is not present.

Active compounds can also affect the regulation of the neuroendocrine juvenile hormone, produced by the corpora cardiaca. These compounds inhibit the formation of the juvenile hormone, which affects insect metamorphosis, causing insects to maintain the juvenile structure. Juvenile hormone content decreases at a critical stage of growth before the insect goes through subsequent molting and deforms into the adult stage. The growth and development of insects are controlled by three enzymes, brain hormone, ecdysone, and juvenile hormone.

The use of secondary metabolites may result in the mortality of larvae by interrupting the flow of Na^+ (sodium) in nerve cells and the production of neurotransmitters at synapses. Active compounds in the nerve can extend the flow of Na^+ into the membrane by slowing or blocking the channel cover. If nerves are maintained in a depolarized state, large quantities of Na^+ will enter the membrane, causing seizures and shaking. If active compounds block channel closure, excess Na^+ on the membrane can eventually result in the inactivation of the nerve, due to difficulty repolarizing, resulting in paralysis.

The existence of active compounds at the synapse can interfere with the chemical transmitters, especially acetylcholine (ACh). Active compounds can increase Ach release and inhibit Ach enzymes, such as acetylcholinesterase. Ach plays an important role, providing permeability properties to the postsynaptic membrane, allowing the displacement of Na^+ ions and depolarization. Ach can be hydrolyzed by acetylcholinesterase, which is normally found in large quantities at the synapse. Active compounds can inhibit enzymes from deactivating Ach, even though Ach continues to be released from the membrane, due to the presence of excess positive ions. Increased Ach concentrations can cause larvae to suffer from paralysis and death (REGNAULT-ROGER 1997). Active compounds in plants, such as coumarin, phenols, terpenoids, polyphenols, saponins, and alkaloids, can also act as antifeedants and inhibit insect growth, causing larvae to experience chronic toxicity and death (KATHIRVELU et al. 2010, GOKULAKRISHNAN et al. 2012). In this study, phenol and flavonoid content were found as active compounds. The findings were confirmed by a previous study from YULIANI et al. (2019). The study conducted by RACHMADIARTI et al. (2019) also found the phenol and flavonoid content in *E. scaber* leaves. The habitat altitude affected the

phenol and flavonoid content of *E. scaber* leaves. The results showed that methanol extract of *E. scaber* leaves in the lowlands was 0.570 mg mL^{-1}, 0.611 mg mL^{-1} in the middle lands, and 0.435 mg mL^{-1} in the highlands. Meanwhile, the levels of flavonoids in the lowlands were 0.93 mg mL^{-1}, the middle lands were 0.83 mg mL^{-1}, and the highlands were 0.87 mg mL^{-1} (RACHMADIARTI et al. 2019).

The use of botanical pesticides during organic farming is expected to conserve natural resources and agricultural productivity in the long term, minimizing environmental impacts, optimizing crop production with minimal chemical inputs, and providing commensurate economic benefits to farmers. Controlling pest growth is necessary to create a balanced agricultural ecosystem and ensure sustainable agriculture. The application of biopesticides containing *E. scaber* methanolic extract may decrease the protein levels of experimental animals. Proteins are macromolecules that play essential roles in all biological processes, including structural proteins, catalysts, transport and storage proteins, and proteins in the immune system (SHAHRAM et al. 2008). Proteins determine the size and structure of cells, contribute to cell signaling, and act as catalysts for various biochemical reactions (FATCHIYAH et al. 2011). Moreover, secondary metabolites from extracts of *E. scaber* can cause a decrease in protein levels in target insects. Secondary metabolites can damage the target insect protein by binding to protein and lipid molecules to affect the physiological function of cell membrane proteins and enzyme proteins. Phenolic compounds derived from plants can form complexes with proteins through hydrogen bonds, thereby inhibiting protein formation (HUANG et al. 2004). It shows good biopesticidal activity. The results showed that protein level in *S. litura* larvae was altered after being exposed to biopesticide due to bioactive compounds in *E. scaber* that induced protein structure damage and inhibited larvae protein formations (SHAHABI et al. 2007). HUANG et al. (2007) and DAWKAR et al. (2019) also showed the pesticide effect of Azadirachtin on the metabolism of *S. litura* larvae, indicated by altered protein levels. Proteins affected by bioactive compounds play a role in various cellular functions and receptors of ecdysone, which regulate insect development and reproduction.

Proteins are influenced by both genotypic and environmental factors (MADIGAN et al. 2003). In addition, environmental changes and stress can influence protein conformations (SHAHRAM et al. 2008). When cells experience environmental changes, signals are sent to the nucleus, which response by performing transcription and translation to produce proteins to respond to environmental changes. However, environmental changes may cause changes in protein conformations, including denaturation and new protein formation.

The use of biopesticides derived from plant secondary metabolites can also influence soil microbial communities living in the habitat, and these effects can be observed by measuring certain parameters, including the number of microbes and microbial diversity. The results of this study indicated that the application of biopesticides derived from *E. scaber* methanolic extracts can reduce the total number of soil microbes (Table 7). HUSSAIN & SALEEM (2009) suggested that biopesticides may negatively influence soil microbial communities by reducing diversity and density. Active compounds likely cause decreases in the total number of microbes with antimicrobial effects. The secondary metabolites identified in *E. scaber* leaves include flavonoids, saponins, terpenoids, and phenols, which can all act as antimicrobials and can inhibit microbial growth, which may eventually lead to microbial death (HUSSAIN & SALEEM 2009, YULIANI et al. 2018). KUMAR et al. (2004) stated that *E. scaber* leaf extracts can act as an antibacterial for various bacteria, including *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacillus subtilis*, and *Escherichia coli*. In addition, terpenoid compounds in *E. scaber* have antibacterial activity against *Staphylococcus aureus* (DAISY et al. 2008). Secondary metabolites can bind with the proteins that form the cell membrane, resulting in the inhibition of cell membrane formation. In addition, the formation of cytoplasmic proteins and nucleic acids can also be inhibited by active compounds, resulting in the disruption of metabolic processes and ATPase binding to the cell membrane. These effects can result in a lack of energy available to bacterial cells, disrupting bacterial growth and development.

Table 7. The number of microbes on the soil following biopesticide treatment.

Treatment	Number of microbes		Mean
	Replication 1	Replication 2	
Control	7.0×10^5	3.51×10^4	3.68×10^5
4%	8.3×10^4	3.9×10^4	6.1×10^4
6%	4.65×10^4	1.55×10^4	3.1×10^4

The active compounds in biopesticides are capable of influencing the soil environment, which further affects soil microbial communities. Microbial responses to environmental changes occur through a complex

combination of adjustment, replacement, and species interactions. Adjustments can be achieved through high adaptability and plasticity, as well as the ability to perform horizontal gene transfer. Replacement occurs when the spread rate of microbes is high (JURG et al. 2015).

Some bacteria are sensitive and are unable to survive in an environment containing antimicrobial chemicals. Therefore, the soil microbial diversity can be influenced by soil structures and plant growth phases, as each phase produces different root exudates. Soil microbes play an important role during plant growth, increasing the ecological health of the host plant by producing beneficial substances that act as bio stimulators. In addition, microbes also secrete compounds that can protect plants against pathogenic microbes.

The results of this study suggested that the use of biopesticides containing plant secondary metabolites can be highly effective, causing greater than 90% pest mortality. However, biopesticides can also influence non-target organisms and soil microbial communities living in the same environment. Therefore, determining the correct plant species and dosages for biopesticide use are necessary, so that target organisms are affected, without affecting non-target organisms. Furthermore, the use of biopesticide is expected to increase the preservation of natural resources and agricultural productivity, facilitating the creation of a balanced and sustainable agriculture ecosystem.

CONCLUSION

The application of biopesticides containing *E. scaber* methanolic extracts influenced the mortality rates and affect the protein content and molecular weight of *S. litura* and *P. xylostella*. The highest concentration of *E. scaber* (12%) can increase the mortality rates of *S. litura* and *P. xylostella* to 93.35% and 96.65%, respectively. The LC50 and LC80 values for *S. litura* were 1.867% and 4.763%, respectively. In contrast, the LC50 and LC80 values for *P. xylostella* were 4.488% and 7.92%, respectively. *E. scaber* extract also affected non-target organisms. *E. scaber* extract affected the decrease in diversity, number of soil microbes, and mortality of earthworms. The higher the concentration of *E. scaber* methanol extract given, the greater the effect on the target and non-target organisms. *E. scaber* extracts, at a concentration of 6%, can kill 60% of earthworm populations over a 20-days period. In addition, *E. scaber* extracts decrease the protein levels and MWs of *S. litura* proteins, with higher concentrations of *E. scaber* extracts resulting in lower protein levels and MWs. Biopesticides containing *E. scaber* extracts decreased the density and diversity of soil microbes. At a concentration of 6%, the *E. scaber* extract reduced the microbial diversity from 12 isolates to six isolates. The recommendation is to use a concentration of 4% *E. scaber* extract, which has a low impact on non-target organisms but is still effective in controlling pests with larval mortality of 80%. The use of *E. scaber* methanol extract in actual conditions in the field still needs to be investigated further to see its effectiveness as a biopesticide.

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