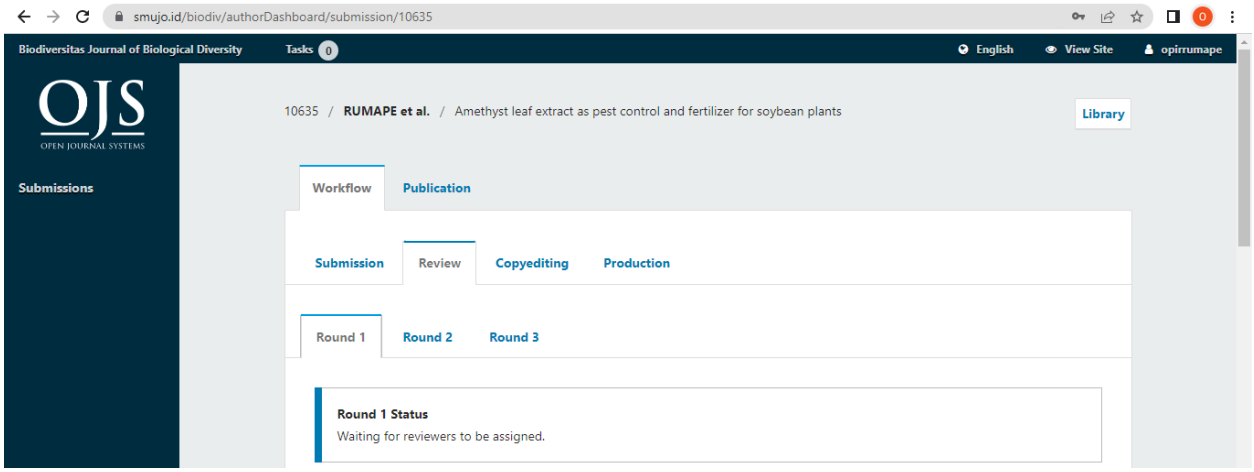
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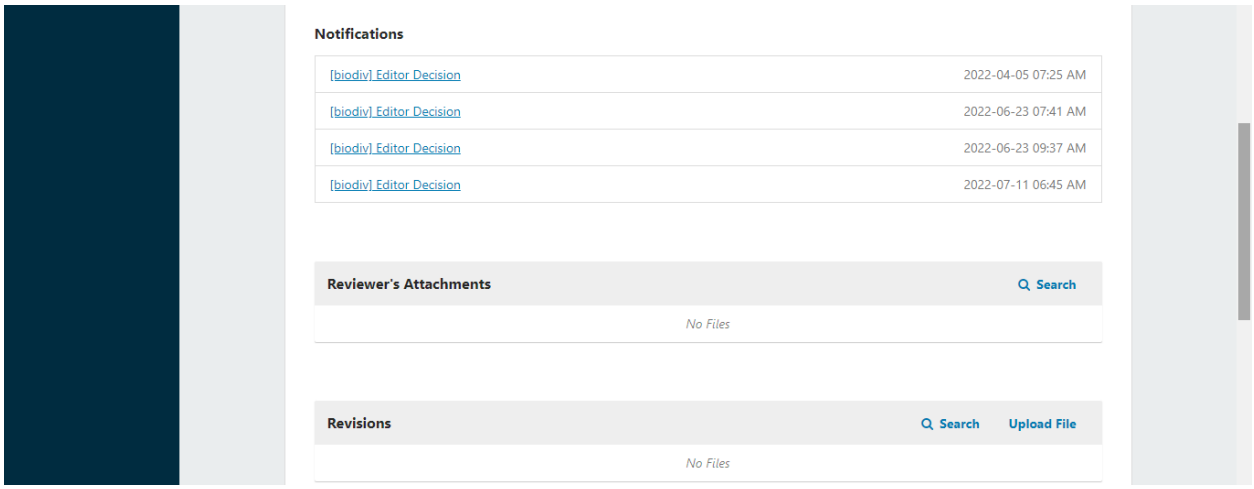
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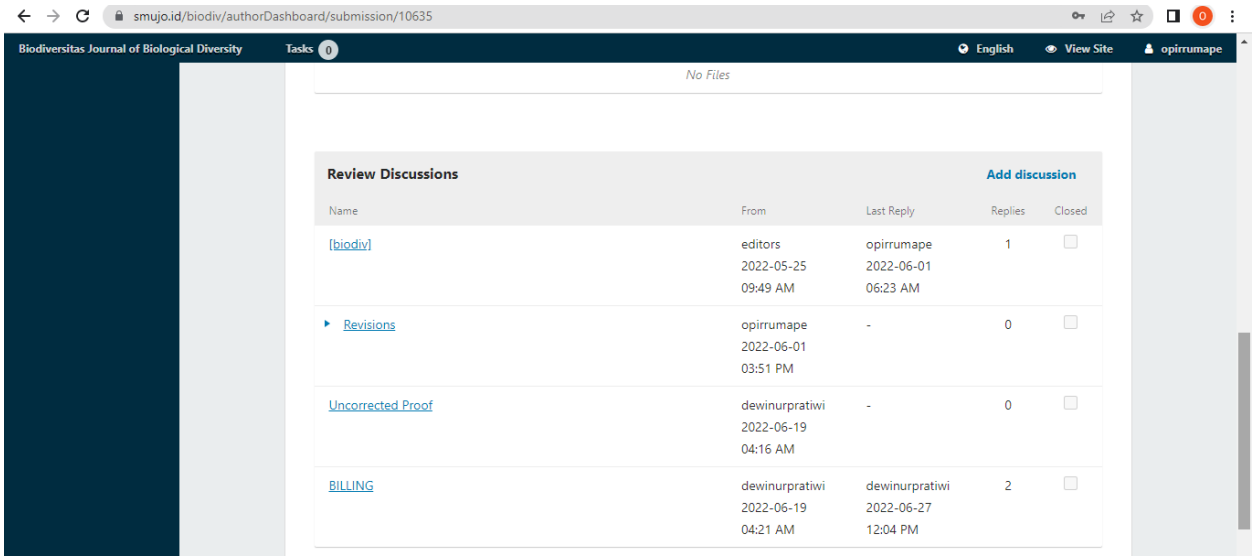
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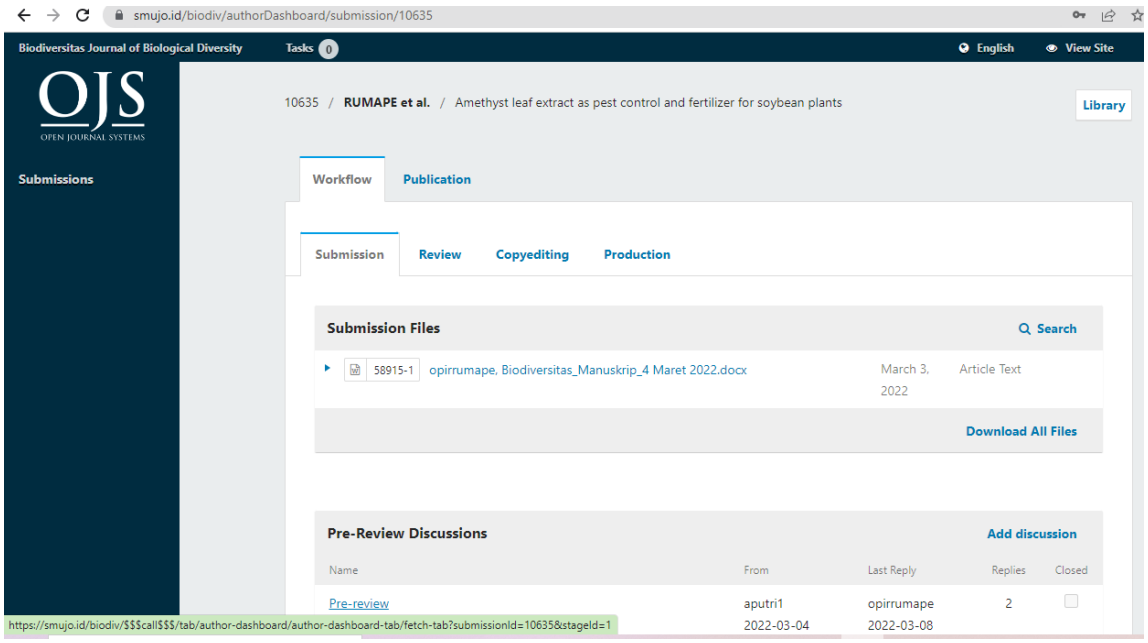
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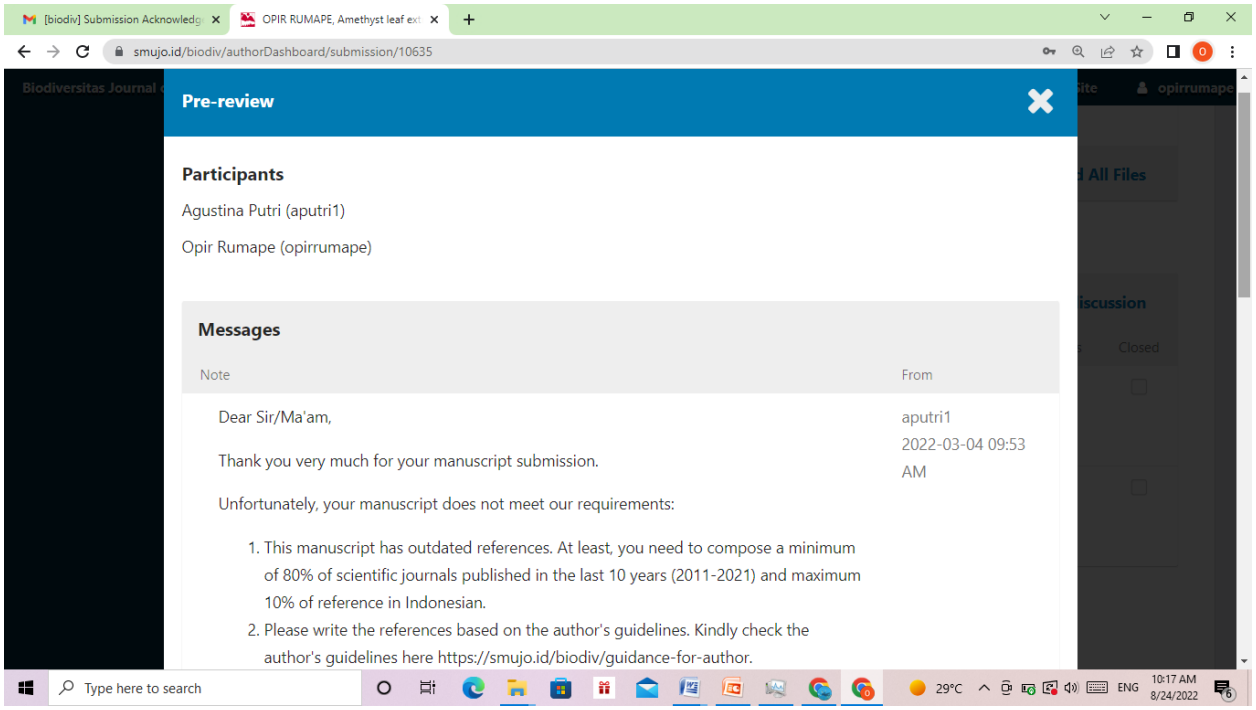
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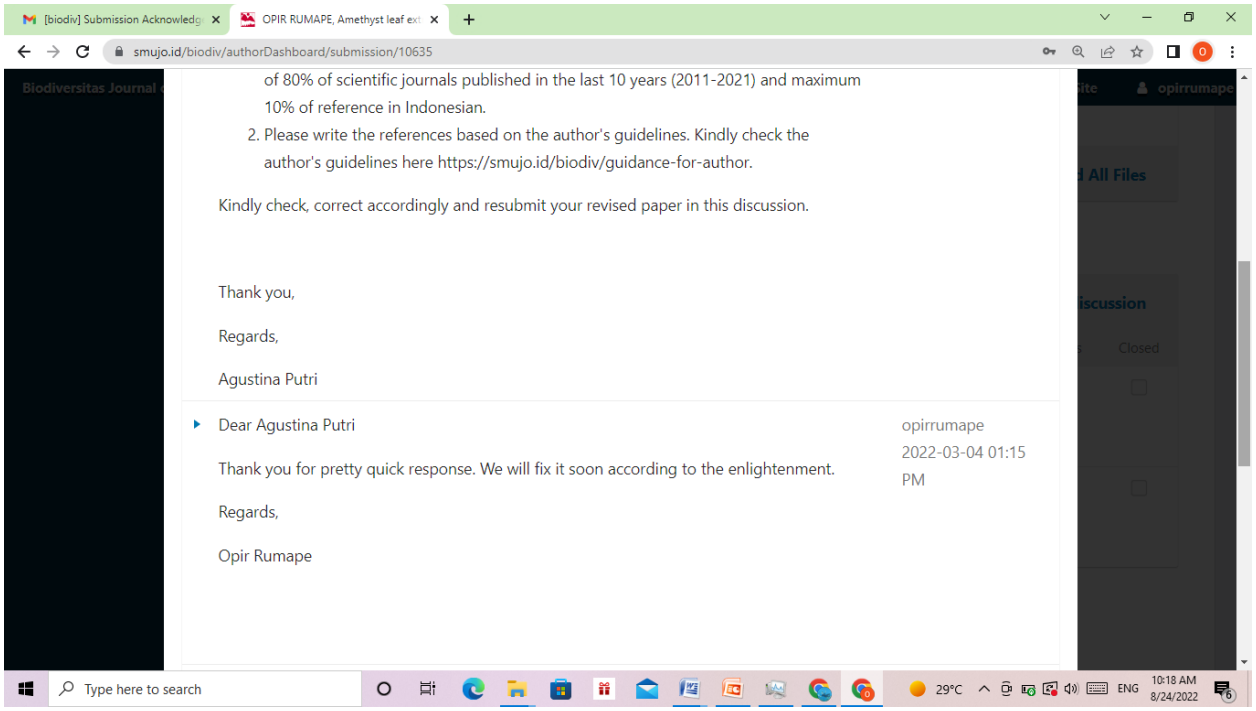
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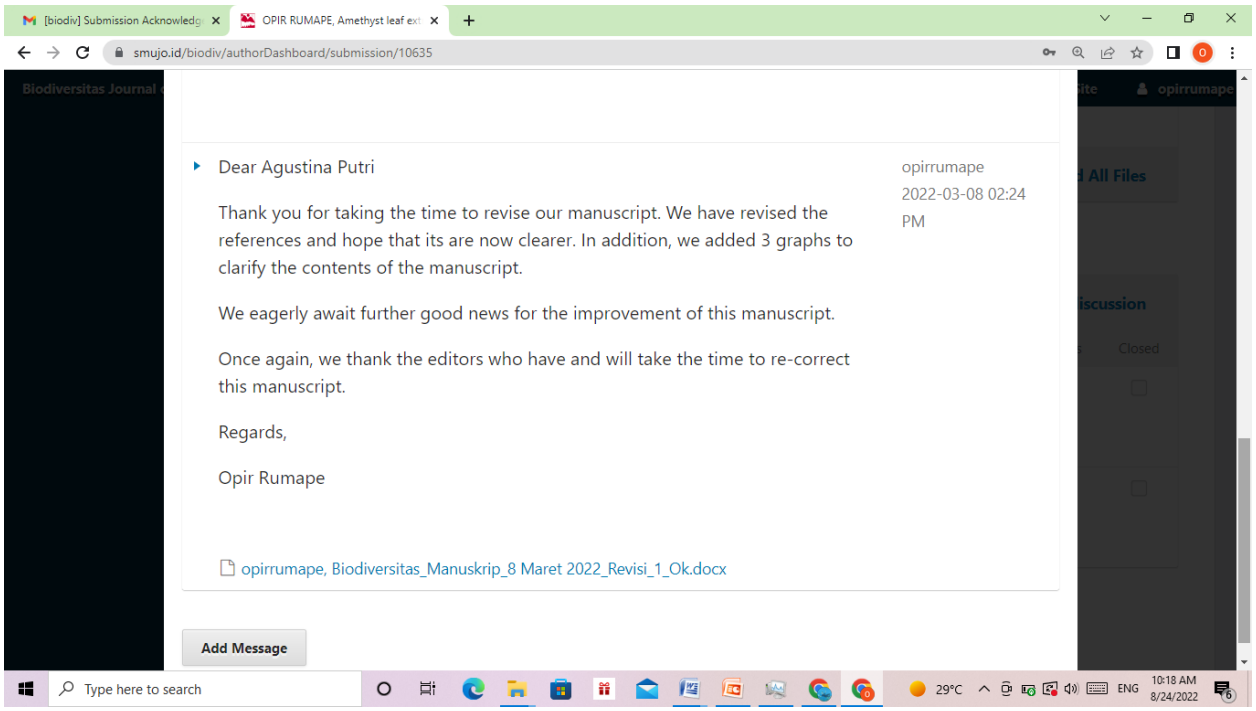
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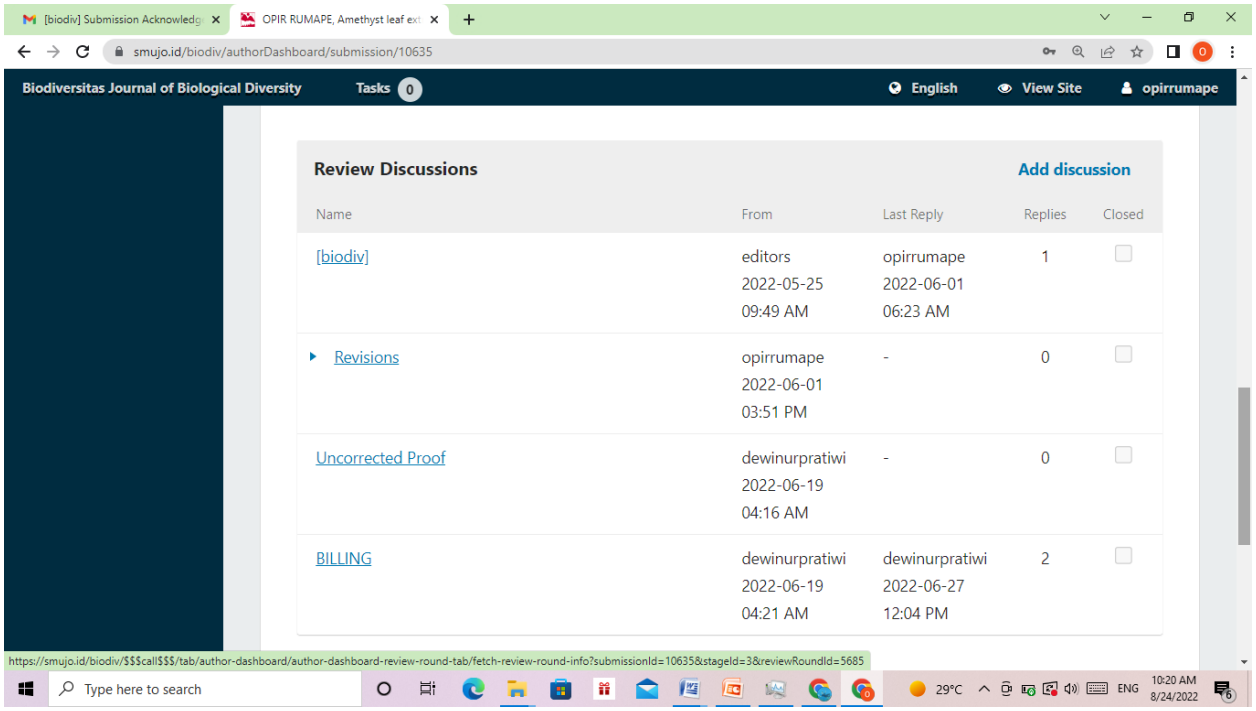
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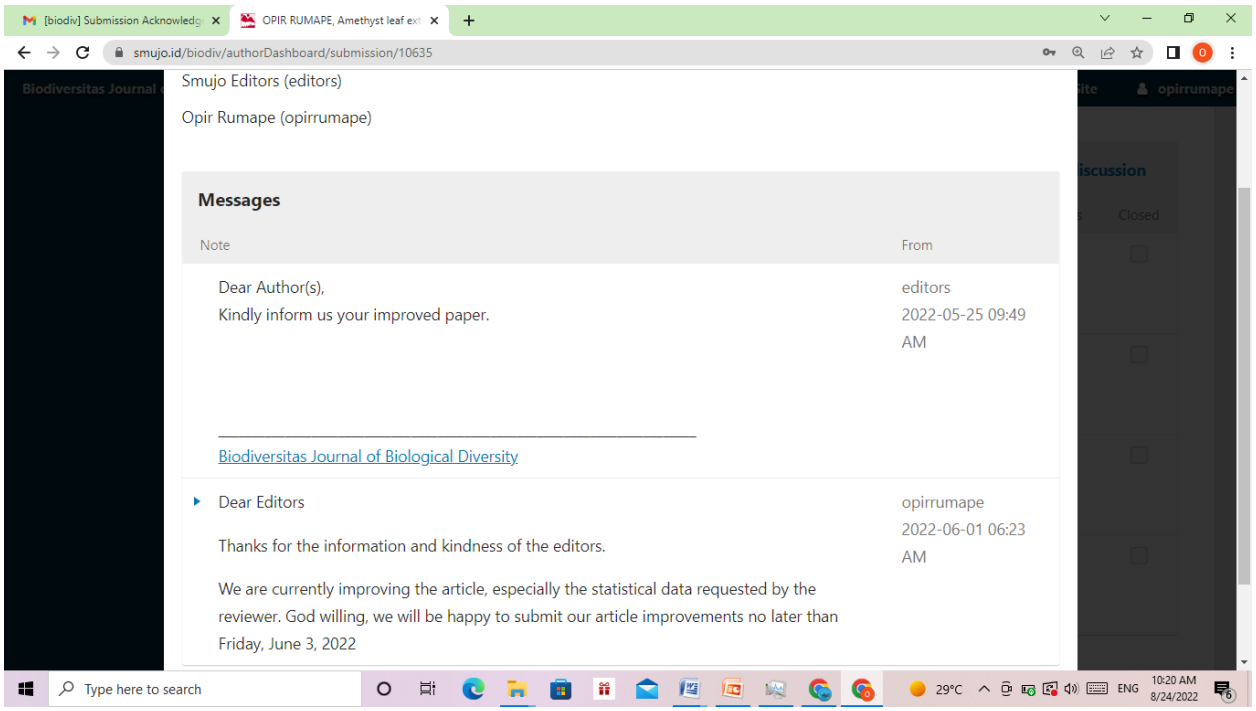
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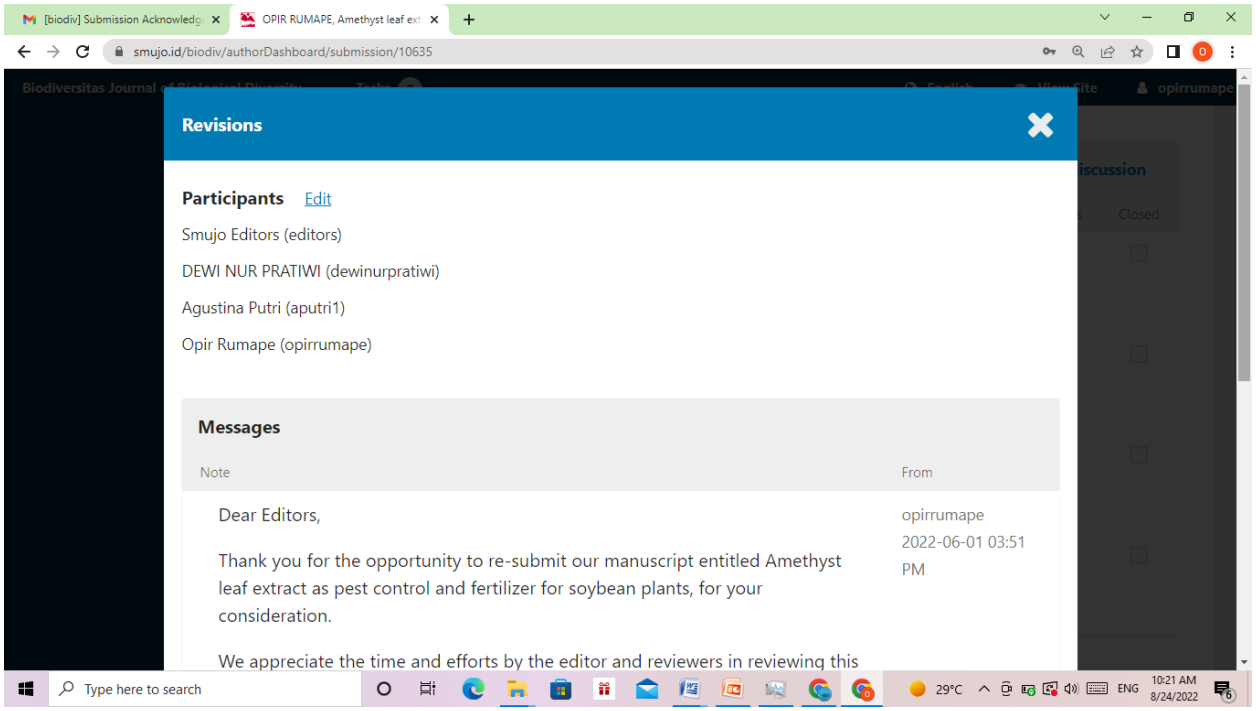
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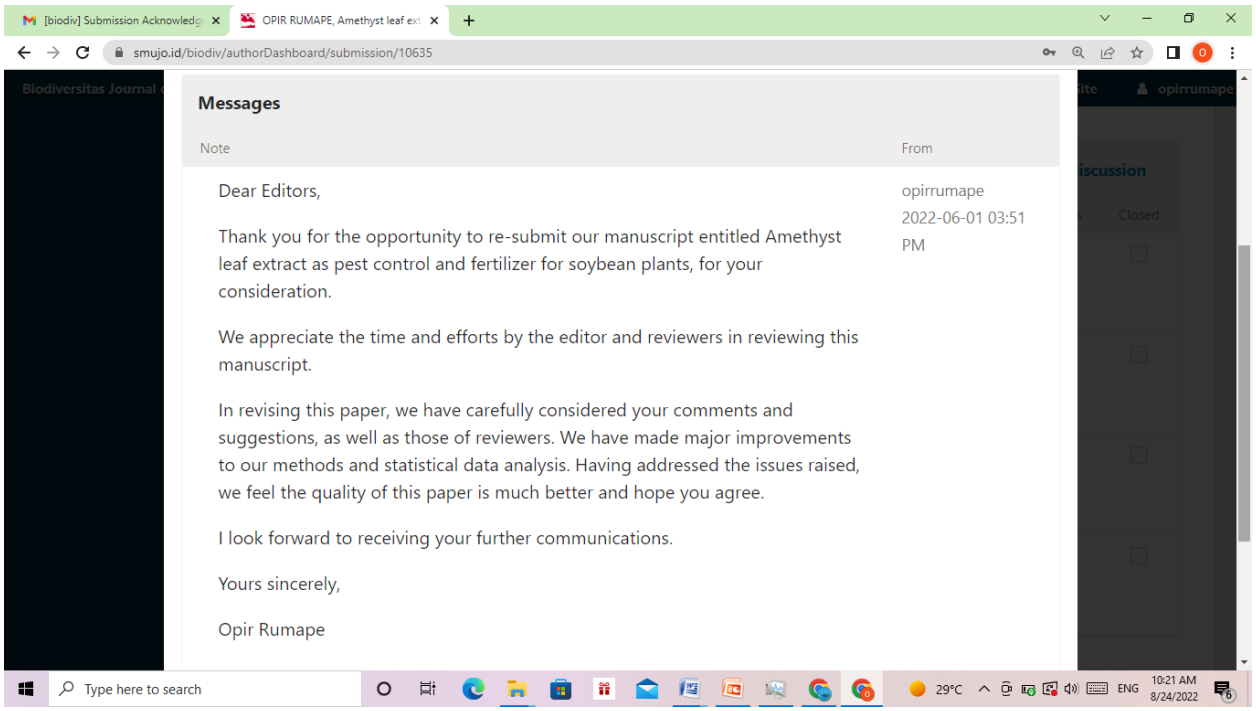
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In revising this paper, we have carefully considered your comments and suggestions, as well as those of reviewers. We have made major improvements to our methods and statistical data analysis. Having addressed the issues raised, we feel the quality of this paper is much better and hope you agree.
I look forward to receiving your further communications.
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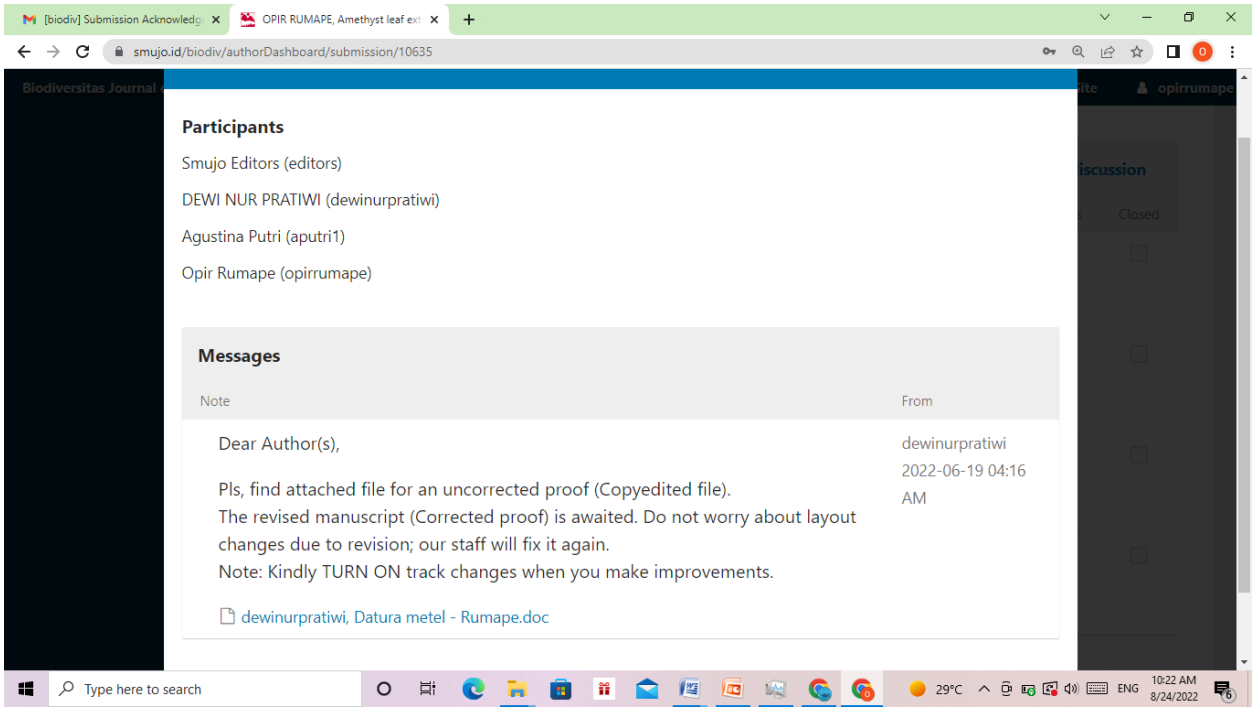
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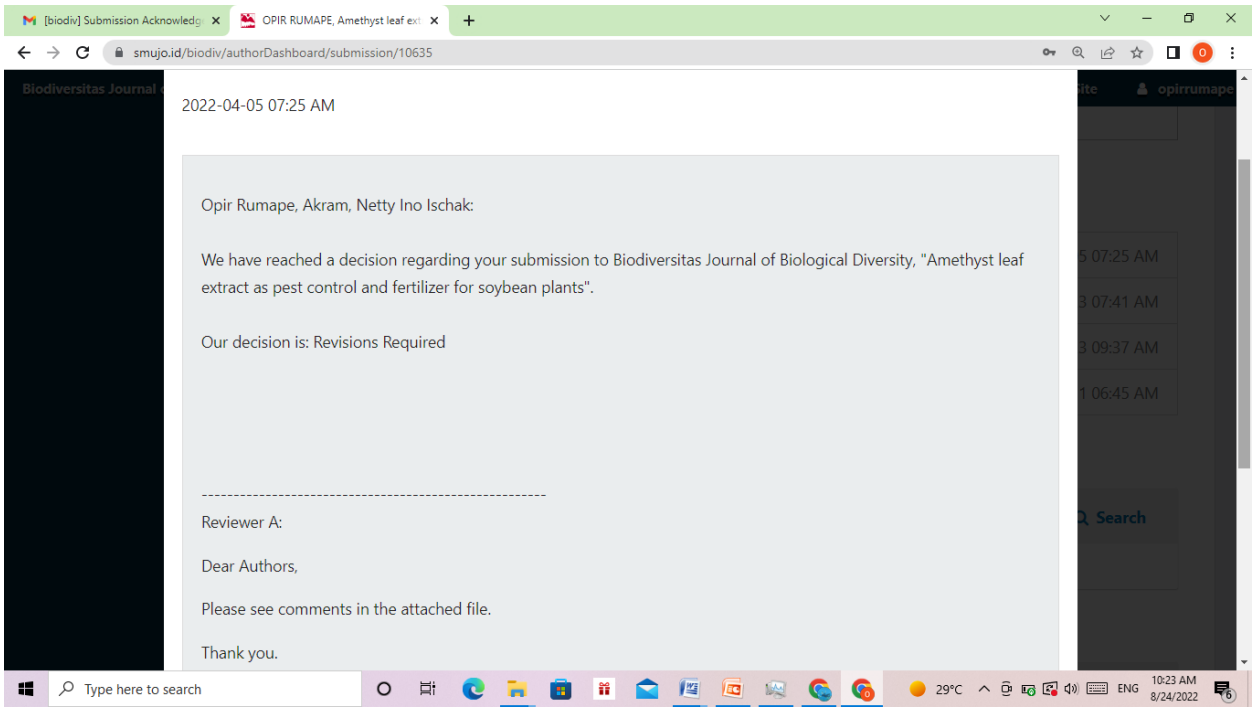
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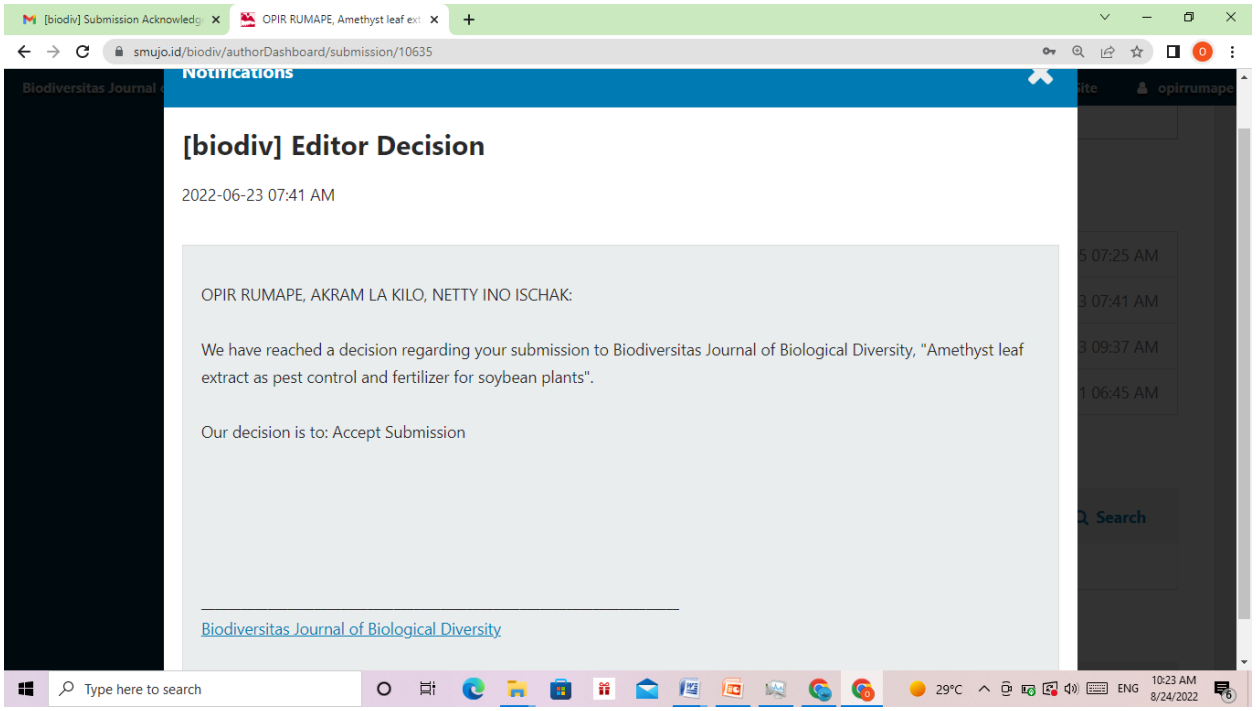
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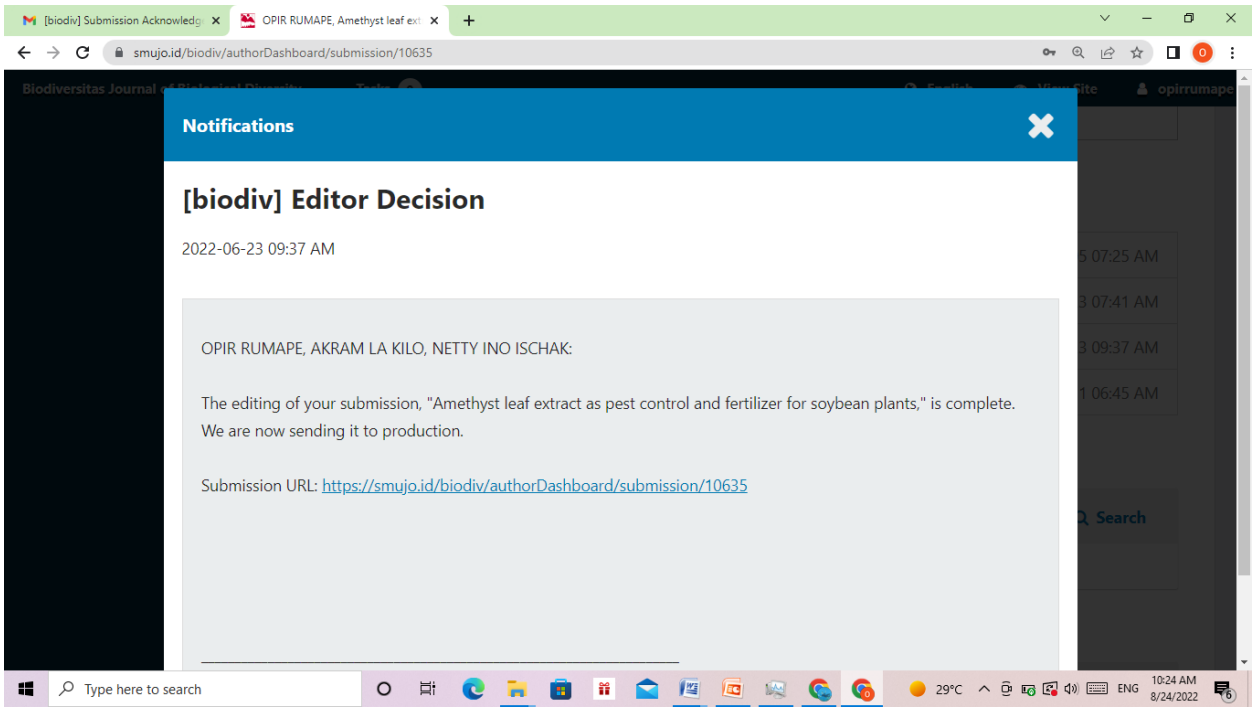
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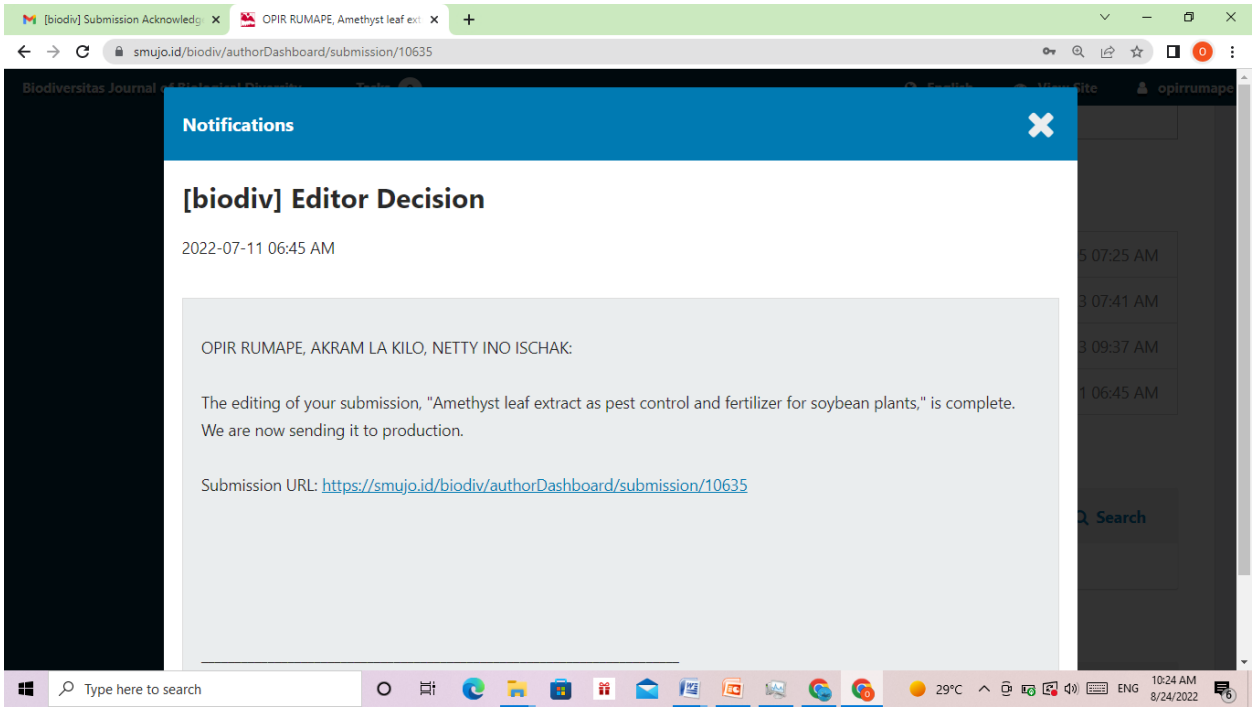
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Department of Chemistry Education, Faculty of Mathematics and Natural Sciences, Universitas Negeri Gorontalo. Jl. Prof. Dr. Ing. B.J Habibie, M.Eng, Jl. Ir. Moutong, Tilongkabila, Kabupaten Bone Bolango 96119, Gorontalo, Indonesia. Tel./Fax. +62 (0435) 821125/ +62(0435) 821752

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Amethyst leaf extract is not only a natural insecticide that has been claimed so far, but we found this extract to be able to fertilize soybean plants grown in gardens. The alkaloid content of this extract which we suspect is responsible for the chlorophyll. For example, yellow soybean leaves, when sprayed with the extract, the leaves become lush green. We found this because the research we did was not only tested in the laboratory, but also in the field/garden. The n-hexane extract of amethyst gave a greater effect as a natural insecticide against Sopdeptoa litura and soybean plant fertilizers, than the methanol and ethyl acetate extracts.

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Opir Rumape

Amethyst leaf extract as pest control and fertilizer for soybean plants

OPIR RUMAPE[1,2,♥], AKRAM LA KILO[1,2], NETTY INO ISCHAK[1,2]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Gorontalo. Jl. Prof. Dr. Ing. B.J Habibie, M.Eng, Jl. Ir. Moutong, Tilongkabila, Kabupaten Bone Bolango 96119, Gorontalo, Indonesia. Tel./Fax. +62 (0435) 821125/ +62(0435) 821752, ♥email: rumapeo@gmail.com

[2]Department of Chemistry Education, Faculty of Mathematics and Natural Sciences, Universitas Negeri Gorontalo. Jl. Prof. Dr. Ing. B.J Habibie, M.Eng, Jl. Ir. Moutong, Tilongkabila, Kabupaten Bone Bolango 96119, Gorontalo, Indonesia. Tel./Fax. +62 (0435) 821125/ +62(0435) 821752

Manuscript received: 01 03 2022 (Date of abstract/manuscript submission). Revision accepted: ..... 2022.

Abstract. Amethyst (Datura Metel L) is a plant that grows and develops in the Gorontalo area, and people use it as traditional medicine. The plant has natural insecticidal activity, but people do not know about it, let alone apply it. So far, amethyst has only been reported as an insecticide on a laboratory scale, not its application in gardens. The purpose of this study was to extract amethyst leaves and apply it as an inhibitor of feeding activity and insect mortality in both the laboratory and soybean gardens. Amethyst leaves were extracted in the laboratory using methanol, n-hexane, and ethyl acetate. The extracts were tested phytochemically to determine the type of secondary metabolite, before applying it. Phytochemical test showed amethyst leaves contain alkaloid, flavonoid, terpenoid, and saponin. The application treatment for the bioactivity used variations in the concentration of amethyst leaf extract of the fractions (methanol, ethyl acetate and n-hexane), namely 1.0, 2.5, 5.0, 7.5, 10%; and 0% as control. In the laboratory, the treatment was applied by contact to 5 insects Spodoptera litura instar III for each concentration treatment with 3 replications. Observation parameters were the percentage decrease in feeding activity and mortality of Spodoptera litura larvae. In the garden, the extracts, with varying concentrations of the same as in the laboratory, ware applied to soybeans treated with the pest Spodoptera litura in a closed container, and the other was sprayed on plants that were left exposed. The results showed that the three extracts could kill pests, but n-hexane extract was the most effective compared to ethyl acetate and methanol extracts. Amazingly, soybean plants whose yellow leaves turn green after being given the extract. This shows that the secondary metabolites of amethyst are not only used as insecticides to control pests, but also as plant fertilizers.

Key words: Datura metel L; antifeedant; natural insecticide; soybean; natural fertilizer; Spodoptera litura

Abbreviations (if any): ME: Methanol Extract of Am

Running title: Amethyst leaf extract to control pest

INTRODUCTION

The use of synthetic pesticides is the main choice of farmers in controlling plant pests, even though they know the bad impact on human health and the environment (Samaria, 2012). They are also aware that chemicals from synthetic pesticides can be exposed to humans through consumption of agricultural products contaminated with pesticides (Ahmed et al., 2000). Only for practical reasons and quickly obtain yields and low costs, farmers ignore the negative effects of these synthetic chemicals.

The use of this fertilizer is inevitable, with the production of synthetic pesticide fertilizers increasing from year to year. This is also exacerbated by the production of very large subsidized fertilizers and is obtained at low prices by farmers. Production of synthetic fertilizers in Indonesia in 2021 has reached 12,235 million tons (Nasution, 2022), with subsidized fertilizers of 8,777 tons (Ramadhan, 2022). The fertilizer is distributed throughout Indonesia, including Gorontalo which gets a quota of 64.162 tons. The allocation of subsidized fertilizer in one Gorontalo district alone has increased to 13,991 tons in 2021 (Eross A, 2021). The use of these pesticides increases agricultural productivity by up to 60% (Gresik, 2020). This shows that farmers' dependence on the use of pesticide fertilizers is very high, and continues to increase the contamination of agricultural products with harmful chemicals of synthetic pesticides. Therefore, it is necessary to find alternative insecticides that are natural and safe for the environment.

The development of natural insecticides is currently more directed at the discovery of secondary metabolite compounds that are not only effective in controlling pests but also have selective activity against certain pests that damage plants. Indonesia has abundant plant resources that produce active compounds as insecticide repellent and antifeedant that are easily decomposed and leave no residue. Here, we conduct research to find plants that grow a lot in Gorontalo and have no known potential applications. This plant has natural compounds that are safe, effective, and environmentally friendly as a substitute for synthetic pesticides which have had a negative impact because they leave residues on plant products and pollute the environment. The plant is amethyst which has important compounds that have the potential as insecticides that

can inhibit feeding activity and can kill insects (Prijono, 2008). Datura metel leaf extract at higher concentrations is more toxic and can be used as an insecticide against grasshoppers and red ants (Kuganathan & Ganeshalingam, 2011). Another use of amethyst leaves is as an antiviral and antifungal (Alam et al., 2021). Unfortunately, as an insecticide and antifungal from amethyst, it has only been tested on a laboratory scale, not in large gardens/land. Until now, amethyst has not been reported that amethyst can fertilize plants as we encountered in this study. Here, we also report the application of amethyst leaf extract in the garden.

The main aim of this study was to apply methanol, n-hexane, and ethyl acetate extracts from amethyst leaves as antifeedant against Spodoptera litura insects on soybean plants. Before applying the extract, the secondary metabolites were tested by phytochemical method. The extract was tested in the laboratory and in the garden. Activity Test of Anti-feeding and Toxicity in the laboratory was carried out on Spodoptera litura. Meanwhile, the application of insecticide efficacy in the garden was carried out on soybean plants against Spodoptera litura pests. These caterpillars can attack soybean plants thereby reducing productivity (Tengkano & Suharsono, 2005).

MATERIALS AND METHODS

Preparation of Amethyst Leaf Extract

Amethyst leaves of 1,256 kg were dried in the open air (Fig. 1C), without direct contact with sunlight, and 625.53 g of dry brownish-green samples were obtained. The sample was mashed with a blender, then macerated with 3 L of methanol for 3×24 hours. Every 1×24 hours, the material is filtered and the residue is macerated again with new methanol. The filtrate was evaporated at 30-40 °C to obtain a concentrated methanol extract of amethyst leave (ME).

ME as much as 50 g was suspended with methanol and water in a ratio of 2/1, and then partitioned with 200 mL of n-hexane. The results were separated using a separatory funnel, and the n-hexane fraction obtained was evaporated at 40°C to obtain a concentrated n-hexane (HE) extract of 17.437 grams. Then the methanol-water fraction was partitioned again with 200 mL of ethyl acetate. The ethyl acetate and water fractions were each evaporated at 40 °C to obtain a blackish red ethyl acetate (EE) extract of 10.9722 grams. The extract was phytochemically tested to determine the types of secondary metabolites of alkaloid, flavonoid, terpenoid, steroid, and saponin. The qualitative test used the method described by (Trease & Evans, 1983), (Harbome, 1998), and (El-Olemy et al., 1994).

Effectiveness Test of Amethyst Leaf Extract

Three amethyst leaf extracts from ME, HE, and EE were tested for their antifungal effectiveness in two locations, namely the laboratory and garden. Each extract with concentrations of 1, 2.5, 5, 7.5, and 10% was applied at both locations under different conditions. The extracts were applied to the leaves to determine the inhibition of the feeding activity (antifeedant) of Spodoptera litura larvae in the laboratory. In addition, toxicity tests were also carried out. In the garden plots, soybeans are planted. The plant was given these extracts to test the insect repellent and fertility of soybeans.

RESULTS AND DISCUSSION

Amethyst Leaf Extract

The selection of plants used in this study is based on data which states that plants have been used empirically as medicine and some of those that have been tested are toxic (Adhana & Chaudhry, 2019; Ko & Ko, 1999). However, the selected plants have not been tested regarding their ability to control pests in the garden on soybeans. The test material used was amethyst leaf (Datura metel L.) as shown in **Figure 1**A. Immediately after being obtained, fresh plants were sorted wet with the aim of separating dirt, foreign materials adhering to plants, unused plant parts, or damaged plant parts from simplicia materials. Then washing was carried out to remove the dirt attached to the simplicia (**Figure 1**B). After washing, the simplicia ingredients are chopped into smaller sizes. This process is done to facilitate the process of drying and pollination.

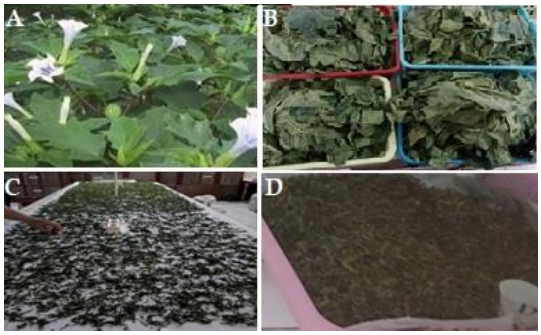

Figure 1. Amethyst Leaves; A. fresh, B. clean, C. dry, and D. smooth.

Amethyst plants were dried in an open air (**Figure 1**C), without direct contact with sunlight to avoid damage to the compounds present in the sample. In addition, the sample can be durable because removing the water content in the sample can facilitate the withdrawal of bioactive compounds during maceration (Cacique et al., 2020). The sample was smoothed (Figure 1D) to expand the touch surface and facilitate the maceration process, where the larger the surface area of the contact area with the solvent, the more effective the extraction process (Aji, 2018; TeGrotenhuis et al., 1999).

The choice of methanol solvent in the sample maceration process is because methanol is a universal solvent that can bind all components of compounds that are polar, semi-polar, and non-polar (Ramdani et al., 2017). In addition, methanol is a solvent that has a high solubility and is harmless or non-toxic. The maceration method was chosen because the characteristics of the active compounds contained in the amethyst leaf sample were not known, so that the extraction method by heating was avoided to prevent the decomposition of compounds that were not heat resistant.

The thick methanol extract (FM) was then partitioned with n-hexane which is nonpolar and ethyl acetate which is semipolar. The extraction process will be efficient if the extraction is carried out repeatedly (Hadi et al., 2022; Khulu et al., 2022). Shaking in the fractionation process aims to expand the contact surface area between the two solvents so that the distribution of solutes between the two can take place properly (Harvey, 2000). The density of n-hexane (0.4 g/mL) and ethyl acetate 0.66 g/mL is smaller than the density of water 1 (g/mL) which shows that the extracts of the two solvents are easy to separate because each is in a solution. The top layer in a water-methanol-containing solution. The yield of n-hexane extract was greater than that of ethyl acetate extract. The higher the yield value show that the raw material has a greater opportunity to be utilized (Kusumawati et al., 2008).

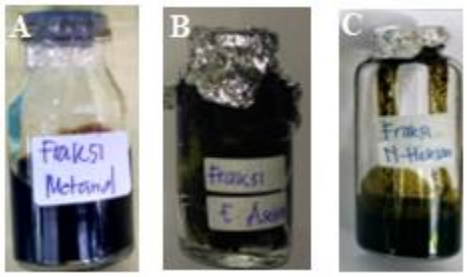

Figure 2. Amethyst leaf extract; A. Methanol, B. Ethyl acetate, and C. n-hexane.

The results of phytochemical screening prove that amethyst leaves contain secondary metabolites of alkaloid, flavonoid, steroid, and terpenoide as shown in **Figure 2**. Alam et al. (2021) reported that the underside of amethyst leaves contains very large chemical compounds such as flavonoid, tropane alkaloid, tannin, saponin, and anolide.

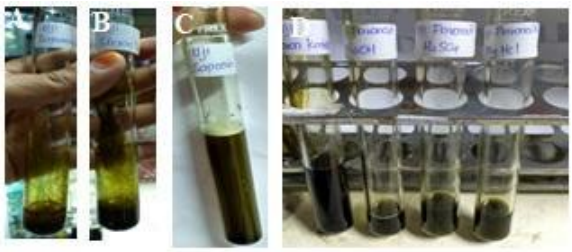

Figure 3. Phytochemical test results of samples of amethyst leave: A. terpenoide, B. steroids C. saponin, D. flavonoid

Test of Amethyst Leaf Extract

The effectiveness of amethyst leaf extract in controlling pests is carried out in two ways, namely by conducting anti-feeding activity and toxicity tests in the laboratory and its application in the garden.

Antifood Activity and Toxicity Test in the Laboratory

Anti-feeding Test

Anti-feeding test is a test carried out to see how much a plant has the power to inhibit the eating activity of a plant-disturbing pest. In the testing process, the insect larvae of Spodoptera litura were fasted for approximately 8 hours. The goal is that the larvae can eat fresh kale leaves provided as a test medium that has been smeared with sample extract (treatment) in various concentrations. If the insect is not fasted first, it is feared that the insect will not eat the treated leaves which can cause the insecticidal activity of the amethyst leaf extract sample to be immeasurable and inferential; whether insects that do not eat are caused by the presence of anti-feeding compounds or the state of insects that are not hungry.

The test results showed that the methanol extract had 100% antifungal effect for the test solution concentrations of 7.5 and 10% as depicted in **Figure 4**. The test solution concentrations of 5%, 2.5%, and 1% each had an anti-eating effect of 75, 62.5, and 38.5%, respectively. The ethyl acetate extract with concentrations of 5.0, 7.5, and 10% had antifungal power of 100%, while the test solution concentrations of 2.5 and 1% had an antifungal effect of about 72 and 58%, respectively. In the n-hexane extract, the concentrations of the test solution 5, 7.5, and 10% had 100% antifungal power, while the test solution concentrations of 2.5 and 1% had an antifungal effect of 63.3 and 43.05%, respectively. This shows that the ethyl acetate extract and the n-hexane extract of amethyst leaf showed an anti-feeding effect of 100% starting from the test solution concentration of 5%, while the methanol extract was 7.5%. There is no standard limit regarding the concentration of an effective test solution for compounds that are antifungal. A plant has effective anti-feeding properties when the level of food inhibition reaches 80-100% (Ambarningrum et al., 2012).

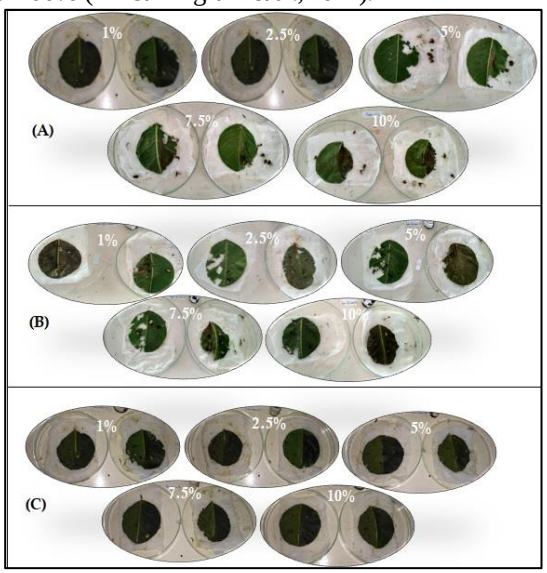

Figure 4. The results of the insect repellent activity test on leaves treated with extracts of (A) methanol, (B) ethyl acetate, and (C) n-hexane with concentrations of 1, 2.5, 7.5, and 10%

The decrease in feeding activity of the test animals was thought to be due to the content of allelochemical compounds contained in the amethyst leaf extract. Insect reactions to certain allelochemical compounds depend on the dose (Hsiao, 1985). Complete inhibition by an antifungal compound may occur over the range of effective and potential doses of the substance. The results of the phytochemical analysis showed that the amethyst leaf extract contained alkaloid, flavonoid, terpenoide, tannin, and saponin.

Compounds that are anti-feeding are mostly found in the secondary metabolite group which can be contact poison and stomach poison (Banwo et al., 2020). Flavonoid compounds are included in the phenolic group which acts as a poison inhibitor of secondary metabolites and a slow-acting nervous system. Insects that die are caused by starvation due to paralysis of the mouth apparatus (Banwo et al., 2020). Flavonoids can reduce the ability to digest food in insects by reducing the activity of protease and amylase enzymes. As a result, insect growth is disrupted (Chen, 2008). Terpenoide is one of the compounds that act as an antifungal because of its unpleasant taste and smell so that insects refuse to eat (Majidi et al.,

2020). At high enough concentrations, terpenoide compounds can reduce insect feeding activity due to the nature of insects that refuse to eat due to the entry of compounds that stimulate chemoreceptors which are continued to the nervous system.

Saponin can reduce the surface tension of the mucous membranes of the digestive tract of larvae so that the walls of the digestive tract are (Aisyafahmi & Wahyuni, 2018; Francis et al., 2001; Rohmah et al., 2020). This is because saponins interact with mucosal cells causing the muscles under the skin surface of the digestive tract to be damaged and paralyzed. The absorption of food that has been contaminated by bioactive saponin compounds will be spread throughout the body through the circulatory system and will damage blood cells through hemolysis reactions so that it will interfere with the physiological processes of the larvae and will die (del Hierro et al., 2018; Francis et al., 2001).

Insect Toxicity Test

Mortality tests were carried out on larvae of the pest Spodoptera litura, with the results showing that the higher the concentration of amethyst leaf extract, the higher the killing power. Leaf extracts with 10% concentration had 100% mortality. The killing power of amethyst leaf extract is caused by toxic secondary metabolites. One of them is an alkaloid compound which is known to have potential as an insecticide. Alkaloids have various effects on organisms. Amethyst leaves found alkaloid compounds with a content of 0.3 - 0.4% (about 85% hyoscyamine and 15% scopolamine as the main content) (Pratama, 2008). Total alkaloid content is 0.426%, mainly as atropine and a small amount of hyoscyamine (Firdaus et al., 2020). Usually the compound hyoscyamine is a racemic compound called atropine that can cause the nervous system of the caterpillar to turn it off. Alkaloids contained in amethyst can stimulate the endocrine glands to produce and increase the ecdysone hormone, causing metamorphosis failure and incomplete growth. In addition, amethyst leaves contain tannins that have a bitter taste and unpleasant odor so that eating activity is reduced and causes death. Spodoptera litura larvae that died due to treatment with amethyst leaf extract experienced stomach poisoning due to sucking the liquid from amethyst leaves which were sprayed on fresh water spinach leaves as a test medium.

Secondary metabolites in plants such as flavonoid glycosides are stomach poisons that work when these compounds enter the insect's body and will interfere with their digestive organs so that these compounds are toxic to pests (Sinaga, 2009). The results showed that the treatment with various concentrations of amethyst showed significant differences in mortality of Spodoptera litura larvae. The control treatment did not show the mortality of Spodoptera litura larvae. In the treatment of methanol extract with concentrations of 1, 2.5, and 5%, the killing power of Spodoptera litura larvae was low, respectively 20, 26.6, and 40%, on the contrary at concentrations of 7.5 and 10%, the killing power was quite effective, that is 60%. For ethyl acetate extract with concentrations of 1 and 2.5%, it had a low killing power of 40%, while at concentrations of 5, 7.5, and 10%, it had an effective killing power of 53, 66, and 86%, respectively. In contrast to the n-hexane extract, it had an effective killing power at concentrations of 1, 2.5, 5, and 7.5%, respectively, namely 53, 60, 80, 86%, and the most effective at a concentration of 10% with the highest killing power of 100. This indicates that the higher the concentration of amethyst leaf extracts, the higher the mortality rate of Spodoptera litura larvae. The higher the concentration, the more active substances that enter the insect (Mulyana, 2002).

Toxic compounds that enter the body will undergo biotransformation to produce compounds that are water-soluble and more polar (Nardina et al., 2021; Valentina, 2021). This metabolic process requires more energy and the toxic compounds that enter the insect's body cause the energy needed for the neutralization process to increase. The amount of energy used to neutralize these toxic compounds causes inhibition of other metabolism so that insects will lack energy and eventually die. The use of n-hexane and ethyl acetate extracts at concentrations of 5, 7.5, and 10% was more precise and effective in killing Spodoptera litura larvae compared to methanol extract. This is in accordance with the ancient Purba (2007) which states that the increase in concentration is directly proportional to the increase in the toxic material so that the killing power is faster. Mardiana et al. (2009) said that the use of amethyst leaf extract at a concentration of 2, 3, and 4% less effective as insecticides. This may be because the alkaloid compounds contained in amethyst leaves are lower than those contained in the roots and seeds, which can reach five times greater than the alkaloid content of the leaves. Mulyana (2002) also stated that the higher the concentration, the faster the insect will die, because the more active substances that enter the insect and conversely, the lower the concentration, the slower the insect will die. Amethyst leaf extract can kill 50% of Spodoptera litura (LC_{50}) larvae at 5% concentration and 95% at concentration of 10%. This showed that the higher the concentration of amethyst leaf extract treatment, the higher the mortality percentage of Spodoptera litura larvae and the faster the time of death.

Application of Insecticide Efficacy

The results of testing the efficacy of botanical insecticides in the laboratory need to be followed up by testing in the field/garden land because the conditions in the laboratory are very different from the conditions in the field. A type of vegetable insecticide that is effective in the laboratory is not necessarily effective in the field, considering that there are many factors that determine the efficacy of a vegetable insecticide in the field such as sunlight, rainfall, and temperature.

Propagation of Test Insects from Spodoptera litura

For the purpose of testing the efficacy of a natural pesticide against insect pests, a sufficient number of test insects is required. Propagation of test insects can be done with artificial feed or natural feed. Propagation by artificial feed requires very expensive costs because it requires various chemicals in the form of vitamins, antibiotics, agar, and other chemicals

that function to stimulate insects to eat and stay healthy. Insect propagation with artificial feed is usually done by researchers with special skills. On the other hand, insect propagation using natural food is relatively inexpensive and relatively easy to implement. Natural feed used is usually in the form of plant parts, such as leaves, fruit, seeds, and stems. The natural feed given was adjusted to the preferences of the test insects to be propagated. For example, Spodoptera litura likes castor leaves, Myzus persicae likes to suck the liquid from young tobacco leaves, and Tribolium sp. likes to eat green bean seeds. Furthermore, the test insect propagation container used a plastic jar with a diameter of 20 cm and a height of 20 cm. To make it easier to understand how to reproduce the test insects, the following describes the steps that must be taken in insect propagation.

Prior to the propagation of the test insects, a container for the reproduction of insects was prepared, namely a type of cage made of gauze. To reproduce Spodoptera litura, the trick is to look for groups of eggs in the field. The eggs of Spodoptera litura are covered with a kind of brown velvet. One egg group consists of hundreds of eggs. This propagation procedure consists of three parts. 1) Take the group of Spodoptera litura eggs carefully by tearing the leaves where the group is found. 2) Placing eggs in a container or cage that has been given fresh castor leaves as feed if at any time the group of eggs hatches. 3). cover the container with gauze. One group of eggs will produce hundreds of Spodoptera litura. Feed regularly every day until the caterpillar reaches the desired size for the purposes of the test insect. The following describes the method of field testing regarding the efficacy of vegetable insecticides isolated from amethyst leaves against Spodoptera litura on soybean plants. The concentration of amethyst leaf extract tested included five concentration levels, namely: 1, 2.5, 5, 7.5 and 10%.

Test of Amethyst Leaf Extract in the Field

The application procedure of amethyst leaf extract test in the garden is as follows:

a. Make research gardens in the form of plots and planted soybeans.
b. Make amethyst leaf vegetable insecticide extract in the form of preparations based on the required concentration, namely 1, 2.5, 5, 7.5, and 10%.
c. Determine 10 sample plants from each plot.
d. In the afternoon, put a Spodoptera litura measuring 0.5-1.0 cm on each sample plant, namely on the leaves. The plants are then covered with a plastic bag that has been perforated with a toothpick.
e. The next morning, the plastic hood was removed and the caterpillars were seen again. If the caterpillar is gone, add the next caterpillar.
f. Spraying methanol extract on plot I (1%), plot II (2.5%), plot III (5%), plot IV (7.5%), and plot V (10%). Each plot measuring 50 cm × 10 m requires 100 mL of extract solution.
g. Do the same with point f for the ethyl acetate and n-hexane fractions.
h. Each extract was repeated three times.
i. Do the capping of soybean plants and put another 5 caterpillars in a plastic bag that has been perforated with a toothpick.
j. Observing caterpillars on each of the sample plants every day. Record the number of dead caterpillars in each plot.
k. Observe the caterpillar's body carefully: Is there a caterpillar that won't eat, a caterpillar that is still fresh, a caterpillar that remains small, or a caterpillar that is very weak.
l. Record the percentage of leaf damage in each plot and count the number of dead caterpillars or larvae.
m. Determine the plot that causes the most caterpillar deaths.

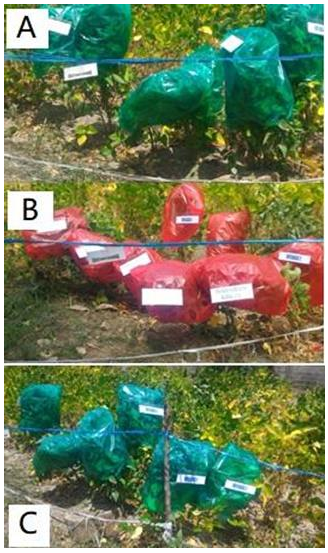

Figure 5. Soybeans given amethyst leaf extract A. methanol, B. ethyl acetate, and C. n-hexane in a plastic bag containing pests.

Tests of amethyst leaf extract, for the methanol fraction, with varying concentrations of 1, 2.5, 5, and 10% respectively gave mortality values of 27, 40, 40, and 60%. In the test of amethyst leaf extract, ethyl acetate extract with various concentrations gave mortality values, respectively: 40, 52, 67, and 87%. Furthermore, in the n-hexane extract test, respectively: 53, 60, 80, 87, and 100%. This showed that n-hexane extract was the most effective in killing pests compared to ethyl acetate and methanol extracts. Previous reports showed that hexane from Datura metel was more effective in controlling the fungus Macrophomina phaseolina, which causes char rot disease in plants (Dhawan & Gupta, 2017).

This amethyst leaf extract not only kills pests but can also fertilize soybean plants as shown in **Figure 6**. Yellow soybean leaves appear, which have not been sprayed with amethyst leaf extract **Figure 5**. Datura metel plant extract is also known to have herbicide activity because the plant methanol extract made from dry leaves can remove unwanted weeds (Mulyana, 2002). This extract also has antifungal activity because it contains pyrrole derivative compounds (Dabur et al., 2004). Nitrogen is the main component of protein, chlorophyll, enzymes, hormones and vitamins. Symptoms of N deficiency in young plants are shown by pale green leaves, and in severe conditions the leaves are pale yellow, the stems are weak and elongated. In older plants, the lower leaves show severe yellowing and eventually fall. Plant growth is stunted, stems are reddish, pod development is inhibited, leaves shrink and have thick walls so that the leaves become rough/hard and fibrous ((Fahmi et al., 2014). Chlorophyll can be increased with NPK fertilizer (Paul, 2001).

Figure 6. Soybean plants, in gardens that are sprayed with amethyst leaf extract, appear greener

The two nitrogen atoms in indole alkaloid are secondary (R_1NH) and tertiary amine (R_2N). The nitrogen atom which has lone electron pair causes the alkaloid to be basic like ammonia. The degree of acidity varies greatly depending on the molecular structure and the presence and location of other functional groups. Like ammonia, the alkaloids are converted to their salts by mineral acids and when the alkaloid salts react with hydroxide ions, the nitrogen releases hydrogen ions and the amines are liberated. The positive charge of the nitrogen ion depends on the number of organic groups covalently bonded to the nitrogen and the positive charge of this ion is balanced by several negative ions [$R_3N^+X^-$]. If the nature of the ammonium ion is such that there are no protons to release, it will not be affected by hydroxide ions. As a result, the compounds will have chemical properties that are very different from those of the amines. Most of the alkaloids are insoluble or slightly so in water but the salts formed after reacting with the acid are usually freely soluble. The N in the alkaloids is what gives the green color of the leaves, and is more influential on chlorophyll compared to P and K (Paul, 2001).

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Amethyst leaf extract as pest control and fertilizer for soybean plants

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Abstract. *Rumape O, Kilo A, Ischak NI. 2022. Amethyst leaf extract as pest control and fertilizer for soybean plants. Biodiversitas 23: xxxx.* Amethyst (*Datura metel* L) is a plant that grows and develops in the Gorontalo area, and people use it as traditional medicine. This plant has a natural insecticidal activity that is not yet known by the general public. So far, the results of research on natural insecticides from amethyst have only been tested on a small scale in the laboratory, not yet applied on a large scale in the garden.-. The purpose of this study was to extract amethyst leaves and apply it as an inhibitor of feeding activity and insect mortality in both the laboratory and soybean gardens. Amethyst leaves were extracted in the laboratory using methanol, n-hexane, and ethyl acetate. The extracts were tested phytochemically to determine the type of secondary metabolite, before applying it. Phytochemical test showed amethyst leaves contain alkaloid, flavonoid, terpenoid, and saponin. The application treatment for the bioactivity used variations in the concentration of amethyst leaf extract of the fractions (methanol, ethyl acetate and n-hexane), namely 1.0, 2.5, 5.0, 7.5, 10%; and 0% as control. In the laboratory, the treatment was applied by contact to 5 insects *Spodoptera litura* instar III for each concentration treatment with 3 replications. Observation parameters were the percentage decrease in feeding activity and mortality of *Spodoptera litura* larvae. In the garden, the extracts, with varying concentrations of the same as in the laboratory, ware applied to soybeans treated with the pest *Spodoptera litura* in a closed container, and the other was sprayed on plants that were left exposed. The results showed that the three extracts could kill pests, but n-hexane extract was the most effective compared to ethyl acetate and methanol extracts. Amazingly, soybean plants whose yellow leaves turn green after being given the extract. This indicates that the secondary metabolites of amethyst are not only used as insecticides to control pests, but also as plant fertilizers.

Key words: *Datura metel* L; antifeedant; natural insecticide; soybean; natural fertilizer; *Spodoptera litura*

INTRODUCTION

The use of synthetic pesticides is the main choice of farmers in controlling plant pests, even though they know the bad impact on human health and the environment (Rani et al. 2021; Rijal et al. 2018). They are also aware that chemicals from synthetic pesticides can be exposed to humans through consumption of agricultural products contaminated with pesticides (Ahmed et al. 2000). Only for practical reasons and quickly obtain yields and low costs, farmers ignore the negative effects of these synthetic chemicals (Damalas and Koutroubas 2018). In developing countries, the use of synthetic pesticides occurs in smallholder farmers who tend to have relatively unsatisfactory of education and restricted access to agricultural edification (Meemken and Qaim 2018). This has shown that the implementation of synthetic pesticides in the field is carried out systematically and widely (Deguine et al. 2021).

The use of this pesticide is inevitable, with the production of synthetic pesticide increasing every year globally (Gyawali 2018). According to the Food and Agriculture Organization (FAO), consumption of chemical pesticides has almost doubled, increasing from 2.3 to 4.1 million tonnes between 1990 and 2018 worldwide where China is the main contributor, followed by the United States, Brazil, Argentina and Canada (Deguine 2021; Fernández 2021). This increase is in line with the industrialization of the agricultural sector which continues to add chemicals to natural ecosystems (Nicolopoulou-Stamati et al. 2016). This is also exacerbated by the production of very large subsidized fertilizers and is obtained at low prices by farmers. Production of synthetic fertilizers in Indonesia in 2021 has reached 12,235 million tons (Nasution 2022), with subsidized fertilizers of 8,777 tons (Ramadhan 2022). The fertilizer is distributed throughout Indonesia, including Gorontalo which gets a quota of 64.162 tons. The allocation of subsidized fertilizer in one Gorontalo district alone has increased to 13,991 tons in 2021 (Eross A 2021). The use of these pesticides increases agricultural productivity by up to 60% (Gresik 2020). This shows that farmers' dependence on the use of pesticide fertilizers is very high, and continues to increase the contamination of agricultural products with harmful chemicals of synthetic pesticides. Therefore, it is necessary to find alternative insecticides that are natural and safe for the environment.

The development of natural insecticides is currently more directed at the discovery of secondary metabolite compounds that are not only effective in controlling pests but also have selective activity against certain pests that damage plants. Indonesia has abundant plant resources that

produce active compounds as insecticide repellent and antifeedant that are easily decomposed and leave no residue (Gurning and others 2020; Simangunsong et al. 2017; Suparman, Rupa, and others 2018). Here, we conduct research to find plants that grow a lot in Gorontalo and have no known potential applications. This plant has natural compounds that are safe, effective, and environmentally friendly as a substitute for synthetic pesticides which have had a negative impact because they leave residues on plant products and pollute the environment. The plant is amethyst which has important compounds that have the potential as insecticides that can inhibit feeding activity and can kill insects (Sreedhar et al. 2020). *Datura metel* leaf extract at higher concentrations is more toxic and can be used as an insecticide against grasshoppers and red ants (Kuganathan and Ganeshalingam 2011). Another use of amethyst leaves is as an antiviral and antifungal (Alam et al. 2021). Unfortunately, as an insecticide and antifungal from amethyst, it has only been tested on a laboratory scale, not in large gardens/land. Until now, amethyst has not been reported that amethyst can fertilize plants as we encountered in this study. Here, we also report the application of amethyst leaf extract in the garden.

The main aim of this study was to apply methanol, n-hexane, and ethyl acetate extracts from amethyst leaves as antifeedant against *Spodoptera litura* insects on soybean plants. Before applying the extract, the secondary metabolites were tested by phytochemical method. The extract was tested in the laboratory and in the garden. Activity Test of Anti-feeding and Toxicity in the laboratory was carried out on *Spodoptera litura*. Meanwhile, the application of insecticide efficacy in the garden was carried out on soybean plants against *Spodoptera litura* pests. These caterpillars can attack soybean plants thereby reducing productivity (Peruca et al. 2018).

MATERIALS AND METHODS

Preparation of amethyst leaf extract

Amethyst leaves of 1,256 kg were dried in the open air (Fig. 1C), without direct contact with sunlight, and 625.53 g of dry brownish-green samples were obtained. The sample was mashed with a blender, then macerated with 3 L of methanol for 3×24 hours. Every 1×24 hours, the material is filtered and the residue is macerated again with new methanol. The filtrate was evaporated at 30-40 °C to obtain a concentrated methanol extract of amethyst leave (ME).

ME as much as 50 g was suspended with methanol and water in a ratio of 2/1, and then partitioned with 200 mL of n-hexane. The results were separated using a separatory funnel, and the n-hexane fraction obtained was evaporated at 40°C to obtain a concentrated n-hexane extract (HE) of 17.437 grams. Then the methanol-water fraction was partitioned again with 200 mL of ethyl acetate. The ethyl acetate and water fractions were each evaporated at 40 °C to obtain a blackish red ethyl acetate extract (EE) of 10.9722 grams. The extract was phytochemically tested to determine the types of secondary metabolites of alkaloid, flavonoid, terpenoid, steroid, and saponin. The qualitative test used the method described by (Trease and Evans 1983), (Harbome 1998), and (El-Olemy, Al-Muhtadi, and Afifi 1994).

Effectiveness test of amethyst leaf extract

Three amethyst leaf extracts from ME, HE, and EE were tested for their antifungal effectiveness in two locations, namely the laboratory and field. Each extract with concentrations of 1, 2.5, 5, 7.5, and 10% was applied at both locations under different conditions.

Test of amethyst leaf extract in laboratory

The test solutions of the extracts that have been prepared with various concentrations are placed in separate containers of the same size. Each treatment used 5 and 15 test larvae, respectively, for testing in the laboratory and garden. All larvae were fasted for 8 hours. Soybean leaf feed was dipped in each of the test solutions and dried. Then put the third instar army larvae in the jar container. Evaluation was carried out every 6 hours after treatment for up to 24 hours to determine the activity of the extract on feeding activity and larval mortality. Three replications were carried out for each treatment with 5 larvae per replication.

Test of amethyst leaf extract in the field

The application procedure of amethyst leaf extract test in the garden is as follows:

a. Make research gardens in the form of plots and planted soybeans.
b. Make amethyst leaf vegetable insecticide extract in the form of preparations based on the required concentration, namely 1.0, 2.5, 5.0, 7.5, and 10%.
c. Determine 10 sample plants from each plot.
d. In the afternoon, put a *Spodoptera litura* measuring 0.5-1.0 cm on each sample plant, namely on the leaves. The plants are then covered with a plastic bag that has been perforated with a toothpick.
e. The next morning, the plastic hood was removed and the caterpillars were seen again. If the caterpillar is gone, add the next caterpillar.
f. Spraying methanol extract on plot I (1%), plot II (2.5%), plot III (5%), plot IV (7.5%), and plot V (10%). Each plot measuring 50 cm × 10 m requires 100 mL of extract solution.
g. Do the same with point f for the ethyl acetate and n-hexane fractions.
h. Each extract was repeated three times.
i. Do the capping of soybean plants and put another 5 caterpillars in a plastic bag that has been perforated with a toothpick.
j. Observing caterpillars on each of the sample plants every day. Record the number of dead caterpillars in each plot.
k. Observe the caterpillar's body carefully: Is there a caterpillar that won't eat, a caterpillar that is still

fresh, a caterpillar that remains small, or a caterpillar that is very weak.

l. Record the percentage of leaf damage in each plot and count the number of dead caterpillars or larvae.

m. Determine the plot that causes the most caterpillar deaths.

Calculation and data analysis

The antifeedant index was calculated by the formula of AFI = (C-T)/(C+T)×100%, where C and T were the weight of leaves consumed in control and treatment, respectively. Percentage of larval mortality was calculated using the Abbott formula of ACM=(PMT-PMC)/(100-PMC)×100, with PMT and PMC represent the percentages of mortality in treatment and control, respectively. Meanwhile, the data analysis used One-way Analysis of Variance (ANOVA) with a confidence level of 5%. Tukey's multiple range test was used to determine significant differences between treatments ($P \leqslant 0.05$).

RESULTS AND DISCUSSION

Amethyst leaf extract

The selection of plants used in this study is based on data which states that plants have been used empirically as medicine and some of those that have been tested are toxic (Adhana and Chaudhry 2019; Ko and Ko 1999). However, the selected plants have not been tested regarding their ability to control pests in the garden on soybeans. The test material used was amethyst leaf (*Datura metel* L.) as shown in **Figure 1**A. Immediately after being obtained, fresh plants were sorted wet with the aim of separating dirt, foreign materials adhering to plants, unused plant parts, or damaged plant parts from simplicia materials. Then washing was carried out to remove the dirt attached to the simplicia (**Figure 1**B). After washing, the simplicia ingredients are chopped into smaller sizes. This process is done to facilitate the process of drying and pollination.

Amethyst plants were dried in an open air (**Figure 1**C), without direct contact with sunlight to avoid damage to the compounds present in the sample. In addition, the sample can be durable because removing the water content in the sample can facilitate the withdrawal of bioactive compounds during maceration (Cacique et al. 2020). The sample was smoothed (Figure 1D) to expand the touch surface and facilitate the maceration process, where the larger the surface area of the contact area with the solvent, the more effective the extraction process (TeGrotenhuis et al. 1999; Yuliani et al. 2019).

The choice of methanol solvent in the sample maceration process is because methanol is a universal solvent that can bind all components of compounds that are polar, semi-polar, and non-polar (Ramdani, Chuzaemi, and others 2017). In addition, methanol is a solvent that has a high solubility and is harmless or non-toxic. The maceration method was chosen because the characteristics of the active compounds contained in the amethyst leaf sample were not known, so that the extraction method by heating was avoided to prevent the decomposition of compounds that were not heat resistant.

The concentrated methanol extract (ME) was then partitioned with n-hexane which is nonpolar and ethyl acetate which is semipolar. The extraction process will be efficient if the extraction is carried out repeatedly (Hadi, Sulistyowati, and Widyaningrum 2022; Khulu et al. 2022). Shaking in the fractionation process aims to expand the contact surface area between the two solvents so that the distribution of solutes between the two can take place properly (Harvey 2000). The density of n-hexane (0.4 g/mL) and ethyl acetate 0.66 g/mL is smaller than the density of water 1 (g/mL) which shows that the extracts of the two solvents are easy to separate because each is in a solution. The top layer in a water-methanol-containing solution. The yield of n-hexane extract was greater than that of ethyl acetate extract. The higher the yield value show that the raw material has a greater opportunity to be utilized (Bhuiya et al. 2020).

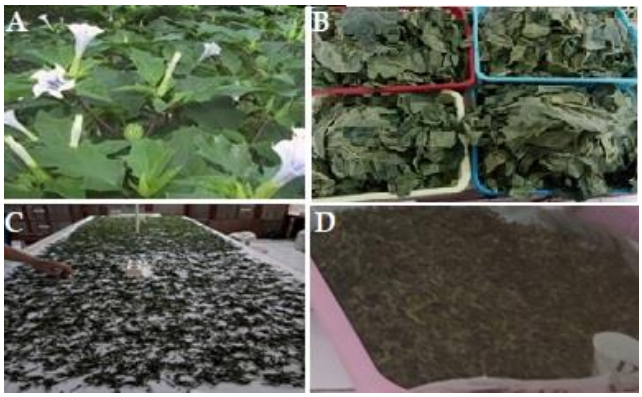

Figure 1. Amethyst Leaves; A. fresh, B. clean, C. dry, and D. Smooth

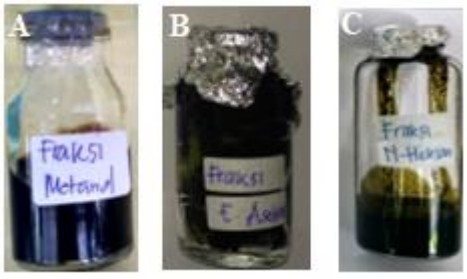

Figure 2. Amethyst leaf extract; A. Methanol, B. Ethyl acetate, and C. n-hexane

The results of phytochemical screening prove that amethyst leaves contain secondary metabolites of alkaloid, flavonoid, steroid, and terpenoide as shown in **Figure 2**. Alam et al. (2021) reported that the underside of amethyst leaves contains very large chemical compounds such as flavonoid, tropane alkaloid, tannin, saponin, and anolide.

Test of amethyst leaf extract

The effectiveness of amethyst leaf extract in controlling pests is carried out in two ways, namely by conducting anti-feeding activity and toxicity tests in the laboratory and its application in the field.

Antifeedant activity and toxicity test in the laboratory

Anti-feeding test

Anti-feeding test is a test carried out to see how much a plant has the power to inhibit the eating activity of a plant-disturbing pest. In the testing process, the insect larvae of *Spodoptera litura* were fasted for approximately 8 hours. The goal is that the larvae can eat fresh soybeanleaves provided as a test medium that has been smeared with sample extract (treatment) in various concentrations. If the insect is not fasted first, it is feared that the insect will not eat the treated leaves which can cause the insecticidal activity of the amethyst leaf extract sample to be immeasurable and inferential; whether insects that do not eat are caused by the presence of anti-feeding compounds or the state of insects that are not hungry. The test results are depicted in **Error! Reference source not found.**.

The test results showed that the methanol extract had 100% antifungal effect for the test solution concentrations of 7.5 and 10% as shown in **Figure 4**. The test solution concentrations of 5%, 2.5%, and 1% each had an anti-eating effect of 75, 62.5, and 38.5%, respectively. The ethyl acetate extract with concentrations of 5.0, 7.5, and 10% had antifungal power of 100%, while the test solution concentrations of 2.5 and 1% had an antifungal effect of about 72 and 58%, respectively. In the n-hexane extract, the concentrations of the test solution 5, 7.5, and 10% had 100% antifungal power, while the test solution concentrations of 2.5 and 1% had an antifungal effect of 63.3 and 43.05%, respectively. This shows that the ethyl acetate extract and the n-hexane extract of amethyst leaf showed an anti-feeding effect of 100% starting from the test solution concentration of 5%, while the methanol extract was 7.5%. There is no standard limit regarding the concentration of an effective test solution for compounds that are antifungal. A plant has effective anti-feeding properties when the level of food inhibition reaches 80-100% (Ambarningrum, Setyowati, and Susatyo 2012). However, statistical data illustrate that the differences in concentration and type of test solution from the extracts did not provide a significant difference to the antifungal activity, as evidenced by the F values which are smaller than the F crit shown in Table 1.

The decrease in feeding activity of the test animals was thought to be due to the content of allelochemical compounds contained in the amethyst leaf extract. Insect reactions to certain allelochemical compounds depend on the dose (Hsiao 1985). Complete inhibition by an antifungal compound may occur over the range of effective and potential doses of the substance. The results of the phytochemical analysis showed that the amethyst leaf extract contained alkaloid, flavonoid, terpenoide, tannin, and saponin.

Compounds that are anti-feeding are mostly found in the secondary metabolite group which can be contact poison and stomach poison (Banwo, Ogunremi, and Sanni 2020). Flavonoid compounds are included in the phenolic group which acts as a poison inhibitor of secondary metabolites and a slow-acting nervous system. Insects that die are caused by starvation due to paralysis of the mouth apparatus (Banwo, Ogunremi, and Sanni 2020). Flavonoids can reduce the ability to digest food in insects by reducing the activity of protease and amylase enzymes. As a result, insect growth is disrupted (Chen 2008). Terpenoide is one of the compounds that act as an antifungal because of its unpleasant taste and smell so that insects refuse to eat (Majidi et al. 2020). At high enough concentrations, terpenoide compounds can reduce insect feeding activity due to the nature of insects that refuse to eat due to the entry of compounds that stimulate chemoreceptors which are continued to the nervous system.

Saponin can reduce the surface tension of the mucous membranes of the digestive tract of larvae so that the walls of the digestive tract are (Aisyafahmi and Wahyuni 2018; Francis, Makkar, and Becker 2001; Rohmah, Subekti, and Rudyanto 2020). This is because saponins interact with mucosal cells causing the muscles under the skin surface of the digestive tract to be damaged and paralyzed. The absorption of food that has been contaminated by bioactive saponin compounds will be spread throughout the body through the circulatory system and will damage blood cells through hemolysis reactions so that it will interfere with the physiological processes of the larvae and will die (Francis, Makkar, and Becker 2001; del Hierro et al. 2018).

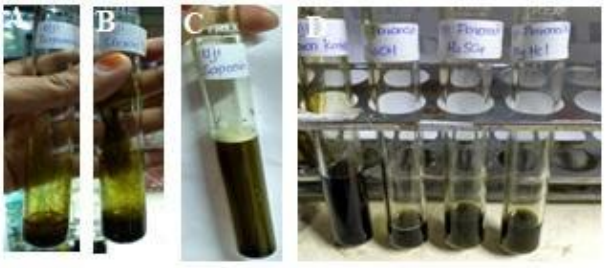

Figure 3. Phytochemical test results of samples of amethyst leave: A. terpenoide, B. steroids C. saponin, D. Flavonoid

Table 1. *F*, *F* crit, and *P* values from the results of the extract solution test on antifungal activity of larvae of *Spodoptera litura*

Test Solution	*F*	*P-value*	*F crit*
ME	2.1063	0.1848	5.3177
EE	0.3534	0.5686	5.3177
HE	0.0115	0.9171	5.3177
Interextract	0.248587	0.78382	3.8853

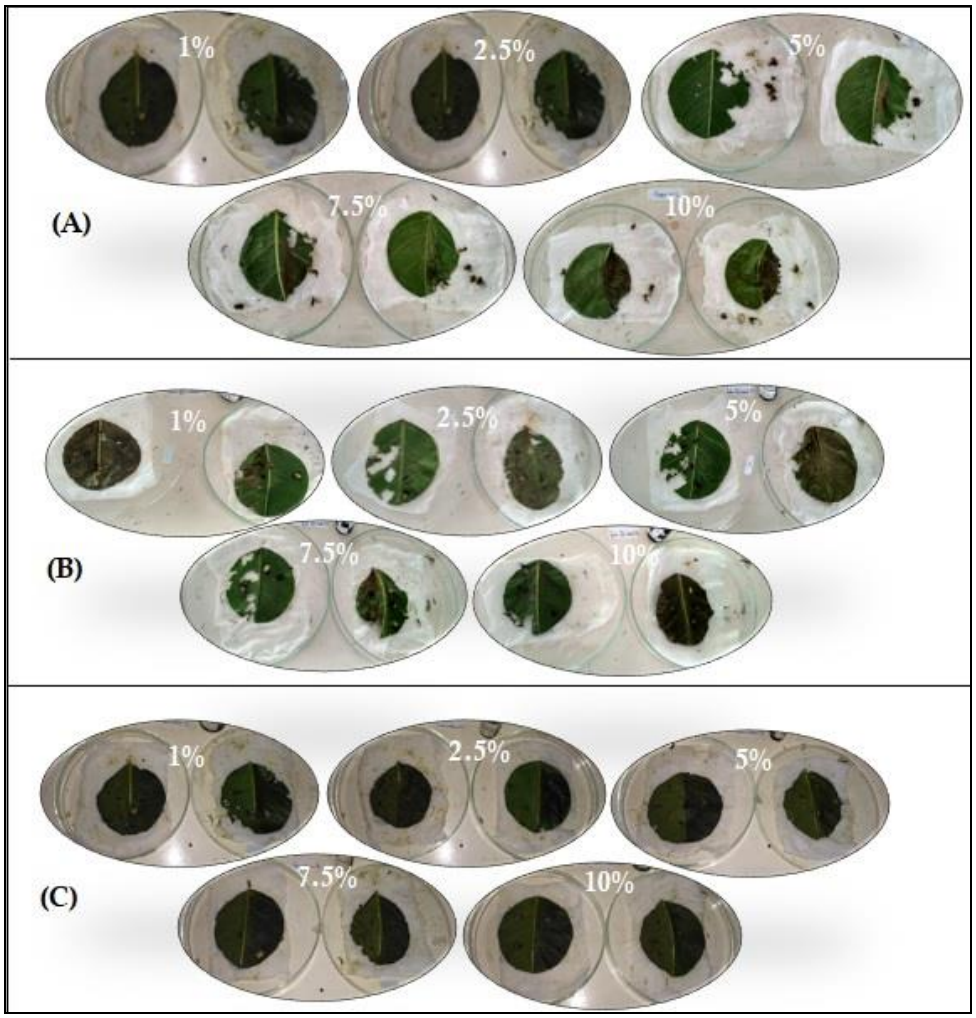

Figure 3. The results of the insect repellent activity test on leaves treated with extracts of (A) methanol, (B) ethyl acetate, and (C) n-hexane with concentrations of 1, 2.5, 7.5, and 10%. The increase in the concentration of the test solution from the extract was followed by an increase in anti-feeding, especially the n-hexane extract (HE).

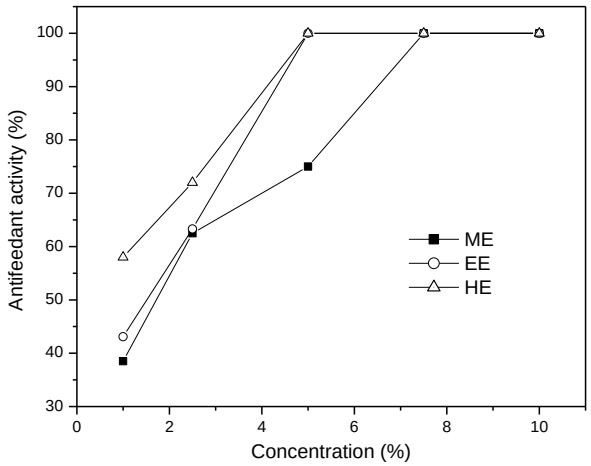

Figure 4. The results of the insect repellent activity test using test solutions of methanol extract (ME), ethyl acetate extract (EE), and n-hexane extract (HE). The anti-feeding activity of larvae against EE and HE at low concentrations was better than ME, otehrwise, at high concentrations the three extracts showed the same antifungal activity.

Insect toxicity test

Mortality tests were carried out on larvae of the pest *Spodoptera litura*, with the results showing that the higher the concentration of amethyst leaf extract, the higher the killing power. Leaf extracts with 10% concentration had 100% mortality. The killing power of amethyst leaf extract is caused by toxic secondary metabolites. One of them is an alkaloid compound which is known to have potential as an insecticide. Alkaloids have various effects on organisms. Amethyst leaves found alkaloid compounds with a content of 0.3 - 0.4% (about 85% hyoscyamine and 15% scopolamine as the main content) (Pratama 2008). Total alkaloid content is 0.426%, mainly as atropine and a small amount of hyoscyamine (Firdaus, Viquar, and Kazmi 2020). Usually the compound hyoscyamine is a racemic compound called atropine that can cause the nervous system of the caterpillar to turn it off. Alkaloids contained in amethyst can stimulate the endocrine glands to produce and increase the ecdysone hormone, causing metamorphosis failure and incomplete growth. In addition, amethyst leaves contain tannins that have a bitter taste and unpleasant odor so that eating activity is reduced and causes death. *Spodoptera litura* larvae that died due to treatment with amethyst leaf extract experienced stomach poisoning due to sucking the liquid from amethyst leaves which were sprayed on fresh water spinach leaves as a test medium.

Secondary metabolites in plants such as flavonoid glycosides are stomach poisons that work when these compounds enter the insect's body and will interfere with their digestive organs so that these compounds are toxic to pests (Ukoroije and Otayor 2020; Weny, Ilyas, and Panggabean 2018; Zhang et al. 2020). The results showed that the treatment with various concentrations of amethyst showed significant differences in mortality of *Spodoptera litura* larvae (**Figure 5**). The control treatment did not show the mortality of *Spodoptera litura* larvae. In the treatment of methanol extract with concentrations of 1, 2.5, and 5%, the killing power of *Spodoptera litura* larvae was low, respectively 20, 26.6, and 40%, on the contrary at concentrations of 7.5 and 10%, the killing power was quite effective, that is 60%. For ethyl acetate extract with concentrations of 1 and 2.5%, it had a low killing power of 40%, while at concentrations of 5, 7.5, and 10%, it had an effective killing power of 53, 66, and 86%, respectively. In contrast to the n-hexane extract, it had an effective killing power at concentrations of 1, 2.5, 5, and 7.5%, respectively, namely 53, 60, 80, 86%, and the most effective at a concentration of 10% with the highest killing power of 100. This indicates that the higher the concentration of amethyst leaf extracts, the higher the mortality rate of *Spodoptera litura* larvae. The higher the concentration, the more active substances that enter the insect (Chowański et al. 2016).

The difference in concentration of each extract did not differ significantly on larval mortality as indicated by a P-value greater than 0.05 (confidence level) or an F value less than F crit (**Table 2**). On the contrary, the extract type had a significant difference on larval mortality, where the F value (4.2419) was greater than the F crit (3.8853). Significant difference especially between n-hexane extract (HE) and methanol extract (ME) as shown with the highest Tukey value compared to others (Table 3).

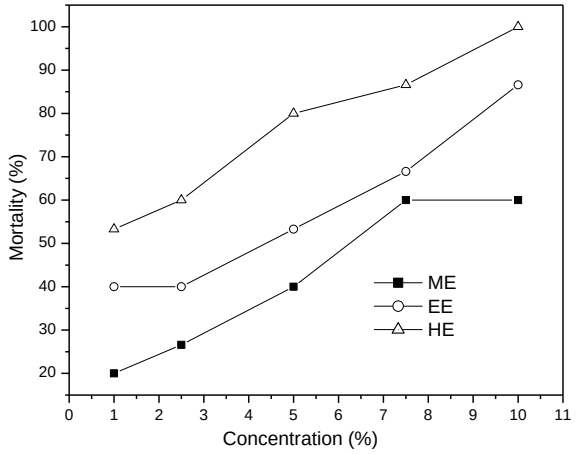

Figure 5. The number of larval deaths after being tested with amethyst leaf extract in various concentrations for 24 hours. The increase in the concentration of the test solutions of methanol extract (ME), ethyl acetate extract (EE), and n-hexane extract (HE) was followed by an increase in mortality, but this did not provide a significant difference. Conversely, the significant difference in mortality was due to the type of test solution, especially between ME and HE.

Table 2. *F*, *F* crit, and *P* values from the results of the extract solution test on mortality of larvae of *Spodoptera litura*

Test Solutions	*F*	*P*-value	*F* crit
ME	0.0609	0.9412	3.8853
EE	0.0073	0.9927	3.8853
HE	0.2000	0.8214	3.8853
Interextract	4.2419	0.0404	3.8853

Table 3. Tukey value between test solutions of extracts on mortality of larvae of *Spodoptera litura*

Test Solutions	Tukey
ME vs EE	1.7583
EE vs HE	2.3468
HE vs ME	4.1051

Toxic compounds that enter the body will undergo biotransformation to produce compounds that are water-soluble and more polar (Gerba 2019; Lushchak et al. 2018). This metabolic process requires more energy and the toxic compounds that enter the insect's body cause the energy needed for the neutralization process to increase. The amount of energy used to neutralize these toxic compounds causes inhibition of other metabolism so that insects will lack energy and eventually die. The use of n-hexane and ethyl acetate extracts at concentrations of 5, 7.5, and 10% was more precise and effective in killing *Spodoptera litura* larvae compared to methanol extract. This is in accordance with Khan et al. (2019) which states that the increase in concentration is directly proportional to the increase in the toxic material so that the killing power is faster. Mardiana et al. (2009) said that the use of amethyst leaf extract at a concentration of 2, 3, and 4% less effective as insecticides. This may be because the alkaloid compounds contained in amethyst leaves are lower than those contained in the roots and seeds, which can reach five times greater than the alkaloid content of the leaves. Mulyana (2002) also stated that the higher the concentration, the faster the insect will die, because the more active substances that enter the insect and conversely, the lower the concentration, the slower the insect will die. Amethyst leaf extract can kill 50% of *Spodoptera litura* (LC_{50}) larvae at 5% concentration and 95% at concentration of 10%. This showed that the higher the concentration of amethyst leaf extract treatment, the higher the mortality percentage of *Spodoptera litura* larvae and the faster the time of death.

Application of insecticide efficacy

The results of testing the efficacy of botanical insecticides in the laboratory need to be followed up by testing in the field/garden land because the conditions in the laboratory are very different from the conditions in the field. A type of vegetable insecticide that is effective in the laboratory is not necessarily effective in the field, considering that there are many factors that determine the efficacy of a vegetable insecticide in the field such as sunlight, rainfall, and temperature.

Propagation of Test Insects from Spodoptera litura

For the purpose of testing the efficacy of a natural pesticide against insect pests, a sufficient number of test insects is required. Propagation of test insects can be done with artificial feed or natural feed. Propagation by artificial feed requires very expensive costs because it requires various chemicals in the form of vitamins, antibiotics, agar, and other chemicals that function to stimulate insects to eat and stay healthy. Insect propagation with artificial feed is usually done by researchers with special skills. On the other hand, insect propagation using natural food is relatively inexpensive and relatively easy to implement. Natural feed used is usually in the form of plant parts, such as leaves, fruit, seeds, and stems. The natural feed given was adjusted to the preferences of the test insects to be propagated. For example, *Spodoptera litura* likes castor leaves, Myzus persicae likes to suck the liquid from young tobacco leaves, and Tribolium sp. likes to eat green bean seeds. Furthermore, the test insect propagation container used a plastic jar with a diameter of 20 cm and a height of 20 cm. To make it easier to understand how to reproduce the test insects, the following describes the steps that must be taken in insect propagation.

Prior to the propagation of the test insects, a container for the reproduction of insects was prepared, namely a type of cage made of gauze. To reproduce *Spodoptera litura*, the trick is to look for groups of eggs in the field. The eggs of *Spodoptera litura* are covered with a kind of brown velvet. One egg group consists of hundreds of eggs. This propagation procedure consists of three parts. 1) Take the group of *Spodoptera litura* eggs carefully by tearing the leaves where the group is found. 2) Placing eggs in a container or cage that has been given fresh castor leaves as feed if at any time the group of eggs hatches. 3). cover the container with gauze. One group of eggs will produce hundreds of *Spodoptera litura*. Feed regularly every day until the caterpillar reaches the desired size for the purposes of the test insect. The following describes the method of field testing regarding the efficacy of vegetable insecticides isolated from amethyst leaves against *Spodoptera litura* on soybean plants. The concentration of amethyst leaf extract tested included five concentration levels, namely: 1, 2.5, 5, 7.5 and 10%.

Tests of amethyst leaf extract, for the methanol fraction, with varying concentrations of 1, 2.5, 5, and 10% respectively gave mortality values of 27, 40, 40, and 60% as shown in **Figure 7**. In the test of amethyst leaf extract, ethyl acetate extract with various concentrations gave mortality values, respectively: 40, 52, 67, and 87%. Furthermore, in the n-hexane extract test, respectively: 53, 60, 80, 87, and 100%. This showed that n-hexane extract was the most effective in killing pests compared to ethyl acetate and methanol extracts. Previous reports showed that hexane from *Datura metel* was more effective in controlling the fungus Macrophomina phaseolina, which causes char rot disease in plants (Dhawan and Gupta 2017).

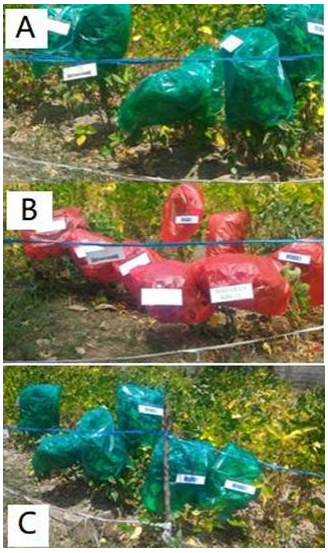

Figure 6. Soybeans given amethyst leaf extract A. methanol, B. ethyl acetate, and C. n-hexane in a plastic bag containing pests.

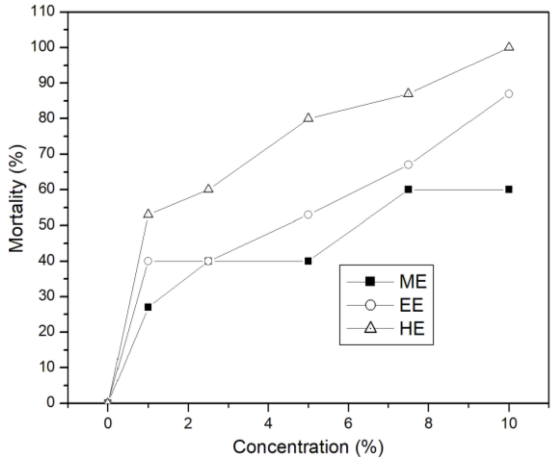

Figure 7. Data Insect mortality in field test of amethyst leaf extract after 24 hours. The increase in the concentration of the test solution from methanol extract (ME), ethyl acetate extract (EE), and n-hexane extract (HE) increased mortality. The increase in mortality due to the effect of increasing the concentration of each extract did not give a significant difference, especially ME.

Figure 8. Soybean plants, in gardens that are sprayed with amethyst leaf extract, appear greene

This amethyst leaf extract not only kills pests but can also fertilize soybean plants as shown in **Figure 8**. Yellow soybean leaves appear, which have not been sprayed with amethyst leaf extract **Figure 6**. *Datura metel* plant extract is also known to have herbicide activity because the plant methanol extract made from dry leaves can remove unwanted weeds (Mulyana 2002). This extract also has antifungal activity because it contains pyrrole derivative compounds (Dabur et al., 2004). Nitrogen is the main component of protein, chlorophyll, enzymes, hormones and vitamins. Symptoms of N deficiency in young plants are shown by pale green leaves, and in severe conditions the leaves are pale yellow, the stems are weak and elongated. In older plants, the lower leaves show severe yellowing and eventually fall. Plant growth is stunted, stems are reddish, pod development is inhibited, leaves shrink and have thick walls so that the leaves become rough/hard and fibrous ((Fahmi, Syamsuddin, and Marliah 2014). Chlorophyll can be increased with NPK fertilizer (Paul 2001).

The two nitrogen atoms in indole alkaloid are secondary (R_1NH) and tertiary amine (R_2N). The nitrogen atom which has lone electron pair causes the alkaloid to be basic like ammonia. The degree of acidity varies greatly depending on the molecular structure and the presence and location of other functional groups. Like ammonia, the alkaloids are converted to their salts by mineral acids and when the alkaloid salts react with hydroxide ions, the nitrogen releases hydrogen ions and the amines are liberated. The positive charge of the nitrogen ion depends on the number of organic groups covalently bonded to the nitrogen and the positive charge of this ion is balanced by several negative ions [$R_3N^+X^-$]. If the nature of the ammonium ion is such that there are no protons to release, it will not be affected by hydroxide ions. As a result, the compounds will have chemical properties that are very different from those of the amines. Most of the alkaloids are insoluble or slightly so in water but the salts formed after reacting with the acid are usually freely soluble. The N in the alkaloids is what gives the green color of the leaves, and is more influential on chlorophyll compared to P and K (Paul 2001).

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Amethyst leaf extract as pest control and fertilizer for soybean plants

Abstract. Amethyst (*Datura metel* L) is a plant that grows and develops in the Gorontalo area, and people use it as traditional medicine. This plant has a natural insecticidal activity that is not yet known by the general public. So far, the results of research on natural insecticides from amethyst have only been tested on a small scale in the laboratory, not yet applied on a large scale in the garden.-. The purpose of this study was to extract amethyst leaves and apply it as an inhibitor of feeding activity and insect mortality in both the laboratory and soybean gardens. Amethyst leaves were extracted in the laboratory using methanol, n-hexane, and ethyl acetate. The extracts were tested phytochemically to determine the type of secondary metabolite, before applying it. Phytochemical test showed amethyst leaves contain alkaloid, flavonoid, terpenoid, and saponin. The application treatment for the bioactivity used variations in the concentration of amethyst leaf extract of the fractions (methanol, ethyl acetate and n-hexane), namely 1.0, 2.5, 5.0, 7.5, 10%; and 0% as control. In the laboratory, the treatment was applied by contact to 5 insects *Spodoptera litura* instar III for each concentration treatment with 3 replications. Observation parameters were the percentage decrease in feeding activity and mortality of *Spodoptera litura* larvae. In the garden, the extracts, with varying concentrations of the same as in the laboratory, ware applied to soybeans treated with the pest *Spodoptera litura* in a closed container, and the other was sprayed on plants that were left exposed. The results showed that the three extracts could kill pests, but n-hexane extract was the most effective compared to ethyl acetate and methanol extracts. Amazingly, soybean plants whose yellow leaves turn green after being given the extract. This indicates that the secondary metabolites of amethyst are not only used as insecticides to control pests, but also as plant fertilizers.

Key words: *Datura metel* L; antifeedant; natural insecticide; soybean; natural fertilizer; *Spodoptera litura*

Abbreviations (if any): ME: Methanol Extract of Am

Running title: Amethyst leaf extract to control pest

INTRODUCTION

The use of synthetic pesticides is the main choice of farmers in controlling plant pests, even though they know the bad impact on human health and the environment (Rani et al. 2021; Rijal et al. 2018). They are also aware that chemicals from synthetic pesticides can be exposed to humans through consumption of agricultural products contaminated with pesticides (Ahmed et al. 2000). Only for practical reasons and quickly obtain yields and low costs, farmers ignore the negative effects of these synthetic chemicals (Damalas and Koutroubas 2018). In developing countries, the use of synthetic pesticides occurs in smallholder farmers who tend to have relatively unsatisfactory of education and restricted access to agricultural edification (Meemken and Qaim 2018). This has shown that the implementation of synthetic pesticides in the field is carried out systematically and widely (Deguine et al. 2021).

The use of this pesticide is inevitable, with the production of synthetic pesticide increasing every year globally (Gyawali 2018). According to the Food and Agriculture Organization (FAO), consumption of chemical pesticides has almost doubled, increasing from 2.3 to 4.1 million tonnes between 1990 and 2018 worldwide where China is the main contributor, followed by the United States, Brazil, Argentina and Canada (Deguine 2021; Fernández 2021). This increase is in line with the industrialization of the agricultural sector which continues to add chemicals to natural ecosystems (Nicolopoulou-Stamati et al. 2016). This is also exacerbated by the production of very large subsidized fertilizers and is obtained at low prices by farmers. Production of synthetic fertilizers in Indonesia in 2021 has reached 12,235 million tons (Nasution 2022), with subsidized fertilizers of 8,777 tons (Ramadhan 2022). The fertilizer is distributed throughout Indonesia, including Gorontalo which gets a quota of 64.162 tons. The allocation of subsidized fertilizer in one Gorontalo district alone has increased to 13,991 tons in 2021 (Eross A 2021). The use of these pesticides increases agricultural productivity by up to 60% (Gresik 2020). This shows that farmers' dependence on the use of pesticide fertilizers is very

high, and continues to increase the contamination of agricultural products with harmful chemicals of synthetic pesticides. Therefore, it is necessary to find alternative insecticides that are natural and safe for the environment.

The development of natural insecticides is currently more directed at the discovery of secondary metabolite compounds that are not only effective in controlling pests but also have selective activity against certain pests that damage plants. Indonesia has abundant plant resources that produce active compounds as insecticide repellent and antifeedant that are easily decomposed and leave no residue (Gurning and others 2020; Simangunsong et al. 2017; Suparman, Rupa, and others 2018). Here, we conduct research to find plants that grow a lot in Gorontalo and have no known potential applications. This plant has natural compounds that are safe, effective, and environmentally friendly as a substitute for synthetic pesticides which have had a negative impact because they leave residues on plant products and pollute the environment. The plant is amethyst which has important compounds that have the potential as insecticides that can inhibit feeding activity and can kill insects (Sreedhar et al. 2020). *Datura metel* leaf extract at higher concentrations is more toxic and can be used as an insecticide against grasshoppers and red ants (Kuganathan and Ganeshalingam 2011). Another use of amethyst leaves is as an antiviral and antifungal (Alam et al. 2021). Unfortunately, as an insecticide and antifungal from amethyst, it has only been tested on a laboratory scale, not in large gardens/land. Until now, amethyst has not been reported that amethyst can fertilize plants as we encountered in this study. Here, we also report the application of amethyst leaf extract in the garden.

The main aim of this study was to apply methanol, n-hexane, and ethyl acetate extracts from amethyst leaves as antifeedant against *Spodoptera litura* insects on soybean plants. Before applying the extract, the secondary metabolites were tested by phytochemical method. The extract was tested in the laboratory and in the garden. Activity Test of Anti-feeding and Toxicity in the laboratory was carried out on *Spodoptera litura*. Meanwhile, the application of insecticide efficacy in the garden was carried out on soybean plants against *Spodoptera litura* pests. These caterpillars can attack soybean plants thereby reducing productivity (Peruca et al. 2018).

MATERIALS AND METHODS

Preparation of Amethyst Leaf Extract

Amethyst leaves of 1,256 kg were dried in the open air (Fig. 1C), without direct contact with sunlight, and 625.53 g of dry brownish-green samples were obtained. The sample was mashed with a blender, then macerated with 3 L of methanol for 3×24 hours. Every 1×24 hours, the material is filtered and the residue is macerated again with new methanol. The filtrate was evaporated at 30-40 °C to obtain a concentrated methanol extract of amethyst leave (ME).

ME as much as 50 g was suspended with methanol and water in a ratio of 2/1, and then partitioned with 200 mL of n-hexane. The results were separated using a separatory funnel, and the n-hexane fraction obtained was evaporated at 40°C to obtain a concentrated n-hexane extract (HE) of 17.437 grams. Then the methanol-water fraction was partitioned again with 200 mL of ethyl acetate. The ethyl acetate and water fractions were each evaporated at 40 °C to obtain a blackish red ethyl acetate extract (EE) of 10.9722 grams. The extract was phytochemically tested to determine the types of secondary metabolites of alkaloid, flavonoid, terpenoid, steroid, and saponin. The qualitative test used the method described by (Trease and Evans 1983), (Harborne 1998), and (El-Olemy, Al-Muhtadi, and Afifi 1994).

Effectiveness Test of Amethyst Leaf Extract

Three amethyst leaf extracts from ME, HE, and EE were tested for their antifungal effectiveness in two locations, namely the laboratory and field. Each extract with concentrations of 1, 2.5, 5, 7.5, and 10% was applied at both locations under different conditions.

Test of Amethyst Leaf Extract in Laboratory

The test solutions of the extracts that have been prepared with various concentrations are placed in separate containers of the same size. Each treatment used 5 and 15 test larvae, respectively, for testing in the laboratory and garden. All larvae were fasted for 8 hours. Soybean leaf feed was dipped in each of the test solutions and dried. Then put the third instar army larvae in the jar container. Evaluation was carried out every 6 hours after treatment for up to 24 hours to determine the activity of the extract on feeding activity and larval mortality. Three replications were carried out for each treatment with 5 larvae per replication.

Test of Amethyst Leaf Extract in the Field

The application procedure of amethyst leaf extract test in the garden is as follows:

a. Make research gardens in the form of plots and planted soybeans.
b. Make amethyst leaf vegetable insecticide extract in the form of preparations based on the required concentration, namely 1.0, 2.5, 5.0, 7.5, and 10%.
c. Determine 10 sample plants from each plot.
d. In the afternoon, put a *Spodoptera litura* measuring 0.5-1.0 cm on each sample plant, namely on the leaves. The plants are then covered with a plastic bag that has been perforated with a toothpick.

e. The next morning, the plastic hood was removed and the caterpillars were seen again. If the caterpillar is gone, add the next caterpillar.
f. Spraying methanol extract on plot I (1%), plot II (2.5%), plot III (5%), plot IV (7.5%), and plot V (10%). Each plot measuring 50 cm × 10 m requires 100 mL of extract solution.
g. Do the same with point f for the ethyl acetate and n-hexane fractions.
h. Each extract was repeated three times.
i. Do the capping of soybean plants and put another 5 caterpillars in a plastic bag that has been perforated with a toothpick.
j. Observing caterpillars on each of the sample plants every day. Record the number of dead caterpillars in each plot.
k. Observe the caterpillar's body carefully: Is there a caterpillar that won't eat, a caterpillar that is still fresh, a caterpillar that remains small, or a caterpillar that is very weak.
l. Record the percentage of leaf damage in each plot and count the number of dead caterpillars or larvae.
m. Determine the plot that causes the most caterpillar deaths.

Calculation and Data Analysis

THE ANTIFEEDANT INDEX WAS CALCULATED BY THE FORMULA OF AFI = (C-T)/(C+T)×100%, WHERE C AND T WERE THE WEIGHT OF LEAVES CONSUMED IN CONTROL AND TREATMENT, RESPECTIVELY. PERCENTAGE OF LARVAL MORTALITY WAS CALCULATED USING THE ABBOTT FORMULA OF ACM=(PMT-PMC)/(100-PMC)×100, WITH PMT AND PMC REPRESENT THE PERCENTAGES OF MORTALITY IN TREATMENT AND CONTROL, RESPECTIVELY. MEANWHILE, THE DATA ANALYSIS USED ONE-WAY ANALYSIS OF VARIANCE (ANOVA) WITH A CONFIDENCE LEVEL OF 5%. TUKEY'S MULTIPLE RANGE TEST WAS USED TO DETERMINE SIGNIFICANT DIFFERENCES BETWEEN TREATMENTS ($P \leqslant 0.05$).RESULTS AND DISCUSSION

Amethyst Leaf Extract

The selection of plants used in this study is based on data which states that plants have been used empirically as medicine and some of those that have been tested are toxic (Adhana and Chaudhry 2019; Ko and Ko 1999). However, the selected plants have not been tested regarding their ability to control pests in the garden on soybeans. The test material used was amethyst leaf (*Datura metel* L.) as shown in **Figure 1**A. Immediately after being obtained, fresh plants were sorted wet with the aim of separating dirt, foreign materials adhering to plants, unused plant parts, or damaged plant parts from simplicia materials. Then washing was carried out to remove the dirt attached to the simplicia (**Figure 1**B). After washing, the simplicia ingredients are chopped into smaller sizes. This process is done to facilitate the process of drying and pollination.

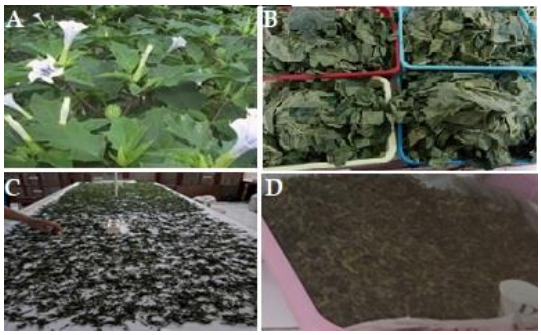

Figure 1. Amethyst Leaves; A. fresh, B. clean, C. dry, and D. smooth.

Amethyst plants were dried in an open air (**Figure 1**C), without direct contact with sunlight to avoid damage to the compounds present in the sample. In addition, the sample can be durable because removing the water content in the sample can facilitate the withdrawal of bioactive compounds during maceration (Cacique et al. 2020). The sample was smoothed (Figure 1D) to expand the touch surface and facilitate the maceration process, where the larger the surface area of the contact area with the solvent, the more effective the extraction process (TeGrotenhuis et al. 1999; Yuliani et al. 2019).

The choice of methanol solvent in the sample maceration process is because methanol is a universal solvent that can bind all components of compounds that are polar, semi-polar, and non-polar (Ramdani, Chuzaemi, and others 2017). In addition, methanol is a solvent that has a high solubility and is harmless or non-toxic. The maceration method was chosen because the characteristics of the active compounds contained in the amethyst leaf sample were not known, so that the extraction method by heating was avoided to prevent the decomposition of compounds that were not heat resistant.

The concentrated methanol extract (ME) was then partitioned with n-hexane which is nonpolar and ethyl acetate which is semipolar. The extraction process will be efficient if the extraction is carried out repeatedly (Hadi, Sulistyowati, and Widyaningrum 2022; Khulu et al. 2022). Shaking in the fractionation process aims to expand the contact surface area between the two solvents so that the distribution of solutes between the two can take place properly (Harvey 2000). The density of n-hexane (0.4 g/mL) and ethyl acetate 0.66 g/mL is smaller than the density of water 1 (g/mL) which shows that the extracts of the two solvents are easy to separate because each is in a solution. The top layer in a water-methanol-containing solution. The yield of n-hexane extract was greater than that of ethyl acetate extract. The higher the yield value show that the raw material has a greater opportunity to be utilized (Bhuiya et al. 2020).

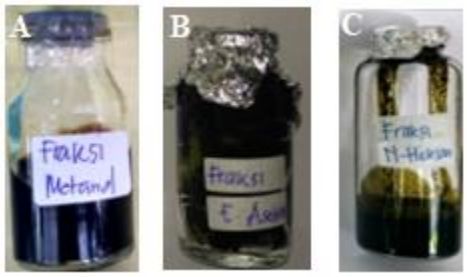

Figure 2. Amethyst leaf extract; A. Methanol, B. Ethyl acetate, and C. n-hexane.

The results of phytochemical screening prove that amethyst leaves contain secondary metabolites of alkaloid, flavonoid, steroid, and terpenoide as shown in **Figure 2**. Alam et al. (2021) reported that the underside of amethyst leaves contains very large chemical compounds such as flavonoid, tropane alkaloid, tannin, saponin, and anolide.

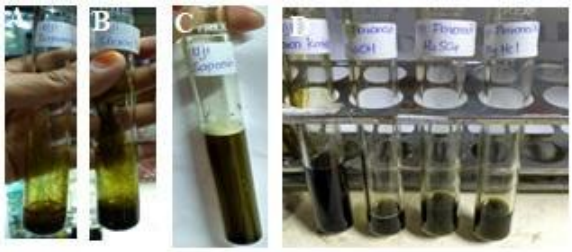

Figure 3. Phytochemical test results of samples of amethyst leave: A. terpenoide, B. steroids C. saponin, D. flavonoid

Test of Amethyst Leaf Extract

The effectiveness of amethyst leaf extract in controlling pests is carried out in two ways, namely by conducting anti-feeding activity and toxicity tests in the laboratory and its application in the field.

Antifeedant Activity and Toxicity Test in the Laboratory

Anti-feeding Test

Anti-feeding test is a test carried out to see how much a plant has the power to inhibit the eating activity of a plant-disturbing pest. In the testing process, the insect larvae of *Spodoptera litura* were fasted for approximately 8 hours. The goal is that the larvae can eat fresh soybeanleaves provided as a test medium that has been smeared with sample extract (treatment) in various concentrations. If the insect is not fasted first, it is feared that the insect will not eat the treated leaves which can cause the insecticidal activity of the amethyst leaf extract sample to be immeasurable and inferential; whether insects that do not eat are caused by the presence of anti-feeding compounds or the state of insects that are not hungry. The test results are depicted in **Figure 4**.

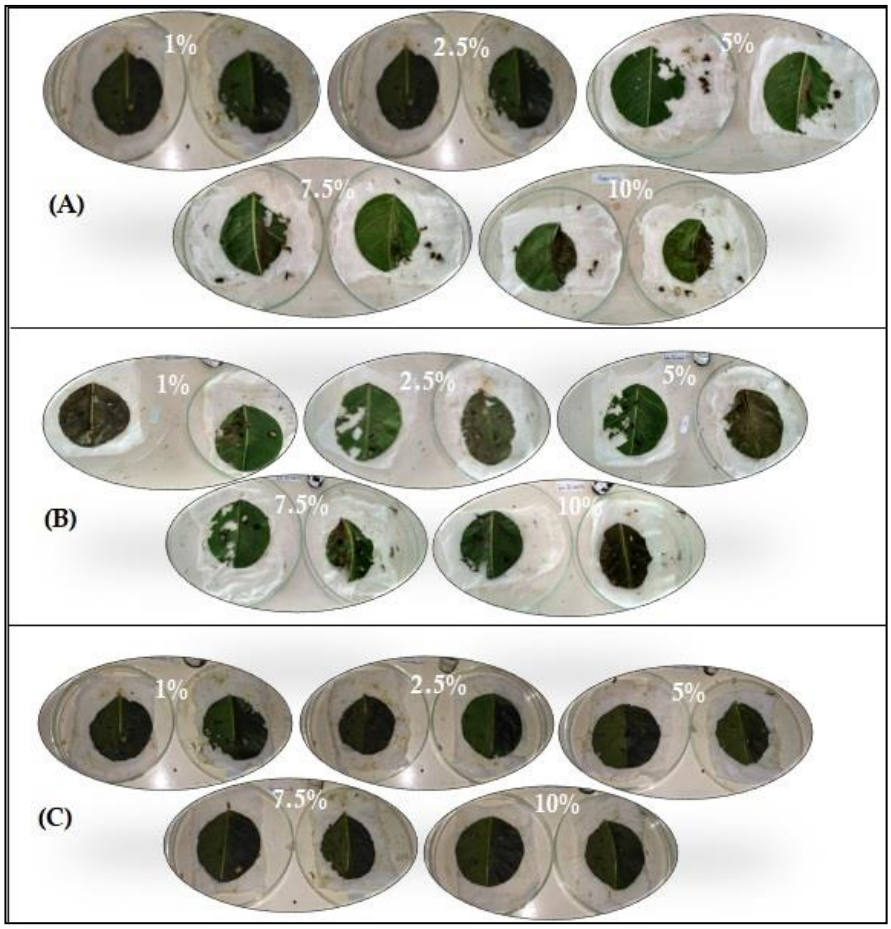

Figure 4. The results of the insect repellent activity test on leaves treated with extracts of (A) methanol, (B) ethyl acetate, and (C) n-hexane with concentrations of 1, 2.5, 7.5, and 10%. The increase in the concentration of the test solution from the extract was followed by an increase in anti-feeding, especially the n-hexane extract (HE).

The test results showed that the methanol extract had 100% antifungal effect for the test solution concentrations of 7.5 and 10% as shown in **Figure 5**. The test solution concentrations of 5%, 2.5%, and 1% each had an anti-eating effect of 75, 62.5, and 38.5%, respectively. The ethyl acetate extract with concentrations of 5.0, 7.5, and 10% had antifungal power of 100%, while the test solution concentrations of 2.5 and 1% had an antifungal effect of about 72 and 58%, respectively. In the n-hexane extract, the concentrations of the test solution 5, 7.5, and 10% had 100% antifungal power, while the test solution concentrations of 2.5 and 1% had an antifungal effect of 63.3 and 43.05%, respectively. This shows that the ethyl acetate extract and the n-hexane extract of amethyst leaf showed an anti-feeding effect of 100% starting from the test solution concentration of 5%, while the methanol extract was 7.5%. There is no standard limit regarding the concentration of an effective test solution for compounds that are antifungal. A plant has effective anti-feeding properties when the level of food inhibition reaches 80-100% (Ambarningrum, Setyowati, and Susatyo 2012). However, statistical data illustrate that the differences in concentration and type of test solution from the extracts did not provide a significant difference to the antifungal activity, as evidenced by the F values which are smaller than the F crit shown in Table 1.

Table 1. *F*, *F* crit, and *P* values from the results of the extract solution test on antifungal activity of larvae of *Spodoptera litura*

Test Solution	*F*	*P-value*	*F crit*
ME	2.1063	0.1848	5.3177
EE	0.3534	0.5686	5.3177

HE	0.0115	0.9171	5.3177
Interextract	0.248587	0.78382	3.8853

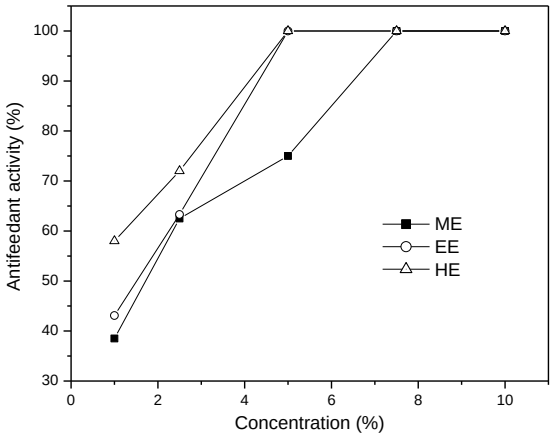

Figure 5 The results of the insect repellent activity test using test solutions of methanol extract (ME), ethyl acetate extract (EE), and n-hexane extract (HE). The anti-feeding activity of larvae against EE and HE at low concentrations was better than ME, otehrwise, at high concentrations the three extracts showed the same antifungal activity.

The decrease in feeding activity of the test animals was thought to be due to the content of allelochemical compounds contained in the amethyst leaf extract. Insect reactions to certain allelochemical compounds depend on the dose (Hsiao 1985). Complete inhibition by an antifungal compound may occur over the range of effective and potential doses of the substance. The results of the phytochemical analysis showed that the amethyst leaf extract contained alkaloid, flavonoid, terpenoide, tannin, and saponin.

Compounds that are anti-feeding are mostly found in the secondary metabolite group which can be contact poison and stomach poison (Banwo, Ogunremi, and Sanni 2020). Flavonoid compounds are included in the phenolic group which acts as a poison inhibitor of secondary metabolites and a slow-acting nervous system. Insects that die are caused by starvation due to paralysis of the mouth apparatus (Banwo, Ogunremi, and Sanni 2020). Flavonoids can reduce the ability to digest food in insects by reducing the activity of protease and amylase enzymes. As a result, insect growth is disrupted (Chen 2008). Terpenoide is one of the compounds that act as an antifungal because of its unpleasant taste and smell so that insects refuse to eat (Majidi et al. 2020). At high enough concentrations, terpenoide compounds can reduce insect feeding activity due to the nature of insects that refuse to eat due to the entry of compounds that stimulate chemoreceptors which are continued to the nervous system.

Saponin can reduce the surface tension of the mucous membranes of the digestive tract of larvae so that the walls of the digestive tract are (Aisyafahmi and Wahyuni 2018; Francis, Makkar, and Becker 2001; Rohmah, Subekti, and Rudyanto 2020). This is because saponins interact with mucosal cells causing the muscles under the skin surface of the digestive tract to be damaged and paralyzed. The absorption of food that has been contaminated by bioactive saponin compounds will be spread throughout the body through the circulatory system and will damage blood cells through hemolysis reactions so that it will interfere with the physiological processes of the larvae and will die (Francis, Makkar, and Becker 2001; del Hierro et al. 2018).

Insect Toxicity Test

Mortality tests were carried out on larvae of the pest *Spodoptera litura*, with the results showing that the higher the concentration of amethyst leaf extract, the higher the killing power. Leaf extracts with 10% concentration had 100% mortality. The killing power of amethyst leaf extract is caused by toxic secondary metabolites. One of them is an alkaloid compound which is known to have potential as an insecticide. Alkaloids have various effects on organisms. Amethyst leaves found alkaloid compounds with a content of 0.3 - 0.4% (about 85% hyoscyamine and 15% scopolamine as the main content) (Pratama 2008). Total alkaloid content is 0.426%, mainly as atropine and a small amount of hyoscyamine (Firdaus, Viquar, and Kazmi 2020). Usually the compound hyoscyamine is a racemic compound called atropine that can cause the nervous system of the caterpillar to turn it off. Alkaloids contained in amethyst can stimulate the endocrine glands to produce and increase the ecdysone hormone, causing metamorphosis failure and incomplete growth. In addition, amethyst leaves contain tannins that have a bitter taste and unpleasant odor so that eating activity is reduced and causes death. *Spodoptera litura* larvae that died due to treatment with amethyst leaf extract experienced stomach poisoning due to sucking the liquid from amethyst leaves which were sprayed on fresh water spinach leaves as a test medium.

Secondary metabolites in plants such as flavonoid glycosides are stomach poisons that work when these compounds enter the insect's body and will interfere with their digestive organs so that these compounds are toxic to pests (Ukoroije and Otayor 2020; Weny, Ilyas, and Panggabean 2018; Zhang et al. 2020). The results showed that the treatment with

various concentrations of amethyst showed significant differences in mortality of *Spodoptera litura* larvae (**Figure 6**). The control treatment did not show the mortality of *Spodoptera litura* larvae. In the treatment of methanol extract with concentrations of 1, 2.5, and 5%, the killing power of *Spodoptera litura* larvae was low, respectively 20, 26.6, and 40%, on the contrary at concentrations of 7.5 and 10%, the killing power was quite effective, that is 60%. For ethyl acetate extract with concentrations of 1 and 2.5%, it had a low killing power of 40%, while at concentrations of 5, 7.5, and 10%, it had an effective killing power of 53, 66, and 86%, respectively. In contrast to the n-hexane extract, it had an effective killing power at concentrations of 1, 2.5, 5, and 7.5%, respectively, namely 53, 60, 80, 86%, and the most effective at a concentration of 10% with the highest killing power of 100. This indicates that the higher the concentration of amethyst leaf extracts, the higher the mortality rate of *Spodoptera litura* larvae. The higher the concentration, the more active substances that enter the insect (Chowański et al. 2016).

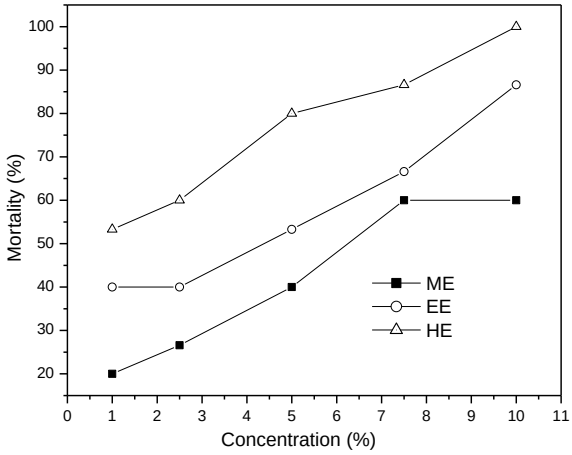

Figure 6. The number of larval deaths after being tested with amethyst leaf extract in various concentrations for 24 hours. The increase in the concentration of the test solutions of methanol extract (ME), ethyl acetate extract (EE), and n-hexane extract (HE) was followed by an increase in mortality, but this did not provide a significant difference. Conversely, the significant difference in mortality was due to the type of test solution, especially between ME and HE.

The difference in concentration of each extract did not differ significantly on larval mortality as indicated by a P-value greater than 0.05 (confidence level) or an F value less than F crit (**Table 2**). On the contrary, the extract type had a significant difference on larval mortality, where the F value (4.2419) was greater than the F crit (3.8853). Significant difference especially between n-hexane extract (HE) and methanol extract (ME) as shown with the highest Tukey value compared to others (**Table 3).**

Table 2. *F*, *F* crit, and *P* values from the results of the extract solution test on mortality of larvae of *Spodoptera litura*

Test Solutions	*F*	*P*-value	*F* crit
ME	0.0609	0.9412	3.8853
EE	0.0073	0.9927	3.8853
HE	0.2000	0.8214	3.8853
Interextract	4.2419	0.0404	3.8853

Table 3. Tukey value between test solutions of extracts on mortality of larvae of *Spodoptera litura*

Test Solutions	Tukey
ME vs EE	1.7583
EE vs HE	2.3468
HE vs ME	4.1051

Toxic compounds that enter the body will undergo biotransformation to produce compounds that are water-soluble and more polar (Gerba 2019; Lushchak et al. 2018). This metabolic process requires more energy and the toxic compounds that enter the insect's body cause the energy needed for the neutralization process to increase. The amount of energy used to neutralize these toxic compounds causes inhibition of other metabolism so that insects will lack energy and eventually die. The use of n-hexane and ethyl acetate extracts at concentrations of 5, 7.5, and 10% was more precise and effective in killing *Spodoptera litura* larvae compared to methanol extract. This is in accordance with Khan et al. (2019) which states that the increase in concentration is directly proportional to the increase in the toxic material so that the killing power is faster. Mardiana et al. (2009) said that the use of amethyst leaf extract at a concentration of 2, 3, and 4% less effective as

insecticides. This may be because the alkaloid compounds contained in amethyst leaves are lower than those contained in the roots and seeds, which can reach five times greater than the alkaloid content of the leaves. Mulyana (2002) also stated that the higher the concentration, the faster the insect will die, because the more active substances that enter the insect and conversely, the lower the concentration, the slower the insect will die. Amethyst leaf extract can kill 50% of *Spodoptera litura* (LC_{50}) larvae at 5% concentration and 95% at concentration of 10%. This showed that the higher the concentration of amethyst leaf extract treatment, the higher the mortality percentage of *Spodoptera litura* larvae and the faster the time of death.

Application of Insecticide Efficacy

The results of testing the efficacy of botanical insecticides in the laboratory need to be followed up by testing in the field/garden land because the conditions in the laboratory are very different from the conditions in the field. A type of vegetable insecticide that is effective in the laboratory is not necessarily effective in the field, considering that there are many factors that determine the efficacy of a vegetable insecticide in the field such as sunlight, rainfall, and temperature.

Propagation of Test Insects from Spodoptera litura

For the purpose of testing the efficacy of a natural pesticide against insect pests, a sufficient number of test insects is required. Propagation of test insects can be done with artificial feed or natural feed. Propagation by artificial feed requires very expensive costs because it requires various chemicals in the form of vitamins, antibiotics, agar, and other chemicals that function to stimulate insects to eat and stay healthy. Insect propagation with artificial feed is usually done by researchers with special skills. On the other hand, insect propagation using natural food is relatively inexpensive and relatively easy to implement. Natural feed used is usually in the form of plant parts, such as leaves, fruit, seeds, and stems. The natural feed given was adjusted to the preferences of the test insects to be propagated. For example, *Spodoptera litura* likes castor leaves, Myzus persicae likes to suck the liquid from young tobacco leaves, and Tribolium sp. likes to eat green bean seeds. Furthermore, the test insect propagation container used a plastic jar with a diameter of 20 cm and a height of 20 cm. To make it easier to understand how to reproduce the test insects, the following describes the steps that must be taken in insect propagation.

Prior to the propagation of the test insects, a container for the reproduction of insects was prepared, namely a type of cage made of gauze. To reproduce *Spodoptera litura*, the trick is to look for groups of eggs in the field. The eggs of *Spodoptera litura* are covered with a kind of brown velvet. One egg group consists of hundreds of eggs. This propagation procedure consists of three parts. 1) Take the group of *Spodoptera litura* eggs carefully by tearing the leaves where the group is found. 2) Placing eggs in a container or cage that has been given fresh castor leaves as feed if at any time the group of eggs hatches. 3). cover the container with gauze. One group of eggs will produce hundreds of *Spodoptera litura*. Feed regularly every day until the caterpillar reaches the desired size for the purposes of the test insect. The following describes the method of field testing regarding the efficacy of vegetable insecticides isolated from amethyst leaves against *Spodoptera litura* on soybean plants. The concentration of amethyst leaf extract tested included five concentration levels, namely: 1, 2.5, 5, 7.5 and 10%.

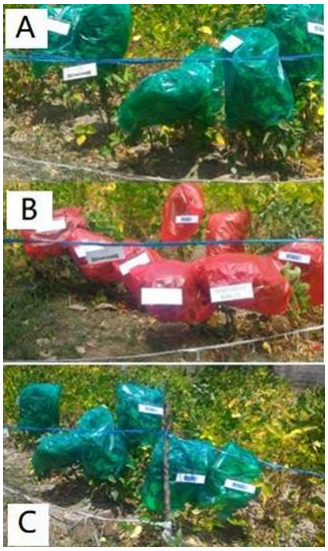

Figure 7. Soybeans given amethyst leaf extract A. methanol, B. ethyl acetate, and C. n-hexane in a plastic bag containing pests.

Tests of amethyst leaf extract, for the methanol fraction, with varying concentrations of 1, 2.5, 5, and 10% respectively gave mortality values of 27, 40, 40, and 60% as shown in **Figure 8**. In the test of amethyst leaf extract, ethyl acetate extract with various concentrations gave mortality values, respectively: 40, 52, 67, and 87%. Furthermore, in the n-hexane extract test, respectively: 53, 60, 80, 87, and 100%. This showed that n-hexane extract was the most effective in killing pests compared to ethyl acetate and methanol extracts. Previous reports showed that hexane from *Datura metel* was more effective in controlling the fungus Macrophomina phaseolina, which causes char rot disease in plants (Dhawan and Gupta 2017).

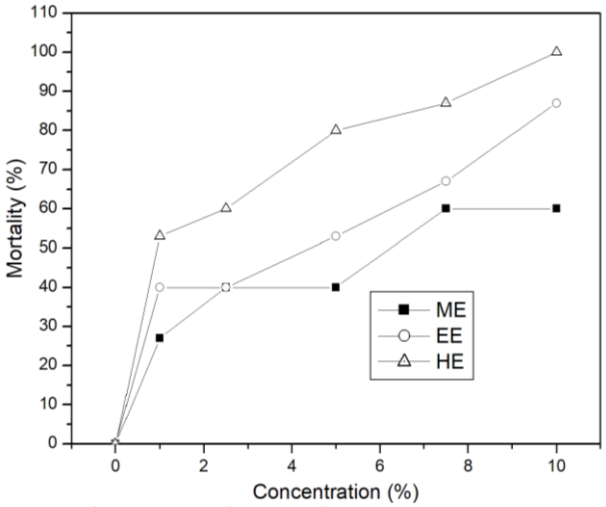

Figure 8. Data Insect mortality in field test of amethyst leaf extract after 24 hours. The increase in the concentration of the test solution from methanol extract (ME), ethyl acetate extract (EE), and n-hexane extract (HE) increased mortality. The increase in mortality due to the effect of increasing the concentration of each extract did not give a significant difference, especially ME.

This amethyst leaf extract not only kills pests but can also fertilize soybean plants as shown in **Figure 9**. Yellow soybean leaves appear, which have not been sprayed with amethyst leaf extract **Figure 7**. *Datura metel* plant extract is also known to have herbicide activity because the plant methanol extract made from dry leaves can remove unwanted weeds (Mulyana 2002). This extract also has antifungal activity because it contains pyrrole derivative compounds (Dabur et al., 2004). Nitrogen is the main component of protein, chlorophyll, enzymes, hormones and vitamins. Symptoms of N deficiency in young plants are shown by pale green leaves, and in severe conditions the leaves are pale yellow, the stems are weak and elongated. In older plants, the lower leaves show severe yellowing and eventually fall. Plant growth is stunted, stems are reddish, pod development is inhibited, leaves shrink and have thick walls so that the leaves become rough/hard and fibrous ((Fahmi, Syamsuddin, and Marliah 2014). Chlorophyll can be increased with NPK fertilizer (Paul 2001).

Figure 9. Soybean plants, in gardens that are sprayed with amethyst leaf extract, appear greener

The two nitrogen atoms in indole alkaloid are secondary (R_1NH) and tertiary amine (R_2N). The nitrogen atom which has lone electron pair causes the alkaloid to be basic like ammonia. The degree of acidity varies greatly depending on the molecular structure and the presence and location of other functional groups. Like ammonia, the alkaloids are converted to their salts by mineral acids and when the alkaloid salts react with hydroxide ions, the nitrogen releases hydrogen ions and the amines are liberated. The positive charge of the nitrogen ion depends on the number of organic groups covalently bonded to the nitrogen and the positive charge of this ion is balanced by several negative ions [$R_3N^+X^-$]. If the nature of the ammonium ion is such that there are no protons to release, it will not be affected by hydroxide ions. As a result, the compounds will have chemical properties that are very different from those of the amines. Most of the alkaloids are insoluble or slightly so in water but the salts formed after reacting with the acid are usually freely soluble. The N in the alkaloids is what gives the green color of the leaves, and is more influential on chlorophyll compared to P and K (Paul 2001).

ACKNOWLEDGEMENTS

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COVERING LETTER

Dear **Editor-in-Chief,**

I herewith enclosed a research article,

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Amethyst leaf extract as pest control and fertilizer for soybean plants

Author(s) name:

Opir Rumape, Akram La Kilo, Netty Ino Ischak

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Amethyst leaf extract is not only a natural insecticide that has been claimed so far, but we found this extract to be able to fertilize soybean plants grown in gardens. The alkaloid content of this extract which we suspect is responsible for the chlorophyll. For example, yellow soybean leaves, when sprayed with the extract, the leaves become lush green. We found this because the research we did was not only tested in the laboratory, but also in the field/garden. The n-hexane extract of amethyst gave a greater effect as a natural insecticide against Sopdeptoa litura and soybean plant fertilizers, than the methanol and ethyl acetate extracts.

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Opir Rumape

Amethyst leaf extract as pest control and fertilizer for soybean plants

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Abstract. Amethyst (Datura Metel L) is a plant that grows and develops in the Gorontalo area, and people use it as traditional medicine. The plant has natural insecticidal activity, but people do not know about it, let alone apply it. So far, amethyst has only been reported as an insecticide on a laboratory scale, not its application in gardens. The purpose of this study was to extract amethyst leaves and apply it as an inhibitor of feeding activity and insect mortality in both the laboratory and soybean gardens. Amethyst leaves were extracted in the laboratory using methanol, n-hexane, and ethyl acetate. The extracts were tested phytochemically to determine the type of secondary metabolite, before applying it. Phytochemical test showed amethyst leaves contain alkaloid, flavonoid, terpenoid, and saponin. The application treatment for the bioactivity used variations in the concentration of amethyst leaf extract of the fractions (methanol, ethyl acetate and n-hexane), namely 1.0, 2.5, 5.0, 7.5, 10%; and 0% as control. In the laboratory, the treatment was applied by contact to 5 insects Spodoptera litura instar III for each concentration treatment with 3 replications. Observation parameters were the percentage decrease in feeding activity and mortality of Spodoptera litura larvae. In the garden, the extracts, with varying concentrations of the same as in the laboratory, ware applied to soybeans treated with the pest Spodoptera litura in a closed container, and the other was sprayed on plants that were left exposed. The results showed that the three extracts could kill pests, but n-hexane extract was the most effective compared to ethyl acetate and methanol extracts. Amazingly, soybean plants whose yellow leaves turn green after being given the extract. This shows that the secondary metabolites of amethyst are not only used as insecticides to control pests, but also as plant fertilizers.

Key words: Datura metel L; antifeedant; natural insecticide; soybean; natural fertilizer; Spodoptera litura

Abbreviations (if any): ME: Methanol Extract of Am

Running title: Amethyst leaf extract to control pest

INTRODUCTION

The use of synthetic pesticides is the main choice of farmers in controlling plant pests, even though they know the bad impact on human health and the environment (Rani et al. 2021; Rijal et al. 2018). They are also aware that chemicals from synthetic pesticides can be exposed to humans through consumption of agricultural products contaminated with pesticides (Ahmed et al. 2000). Only for practical reasons and quickly obtain yields and low costs, farmers ignore the negative effects of these synthetic chemicals (Damalas and Koutroubas 2018). In developing countries, the use of synthetic pesticides occurs in smallholder farmers who tend to have relatively unsatisfactory of education and restricted access to agricultural edification (Meemken and Qaim 2018). This has shown that the implementation of synthetic pesticides in the field is carried out systematically and widely (Deguine et al. 2021).

The use of this pesticide is inevitable, with the production of synthetic pesticide increasing every year globally (Gyawali 2018). According to the Food and Agriculture Organization (FAO), consumption of chemical pesticides has almost doubled, increasing from 2.3 to 4.1 million tonnes between 1990 and 2018 worldwide where China is the main contributor, followed by the United States, Brazil, Argentina and Canada (Deguine 2021; Fernández 2021). This increase is in line with the industrialization of the agricultural sector which continues to add chemicals to natural ecosystems (Nicolopoulou-Stamati et al. 2016). This is also exacerbated by the production of very large subsidized fertilizers and is obtained at low prices by farmers. Production of synthetic fertilizers in Indonesia in 2021 has reached 12,235 million tons (Nasution 2022), with subsidized fertilizers of 8,777 tons (Ramadhan 2022). The fertilizer is distributed throughout Indonesia, including Gorontalo which gets a quota of 64.162 tons. The allocation of subsidized fertilizer in one Gorontalo district alone has increased to 13,991 tons in 2021 (Eross A 2021). The use of these pesticides increases agricultural productivity by up to 60% (Gresik 2020). This shows that farmers' dependence on the use of pesticide fertilizers is very high, and continues to increase the contamination of agricultural products with harmful chemicals of synthetic pesticides. Therefore, it is necessary to find alternative insecticides that are natural and safe for the environment.

The development of natural insecticides is currently more directed at the discovery of secondary metabolite compounds that are not only effective in controlling pests but also have selective activity against certain pests that damage plants. Indonesia has abundant plant resources that produce active compounds as insecticide repellent and antifeedant that are easily decomposed and leave no residue (Gurning and others 2020; Simangunsong et al. 2017; Suparman, Rupa, and others 2018). Here, we conduct research to find plants that grow a lot in Gorontalo and have no known potential applications. This plant has natural compounds that are safe, effective, and environmentally friendly as a substitute for synthetic pesticides which have had a negative impact because they leave residues on plant products and pollute the environment. The plant is amethyst which has important compounds that have the potential as insecticides that can inhibit feeding activity and can kill insects (Sreedhar et al. 2020). Datura metel leaf extract at higher concentrations is more toxic and can be used as an insecticide against grasshoppers and red ants (Kuganathan and Ganeshalingam 2011). Another use of amethyst leaves is as an antiviral and antifungal (Alam et al. 2021). Unfortunately, as an insecticide and antifungal from amethyst, it has only been tested on a laboratory scale, not in large gardens/land. Until now, amethyst has not been reported that amethyst can fertilize plants as we encountered in this study. Here, we also report the application of amethyst leaf extract in the garden.

The main aim of this study was to apply methanol, n-hexane, and ethyl acetate extracts from amethyst leaves as antifeedant against Spodoptera litura insects on soybean plants. Before applying the extract, the secondary metabolites were tested by phytochemical method. The extract was tested in the laboratory and in the garden. Activity Test of Anti-feeding and Toxicity in the laboratory was carried out on Spodoptera litura. Meanwhile, the application of insecticide efficacy in the garden was carried out on soybean plants against Spodoptera litura pests. These caterpillars can attack soybean plants thereby reducing productivity (Peruca et al. 2018).

MATERIALS AND METHODS

Preparation of Amethyst Leaf Extract

Amethyst leaves of 1,256 kg were dried in the open air (Fig. 1C), without direct contact with sunlight, and 625.53 g of dry brownish-green samples were obtained. The sample was mashed with a blender, then macerated with 3 L of methanol for 3×24 hours. Every 1×24 hours, the material is filtered and the residue is macerated again with new methanol. The filtrate was evaporated at 30-40 °C to obtain a concentrated methanol extract of amethyst leave (ME).

ME as much as 50 g was suspended with methanol and water in a ratio of 2/1, and then partitioned with 200 mL of n-hexane. The results were separated using a separatory funnel, and the n-hexane fraction obtained was evaporated at 40°C to obtain a concentrated n-hexane extract (HE) of 17.437 grams. Then the methanol-water fraction was partitioned again with 200 mL of ethyl acetate. The ethyl acetate and water fractions were each evaporated at 40 °C to obtain a blackish red ethyl acetate (EE) extract of 10.9722 grams. The extract was phytochemically tested to determine the types of secondary metabolites of alkaloid, flavonoid, terpenoid, steroid, and saponin. The qualitative test used the method described by (Trease and Evans 1983), (Harbome 1998), and (El-Olemy, Al-Muhtadi, and Afifi 1994).

Effectiveness Test of Amethyst Leaf Extract

Three amethyst leaf extracts from ME, HE, and EE were tested for their antifungal effectiveness in two locations, namely the laboratory and garden. Each extract with concentrations of 1, 2.5, 5, 7.5, and 10% was applied at both locations under different conditions. The extracts were applied to the leaves to determine the inhibition of the feeding activity (antifeedant) of Spodoptera litura larvae in the laboratory. In addition, toxicity tests were also carried out. In the garden plots, soybeans are planted. The plant was given these extracts to test the insect repellent and fertility of soybeans.

RESULTS AND DISCUSSION

Amethyst Leaf Extract

The selection of plants used in this study is based on data which states that plants have been used empirically as medicine and some of those that have been tested are toxic (Adhana and Chaudhry 2019; Ko and Ko 1999). However, the selected plants have not been tested regarding their ability to control pests in the garden on soybeans. The test material used was amethyst leaf (Datura metel L.) as shown in **Figure 1**A. Immediately after being obtained, fresh plants were sorted wet with the aim of separating dirt, foreign materials adhering to plants, unused plant parts, or damaged plant parts from simplicia materials. Then washing was carried out to remove the dirt attached to the simplicia (**Figure 1**B). After washing, the simplicia ingredients are chopped into smaller sizes. This process is done to facilitate the process of drying and pollination.

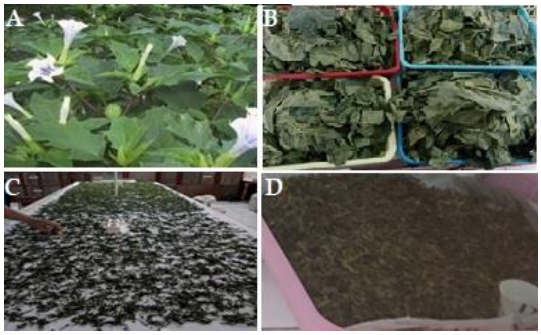

Figure 1. Amethyst Leaves; A. fresh, B. clean, C. dry, and D. smooth.

Amethyst plants were dried in an open air (**Figure 1**C), without direct contact with sunlight to avoid damage to the compounds present in the sample. In addition, the sample can be durable because removing the water content in the sample can facilitate the withdrawal of bioactive compounds during maceration (Cacique et al. 2020). The sample was smoothed (Figure 1D) to expand the touch surface and facilitate the maceration process, where the larger the surface area of the contact area with the solvent, the more effective the extraction process (TeGrotenhuis et al. 1999; Yuliani et al. 2019).

The choice of methanol solvent in the sample maceration process is because methanol is a universal solvent that can bind all components of compounds that are polar, semi-polar, and non-polar (Ramdani, Chuzaemi, and others 2017). In addition, methanol is a solvent that has a high solubility and is harmless or non-toxic. The maceration method was chosen because the characteristics of the active compounds contained in the amethyst leaf sample were not known, so that the extraction method by heating was avoided to prevent the decomposition of compounds that were not heat resistant.

The concentrated methanol extract (ME) was then partitioned with n-hexane which is nonpolar and ethyl acetate which is semipolar. The extraction process will be efficient if the extraction is carried out repeatedly (Hadi, Sulistyowati, and Widyaningrum 2022; Khulu et al. 2022). Shaking in the fractionation process aims to expand the contact surface area between the two solvents so that the distribution of solutes between the two can take place properly (Harvey 2000). The density of n-hexane (0.4 g/mL) and ethyl acetate 0.66 g/mL is smaller than the density of water 1 (g/mL) which shows that the extracts of the two solvents are easy to separate because each is in a solution. The top layer in a water-methanol-containing solution. The yield of n-hexane extract was greater than that of ethyl acetate extract. The higher the yield value show that the raw material has a greater opportunity to be utilized (Bhuiya et al. 2020).

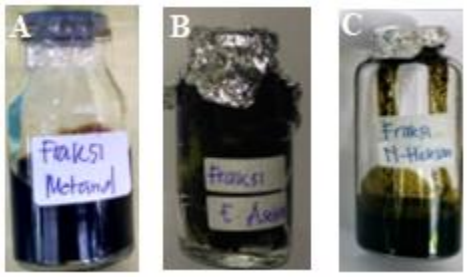

Figure 2. Amethyst leaf extract; A. Methanol, B. Ethyl acetate, and C. n-hexane.

The results of phytochemical screening prove that amethyst leaves contain secondary metabolites of alkaloid, flavonoid, steroid, and terpenoide as shown in **Figure 2**. Alam et al. (2021) reported that the underside of amethyst leaves contains very large chemical compounds such as flavonoid, tropane alkaloid, tannin, saponin, and anolide.

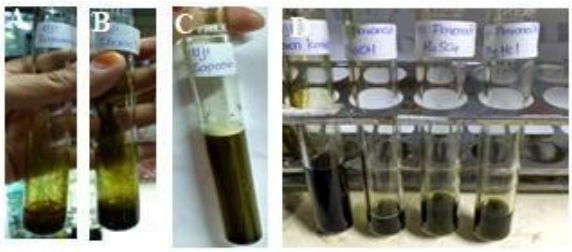

Figure 3. Phytochemical test results of samples of amethyst leave: A. terpenoide, B. steroids C. saponin, D. flavonoid

Test of Amethyst Leaf Extract

The effectiveness of amethyst leaf extract in controlling pests is carried out in two ways, namely by conducting anti-feeding activity and toxicity tests in the laboratory and its application in the garden.

Antifeedant Activity and Toxicity Test in the Laboratory

Anti-feeding Test

Anti-feeding test is a test carried out to see how much a plant has the power to inhibit the eating activity of a plant-disturbing pest. In the testing process, the insect larvae of Spodoptera litura were fasted for approximately 8 hours. The goal is that the larvae can eat fresh kale leaves provided as a test medium that has been smeared with sample extract (treatment) in various concentrations. If the insect is not fasted first, it is feared that the insect will not eat the treated leaves which can cause the insecticidal activity of the amethyst leaf extract sample to be immeasurable and inferential; whether insects that do not eat are caused by the presence of anti-feeding compounds or the state of insects that are not hungry. The test results are depicted in **Figure 4**.

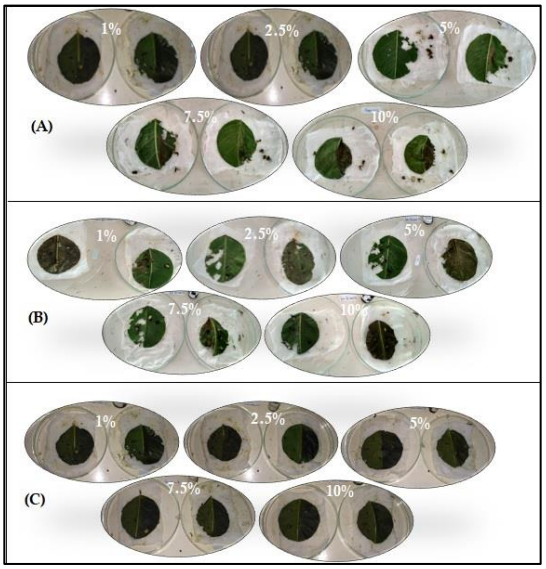

Figure 4. The results of the insect repellent activity test on leaves treated with extracts of (A) methanol, (B) ethyl acetate, and (C) n-hexane with concentrations of 1, 2.5, 7.5, and 10%

The test results showed that the methanol extract had 100% antifungal effect for the test solution concentrations of 7.5 and 10% as shown in **Figure 5**. The test solution concentrations of 5%, 2.5%, and 1% each had an anti-eating effect of 75, 62.5, and 38.5%, respectively. The ethyl acetate extract with concentrations of 5.0, 7.5, and 10% had antifungal power of 100%, while the test solution concentrations of 2.5 and 1% had an antifungal effect of about 72 and 58%, respectively. In the n-hexane extract, the concentrations of the test solution 5, 7.5, and 10% had 100% antifungal power, while the test solution concentrations of 2.5 and 1% had an antifungal effect of 63.3 and 43.05%, respectively. This shows that the ethyl acetate extract and the n-hexane extract of amethyst leaf showed an anti-feeding effect of 100% starting from the test solution concentration of 5%, while the methanol extract was 7.5%. There is no standard limit regarding the concentration of an effective test solution for compounds that are antifungal. A plant has effective anti-feeding properties when the level of food inhibition reaches 80-100% (Ambarningrum, Setyowati, and Susatyo 2012).

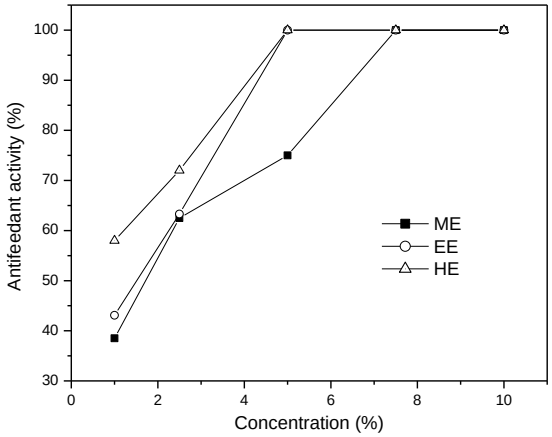

Figure 5 The results of the insect repellent activity test.

The decrease in feeding activity of the test animals was thought to be due to the content of allelochemical compounds contained in the amethyst leaf extract. Insect reactions to certain allelochemical compounds depend on the dose (Hsiao 1985). Complete inhibition by an antifungal compound may occur over the range of effective and potential doses of the substance. The results of the phytochemical analysis showed that the amethyst leaf extract contained alkaloid, flavonoid, terpenoide, tannin, and saponin.

Compounds that are anti-feeding are mostly found in the secondary metabolite group which can be contact poison and stomach poison (Banwo, Ogunremi, and Sanni 2020). Flavonoid compounds are included in the phenolic group which acts as a poison inhibitor of secondary metabolites and a slow-acting nervous system. Insects that die are caused by starvation due to paralysis of the mouth apparatus (Banwo, Ogunremi, and Sanni 2020). Flavonoids can reduce the ability to digest food in insects by reducing the activity of protease and amylase enzymes. As a result, insect growth is disrupted (Chen 2008). Terpenoide is one of the compounds that act as an antifungal because of its unpleasant taste and smell so that insects refuse to eat (Majidi et al. 2020). At high enough concentrations, terpenoide compounds can reduce insect feeding activity due to the nature of insects that refuse to eat due to the entry of compounds that stimulate chemoreceptors which are continued to the nervous system.

Saponin can reduce the surface tension of the mucous membranes of the digestive tract of larvae so that the walls of the digestive tract are (Aisyafahmi and Wahyuni 2018; Francis, Makkar, and Becker 2001; Rohmah, Subekti, and Rudyanto 2020). This is because saponins interact with mucosal cells causing the muscles under the skin surface of the digestive tract to be damaged and paralyzed. The absorption of food that has been contaminated by bioactive saponin compounds will be spread throughout the body through the circulatory system and will damage blood cells through hemolysis reactions so that it will interfere with the physiological processes of the larvae and will die (Francis, Makkar, and Becker 2001; del Hierro et al. 2018).

Insect Toxicity Test

Mortality tests were carried out on larvae of the pest Spodoptera litura, with the results showing that the higher the concentration of amethyst leaf extract, the higher the killing power. Leaf extracts with 10% concentration had 100% mortality. The killing power of amethyst leaf extract is caused by toxic secondary metabolites. One of them is an alkaloid compound which is known to have potential as an insecticide. Alkaloids have various effects on organisms. Amethyst leaves found alkaloid compounds with a content of 0.3 - 0.4% (about 85% hyoscyamine and 15% scopolamine as the main content) (Pratama 2008). Total alkaloid content is 0.426%, mainly as atropine and a small amount of hyoscyamine (Firdaus, Viquar, and Kazmi 2020). Usually the compound hyoscyamine is a racemic compound called atropine that can cause the nervous system of the caterpillar to turn it off. Alkaloids contained in amethyst can stimulate the endocrine glands to produce and increase the ecdysone hormone, causing metamorphosis failure and incomplete growth. In addition, amethyst leaves contain tannins that have a bitter taste and unpleasant odor so that eating activity is reduced and causes death. Spodoptera litura larvae that died due to treatment with amethyst leaf extract experienced stomach poisoning due to sucking the liquid from amethyst leaves which were sprayed on fresh water spinach leaves as a test medium.

Secondary metabolites in plants such as flavonoid glycosides are stomach poisons that work when these compounds enter the insect's body and will interfere with their digestive organs so that these compounds are toxic to pests (Ukoroije and Otayor 2020; Weny, Ilyas, and Panggabean 2018; Zhang et al. 2020). The results showed that the treatment with various concentrations of amethyst showed significant differences in mortality of Spodoptera litura larvae (**Figure 6**). The control treatment did not show the mortality of Spodoptera litura larvae. In the treatment of methanol extract with concentrations of 1, 2.5, and 5%, the killing power of Spodoptera litura larvae was low, respectively 20, 26.6, and 40%, on the contrary at concentrations of 7.5 and 10%, the killing power was quite effective, that is 60%. For ethyl acetate extract with concentrations of 1 and 2.5%, it had a low killing power of 40%, while at concentrations of 5, 7.5, and 10%, it had an effective killing power of 53, 66, and 86%, respectively. In contrast to the n-hexane extract, it had an effective killing

power at concentrations of 1, 2.5, 5, and 7.5%, respectively, namely 53, 60, 80, 86%, and the most effective at a concentration of 10% with the highest killing power of 100. This indicates that the higher the concentration of amethyst leaf extracts, the higher the mortality rate of Spodoptera litura larvae. The higher the concentration, the more active substances that enter the insect (Chowański et al. 2016).

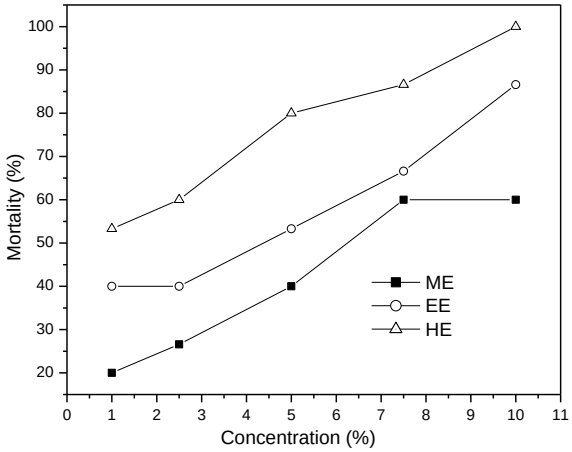

Figure 6. The number of larval deaths after being tested with amethyst leaf extract in various concentrations for 24 hours.

Toxic compounds that enter the body will undergo biotransformation to produce compounds that are water-soluble and more polar (Gerba 2019; Lushchak et al. 2018). This metabolic process requires more energy and the toxic compounds that enter the insect's body cause the energy needed for the neutralization process to increase. The amount of energy used to neutralize these toxic compounds causes inhibition of other metabolism so that insects will lack energy and eventually die. The use of n-hexane and ethyl acetate extracts at concentrations of 5, 7.5, and 10% was more precise and effective in killing Spodoptera litura larvae compared to methanol extract. This is in accordance with Khan et al. (2019) which states that the increase in concentration is directly proportional to the increase in the toxic material so that the killing power is faster. Mardiana et al. (2009) said that the use of amethyst leaf extract at a concentration of 2, 3, and 4% less effective as insecticides. This may be because the alkaloid compounds contained in amethyst leaves are lower than those contained in the roots and seeds, which can reach five times greater than the alkaloid content of the leaves. Mulyana (2002) also stated that the higher the concentration, the faster the insect will die, because the more active substances that enter the insect and conversely, the lower the concentration, the slower the insect will die. Amethyst leaf extract can kill 50% of Spodoptera litura (LC_{50}) larvae at 5% concentration and 95% at concentration of 10%. This showed that the higher the concentration of amethyst leaf extract treatment, the higher the mortality percentage of Spodoptera litura larvae and the faster the time of death.

Application of Insecticide Efficacy

The results of testing the efficacy of botanical insecticides in the laboratory need to be followed up by testing in the field/garden land because the conditions in the laboratory are very different from the conditions in the field. A type of vegetable insecticide that is effective in the laboratory is not necessarily effective in the field, considering that there are many factors that determine the efficacy of a vegetable insecticide in the field such as sunlight, rainfall, and temperature.

Propagation of Test Insects from Spodoptera litura

For the purpose of testing the efficacy of a natural pesticide against insect pests, a sufficient number of test insects is required. Propagation of test insects can be done with artificial feed or natural feed. Propagation by artificial feed requires very expensive costs because it requires various chemicals in the form of vitamins, antibiotics, agar, and other chemicals that function to stimulate insects to eat and stay healthy. Insect propagation with artificial feed is usually done by researchers with special skills. On the other hand, insect propagation using natural food is relatively inexpensive and relatively easy to implement. Natural feed used is usually in the form of plant parts, such as leaves, fruit, seeds, and stems. The natural feed given was adjusted to the preferences of the test insects to be propagated. For example, Spodoptera litura likes castor leaves, Myzus persicae likes to suck the liquid from young tobacco leaves, and Tribolium sp. likes to eat green bean seeds. Furthermore, the test insect propagation container used a plastic jar with a diameter of 20 cm and a height of 20 cm. To make it easier to understand how to reproduce the test insects, the following describes the steps that must be taken in insect propagation.

Prior to the propagation of the test insects, a container for the reproduction of insects was prepared, namely a type of cage made of gauze. To reproduce Spodoptera litura, the trick is to look for groups of eggs in the field. The eggs of Spodoptera litura are covered with a kind of brown velvet. One egg group consists of hundreds of eggs. This propagation procedure consists of three parts. 1) Take the group of Spodoptera litura eggs carefully by tearing the leaves where the group is found. 2) Placing eggs in a container or cage that has been given fresh castor leaves as feed if at any time the group of eggs hatches. 3). cover the container with gauze. One group of eggs will produce hundreds of Spodoptera litura.

Feed regularly every day until the caterpillar reaches the desired size for the purposes of the test insect. The following describes the method of field testing regarding the efficacy of vegetable insecticides isolated from amethyst leaves against Spodoptera litura on soybean plants. The concentration of amethyst leaf extract tested included five concentration levels, namely: 1, 2.5, 5, 7.5 and 10%.

Test of Amethyst Leaf Extract in the Field

The application procedure of amethyst leaf extract test in the garden is as follows:

a. Make research gardens in the form of plots and planted soybeans.
b. Make amethyst leaf vegetable insecticide extract in the form of preparations based on the required concentration, namely 1, 2.5, 5, 7.5, and 10%.
c. Determine 10 sample plants from each plot.
d. In the afternoon, put a Spodoptera litura measuring 0.5-1.0 cm on each sample plant, namely on the leaves. The plants are then covered with a plastic bag that has been perforated with a toothpick.
e. The next morning, the plastic hood was removed and the caterpillars were seen again. If the caterpillar is gone, add the next caterpillar.
f. Spraying methanol extract on plot I (1%), plot II (2.5%), plot III (5%), plot IV (7.5%), and plot V (10%). Each plot measuring 50 cm × 10 m requires 100 mL of extract solution.
g. Do the same with point f for the ethyl acetate and n-hexane fractions.
h. Each extract was repeated three times.
i. Do the capping of soybean plants and put another 5 caterpillars in a plastic bag that has been perforated with a toothpick.
j. Observing caterpillars on each of the sample plants every day. Record the number of dead caterpillars in each plot.
k. Observe the caterpillar's body carefully: Is there a caterpillar that won't eat, a caterpillar that is still fresh, a caterpillar that remains small, or a caterpillar that is very weak.
l. Record the percentage of leaf damage in each plot and count the number of dead caterpillars or larvae.
m. Determine the plot that causes the most caterpillar deaths.

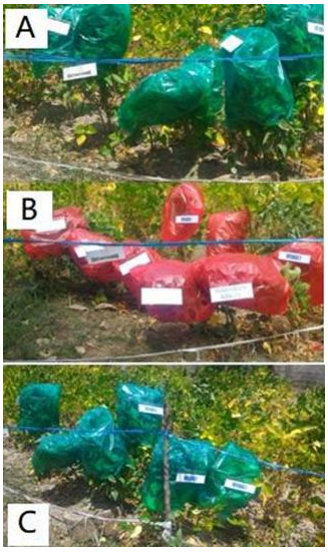

Figure 7. Soybeans given amethyst leaf extract A. methanol, B. ethyl acetate, and C. n-hexane in a plastic bag containing pests.

Tests of amethyst leaf extract, for the methanol fraction, with varying concentrations of 1, 2.5, 5, and 10% respectively gave mortality values of 27, 40, 40, and 60% as shown in **Figure 8** . In the test of amethyst leaf extract, ethyl acetate extract with various concentrations gave mortality values, respectively: 40, 52, 67, and 87%. Furthermore, in the n-hexane extract test, respectively: 53, 60, 80, 87, and 100%. This showed that n-hexane extract was the most effective in killing pests compared to ethyl acetate and methanol extracts. Previous reports showed that hexane from Datura metel was more effective in controlling the fungus Macrophomina phaseolina, which causes char rot disease in plants (Dhawan and Gupta 2017).

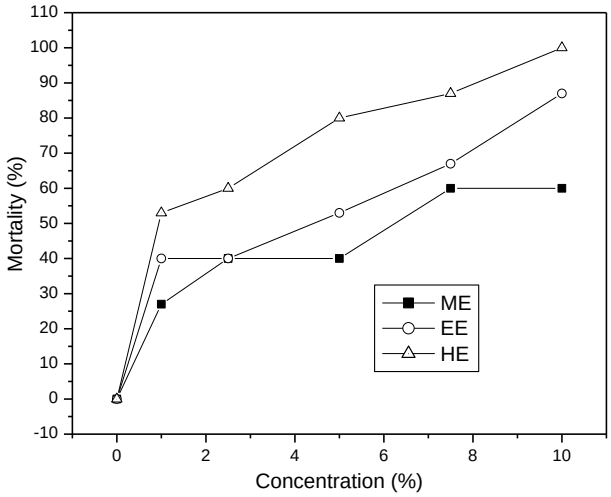

Figure 8. Data Insect mortality in field test of amethyst leaf extract after 24 hours.

This amethyst leaf extract not only kills pests but can also fertilize soybean plants as shown in **Figure 9**. Yellow soybean leaves appear, which have not been sprayed with amethyst leaf extract **Figure 7**. Datura metel plant extract is also known to have herbicide activity because the plant methanol extract made from dry leaves can remove unwanted weeds (Mulyana 2002). This extract also has antifungal activity because it contains pyrrole derivative compounds (Dabur et al., 2004). Nitrogen is the main component of protein, chlorophyll, enzymes, hormones and vitamins. Symptoms of N deficiency in young plants are shown by pale green leaves, and in severe conditions the leaves are pale yellow, the stems are weak and elongated. In older plants, the lower leaves show severe yellowing and eventually fall. Plant growth is stunted, stems are reddish, pod development is inhibited, leaves shrink and have thick walls so that the leaves become rough/hard and fibrous ((Fahmi, Syamsuddin, and Marliah 2014). Chlorophyll can be increased with NPK fertilizer (Paul 2001).

Figure 9. Soybean plants, in gardens that are sprayed with amethyst leaf extract, appear greener

The two nitrogen atoms in indole alkaloid are secondary (R_1NH) and tertiary amine (R_2N). The nitrogen atom which has lone electron pair causes the alkaloid to be basic like ammonia. The degree of acidity varies greatly depending on the

molecular structure and the presence and location of other functional groups. Like ammonia, the alkaloids are converted to their salts by mineral acids and when the alkaloid salts react with hydroxide ions, the nitrogen releases hydrogen ions and the amines are liberated. The positive charge of the nitrogen ion depends on the number of organic groups covalently bonded to the nitrogen and the positive charge of this ion is balanced by several negative ions [$R_3N^+X^-$]. If the nature of the ammonium ion is such that there are no protons to release, it will not be affected by hydroxide ions. As a result, the compounds will have chemical properties that are very different from those of the amines. Most of the alkaloids are insoluble or slightly so in water but the salts formed after reacting with the acid are usually freely soluble. The N in the alkaloids is what gives the green color of the leaves, and is more influential on chlorophyll compared to P and K (Paul 2001).

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