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Analysis of the Phytochemical Performance of the Neem Plant (*Azadirachta indica*) Sumbawa Phenotype as a Natural Insecticide

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Abstract: Excessive use of chemical insecticides on plants has harmed the environment and human health. To overcome this, plant-based pesticides are an attractive alternative, but they are not optimal in Indonesia. This research aims to study the potential use of neem leaf extract (*Azadirachta indica*) as a plant insecticide, focusing on local knowledge of the Samawa tribe on Sumbawa Island. Extraction methods and phytochemical analysis were carried out, as well as insecticidal power tests against several common pest species in the area. The research results show that neem leaf extract has the potential to be an effective natural insecticide in controlling pest populations. Factors such as extract concentration and drying method of neem leaves influence its effectiveness. Using neem leaf extract at an 80 gr/liter dose has proven more effective than the Dane brand of vegetable pesticide at three times the dose. However, synthetic pesticides at the recommended dosage still show faster and more effective results in controlling pests. This research contributes to developing environmentally friendly and effective plant protection materials and expands understanding of the potential of local natural resources for sustainable agriculture.

Keywords: Natural Pesticides; Neem; Phytochemical Analysis; Sumbawa.

Introduction

Pest and plant disease control is a critical factor in agricultural development in Indonesia. The significant losses caused by pest attacks have motivated researchers to find ways to reduce the rate of pest and plant disease infestations. The use of synthetic pesticides still dominates pest and plant disease control methods. While synthetic pesticides effectively control pests and plant diseases, their excessive use negatively impacts the environment and human health, including harming non-target organisms and causing resistance in pests. Additionally, air, water, and soil pollution are difficult to degrade naturally in the soil, leading to the accumulation of toxins in humans through food derived from animals and plants. The World Health Organization (WHO) estimates that 20,000 deaths occur each year due to synthetic pesticide poisoning. Other adverse effects of pesticide use include triggering cancer cells, causing physical deformities, infertility, and liver disease due to the accumulation of chemical substances from botanical pesticides in animals, plants, water, and soil [1]

The excessive use of insecticides on crops leaves harmful residues in the environment, including pollution that can kill soil microorganisms, threaten aquatic life, poison non-target organisms such as birds, and lead to pest resistance. Controlling the use of pesticides remains challenging, especially at the grassroots level, i.e., among farmers. According to [2], 96% of pesticides used nationally are chemical-based, posing a threat to environmental sustainability. Efforts to reduce chemical pesticides or convert to botanical pesticides have not been maximized. This is due to weak infrastructure readiness and limited knowledge and innovation in natural-based pesticides [3]. Therefore, strategic and concrete steps are needed to optimize the use of botanical pesticides based on the local knowledge of traditional communities, such as the Samawa tribe on Sumbawa Island, who utilize the neem plant (*Azadirachta indica*) to treat various ailments [4].

Neem, belonging to the family Meliaceae, can be found in several provinces in Indonesia, including Banten, East Java, Bali, and West Nusa Tenggara (NTB) [5]. Mentioned that a tannin test on the growth of Salmonella typhi showed a potent inhibition with a zone of 21.8 mm (based on the classification by Davis and Stout). This ability of neem leaf extract can be developed as a future treatment for typhoid. Research by [6] indicated the effectiveness of neem seeds as a larvicide against *Culex sp.* mosquitoes. Neem leaves contain various active compounds, particularly secondary metabolites such as limonoids, triterpenoids, and flavonoids, which are known to have potential as natural insecticides [7]. However, the levels and types of these compounds can vary depending on various factors, including genetics, growing environment, and extraction and processing methods [8].

Sumbawa Island is an island in Indonesia with high floral diversity and various potentials, including neem. However, studies on this plant's growth characteristics and secondary metabolite content are still rarely conducted,

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especially its potential as a botanical insecticide. Therefore, this research aims to further analyze the secondary metabolites in the neem leaf extract from phenotypes grown on Sumbawa Island. Additionally, this study will test the performance of neem leaf extract as a natural insecticide against several pest species that commonly infect crops in the region [9].

Neem extract has been reported to affect approximately 400 insects [10], but testing on the leaf miner pest in Podocarpus neriifolius has not yet been conducted. This research aims to determine the effectiveness of neem extract in controlling leaf miner caterpillars on *P. neriifolius* compared to Dane brand botanical pesticides. It compares the efficacy of fresh neem leaves and dried neem leaves. This data will benefit the treatment process of materials in producing neem-based botanical pesticides. Additionally, it aims to determine the effective dosage for controlling pests.

With a deeper understanding of the secondary metabolite profile of neem leaves from Sumbawa Island and their potential as a natural insecticide, this research is expected to significantly contribute to developing environmentally friendly and effective plant protection materials for farmers. Additionally, it aims to open new opportunities for utilizing local natural resources in sustainable agriculture**.**

Research Methods

Neem leaf samples were obtained from the rice field area in Pernek Village, Moyo Hulu Subdistrict, Sumbawa Regency. The selection of leaves consisted of mature leaves due to their level of maturity and high amount of secondary metabolites. One kilogram of leaf samples was collected and then dried without sunlight exposure. The dried neem leaf samples were then ground using a blender. The extraction process was conducted in the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, University of Mataram. The research was carried out on March 11, 2024. The tools and materials used in this research included.

Equipment

1 L beaker, 1 L graduated cylinder, Erlenmeyer flask, 1 L round-bottom flask, filter paper, glass funnel, separatory funnel and stand, oven, blender, rotary evaporator, powder sieve, aluminum foil.

Materials

1 L ethanol, 1 L n-hexane, 1 L ethyl acetate, 500 g neem leaf powder, distilled water, ice cubes, and larvae.

Preparation of Extract

The initial phase of this research involved selecting dark green leaves and neem bark (*Azadirachta indica*). After selection, the leaves and bark were dried without exposure to sunlight. Once dried, they were ground into powder using a blender. This powder was then placed into a 1-liter beaker containing 80% ethanol. The maceration process lasted for two days (48 hours) with a solvent-to-solute ratio of 1:3, where the sample was kept covered with aluminum foil and stirred using a shaker. After maceration, the sample was filtered through filter paper to separate the filtrate. The collected filtrate was then processed using a rotary evaporator at 40°C until a thick extract was obtained. This thick extract was subsequently partitioned using n-hexane and ethyl acetate for further analysis.

The materials used in this study include A. indica leaves, methanol, Tween, adhesive, and sterile water. The equipment comprises a blender, scales, plastic containers, measuring glasses, micropipettes, glass bottles, knives, and cutting boards. The leaves were extracted using two different methods: wet extraction and dry extraction. Consequently, four types of extracts were obtained during the treatment: damp and dry. The formulation of the wet extract involves blending 200 grams of neem leaves mixed with approximately 500 ml of sterile water. The blended mixture is then combined with 25 ml of Tween and 100 ml of methanol. This mixture is stored in a closed container, kept for three nights, and occasionally stirred. After three nights, the solution is filtered and squeezed to remove the residue. Then 25 ml of adhesive and more sterile water are added until the volume reaches 1 liter, resulting in a 200 grams per liter extract formulation. This formulation can be diluted with water to achieve the desired concentration variations. For the dry extract formulation, 200 grams of neem leaves are chopped and air-dried for approximately three days, occasionally stirred to ensure even drying. About 100 grams of dried material is obtained from the initial 200 grams of wet material. The dried material is then mixed with 500 ml of methanol, stored in a closed container for three nights, and occasionally stirred. After three nights, the solution is filtered to remove the residue. This solution is mixed with 25 ml of Tween, 25 ml of adhesive, and more methanol is added until the volume reaches 500 ml, resulting in a 200 grams per 500 ml extract formulation. This formulation can also be diluted with water to achieve the desired concentration variations. As a comparison in this experiment, tests were also conducted on synthetic and other botanical pesticides available in the market. The botanical pesticide is branded as Dane, which contains the active ingredients azadirachtin and euphorbin. The recommended dosage used is 10 ml per liter and its multiples. The experiment was conducted by placing 20 pest larvae, and P. neriifolius leaves as their food into cages. The cages were made from plastic containers with mesh holes for ventilation. Each cage was treated differently, with each treatment as follows:

K0 (Healthy Control)

The pest larvae are left untreated to remain healthy and active. This serves as a reference point for comparing the condition of the treated pests [9].

K1 and K2 (Solvent Controls):

The pest larvae are treated with solvent solutions used in the extract formulation, with varying concentrations. The solvent control aims to evaluate whether the solvent affects the pests [9].

DK1, DK2, and DK3 (Dry Leaf Formulations):

The pest larvae are treated with diluted extracts of dry leaves with varying concentrations. This aims to test the effects of dry leaf extracts with different concentrations on the pest larvae [9].

N1, PN2, and PN3 (Commercial Botanical Pesticides):

The pest larvae are treated with the botanical pesticide brand DANE, with different dosage variations. The aim is to evaluate the effectiveness of the botanical pesticide against the pest larvae [9].

PK (Synthetic Pesticide):

The pest larvae are treated with synthetic pesticides according to the recommended dosage. This compares synthetic and botanical pesticides' effectiveness against the pest larvae [9].

Phytochemical Analysis

Tannins

Tannin test using the method used by [10]. The identification process of secondary metabolite compounds in the tannin group begins by weighing 0.5 grams of neem leaves and bark extracts using an analytical balance. The extracts are placed into separate beakers containing 20 ml of distilled water. Next, the samples are heated to boiling and filtered to obtain the filtrate. The resulting filtrate is mixed with a 0.1% $FeCl_3$ solution and observed for any colour change. A colour change in the filtrate indicates the presence of tannin compounds in the sample.

Saponin

Saponin test using the method used by [10]. To identify secondary metabolites in neem samples, the first step involves weighing 2 grams of neem sample, both leaves and bark, using an analytical balance. Subsequently, both samples are placed into glassware and supplemented with 20 ml of distilled water. The samples are then heated and filtered using filter paper to obtain the filtrate. The resulting filtrate is mixed with 5 ml of distilled water and shaken to form foam. Next, three drops of olive oil are added to the foam produced, and the shaking process is continued to observe the formation of an emulsion.

Flavonoid

Flavonoid test using the method used by [10]. The first step in identifying flavonoid-type secondary metabolites is adding 2 grams of neem leaf and bark samples into glassware, mixing them with 20 ml of distilled water, and heating them to boiling. Afterwards, the solution is filtered. The next step involves mixing 0.5 ml of the obtained filtrate with 5 ml of dilute ammonia and 5 ml of concentrated sulfuric acid. A colour change from yellow-orange to dark red within 3 minutes indicates a positive result for the presence of flavonoids.

Triterpenoid and Steroid

Triterpenoid and steroid tests using the method used by [11]. Identifying secondary metabolites from the triterpenoid and steroid groups begins by dissolving the thick extract of leaves or bark in a test tube. Next, 0.5 ml of glacial acetic acid and 0.5 ml of chloroform are added to the solution. Subsequently, 1 ml to 2 ml of concentrated sulfuric acid is carefully added to the test tube.

At this stage, the formation of a brownish or violet ring at the interface between the two solvents indicates the presence of triterpenoids. Meanwhile, the formation of a bluish-green color suggests the presence of steroids. This provides initial clues regarding each group of secondary metabolites in the analyzed sample.

Alkaloid

Alkaloid test using the method used by [11]. Identifying alkaloid compounds in neem leaf and bark samples begins with weighing 10 mg of the extract and placing it in a test tube. Then, 3 ml of 2 N hydrochloric acid and distilled water are added to the tube. Next, the tube is heated in a water bath and cooled before filtering. The obtained filtrate is then mixed with Mayer's reagent until a white or yellowish precipitate forms. Alternatively, three drops of the filtrate can be added to Dragendorff's reagent, resulting in an orange-reprecipitate. Additionally, identification can be done using Bouchardat's reagent, where three drops of the sample are mixed with this reagent, producing a brown-to-black precipitate. These three methods can be used to identify the presence of alkaloids in neem leaf and bark samples, each providing different indications depending on the specific alkaloid compounds present in the sample.

Insecticidal Efficacy Test on Leaf Miner Pests

After the treatment is applied to the pest larvae in the enclosure, the next step is to observe the response or effects of the treatment. Observations include the mortality rate of the pest larvae, behavioral changes such as feeding patterns and activity, and impact on the growth and development of the larvae. Additionally, it is essential to note the treatment's effects on the condition of the plant leaves that serve as the larvae's food. By considering all these factors, researchers can evaluate the effectiveness and potential toxicity of the pesticides being tested, whether synthetic or botanical. This evaluation is crucial to ensure pesticides' safe and effective use in plant pest control [11]. This aligns with findings from [12], who reported that methanol extract from neem leaves effectively killed the brown planthopper with a mortality rate of 95% at DM25% and DM50% treatments. Similarly, [13] found that neem leaf extract at a concentration of 60% killed an average of 2.75 brown planthoppers.

Soursop leaf extract also caused mortality in test insects by 53% at a concentration of 30% [14]. Neem plants can kill insects due to their azadirachtin content, which kills insects through mechanisms like feeding deterrence and disruption of insect growth and reproduction [15]. [16] alkaloid and flavonoid content act as stomach poisons, inhibiting digestion processes when these compounds enter the insect's body, thus proving toxic to insects. In this experiment, various treatments were applied to pest larvae in the enclosure to evaluate their effects on the condition and behavior of the pests. Below is a brief description of each treatment:

Results and Discussion

Results of Phytochemical Screening of Neem Leaves

From the results of the phytochemical screening test of neem leaves, six groups of secondary metabolite compounds were obtained, as seen in Table 1.

The results of the phytochemical test showed that components of the secondary metabolite group could act as insecticides. This aligns with the research results [16,17], which state that methanol extract from neem leaves can kill leaf borer larvae.

Table 1. Phytochemical test of neem leaf extract

Compounds	Test result	Methods
Tanin	++	Ferric Chloride
Triterpenoid dan steroid	++	Libermann Burchard
Alkaloid	++	Meyer & Wagner
Flavonoid	+	Alkaline Reagent
Saponin	+++	Foam test

Test Results Of Neem Leaf Extract In Controlling Pest Larvae

Based on the results of tests on the ability of neem leaf extract to control pest larvae, the data in Table 2 below is obtained.

Table 2. Extract test against leaf borer larvae

Number of Dead Larvae/Hour	C0	C1	C2	DB	DB	DB	DK	DK	DK
0.25	0	0	0	0	4	7	0	6	11
0.5	0	0	0	3	5	8	2	6	11
1	0	0	0	3	5	8	2	6	14
2	0	0	0	3	7	11	2	6	16
4	0	0	0	3	7	11	2	6	16
8	0	0	0	3	7	11	2	6	16
12	0	0	0	3	7	12	2	6	16
24	0	0	0	3	8	12	2	6	16

Table 3. Advanced

Number of Dead Larvae/Hour	PN	PN	PN
0.25	0	3	3
0.5	0	10	5
1	0	11	6
2	0	11	6
4	0	11	6
8	0	11	6
12	0	11	10
24	0	11	10

The observation results indicate that the healthy control and solvent control had no impact on the pests, nor did they cause any pest mortality. This is due to the low solvent concentration in the formulation, which was not strong enough to kill the pests. However, wet and dry neem bark and leaf extracts at a concentration of 20 grams per liter showed some ability to kill the pests, although the effect was still limited. This suggests that lower concentrations of the extract had no significant impact on the pests.

The ability to control pest populations is evident in both extracts at a concentration of 40 grams per liter. However, the level of control only reaches 50% of the total pests. However, both extracts demonstrate a better ability to effectively control pests (70% pest mortality) at a concentration of 80 grams per liter during a 2 x 24-hour observation period. This is due to tetranorterpenoid limonoid compounds in the extract, which act as insecticides [17].

This aligns with findings from [18], who reported that methanol extract from neem leaves effectively killed the brown planthopper with a mortality rate of 95% at DM25% and DM50% treatments. Similarly, [19] found that neem leaf extract at a concentration of 60% killed an average of 2.75 brown planthoppers. Soursop leaf extract also caused mortality in test insects by 53% at a concentration of 30% [20].

Neem plants can kill insects due to their azadirachtin content, which kills insects through mechanisms like feeding deterrence and disruption of insect growth and reproduction [21]. Alkaloid and flavonoid content act as stomach poisons, inhibiting digestion processes when these compounds enter the insect's body, thus proving toxic to insects [22]. In this experiment, various treatments were applied to pest larvae in the enclosure to evaluate their effects on the condition and behavior of the pests. Below is a brief description of each treatment.

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The recommended dosage of the DANE botanical pesticide brand (10 ml per liter) does not show significant effects on pests, possibly because the dosage is too low [25]. However, at a dosage of 20 ml per liter, the effectiveness in killing pests reaches 50%. It increases with dosage, with 40 ml per liter capable of killing 75% of the pest population. However, a maximum pest mortality of 75% occurs on the second day [26].

Synthetic pesticides at the recommended dosage show excellent results in killing pests, with the ability to kill 80% of the population within 15 minutes and all pests within 30 minutes. [27] However, synthetic pesticides must be used carefully as they can damage the environment. Nevertheless, pest control using wet and dry neem leaf extracts at 20 grams per liter has been proven to be better than pest control using the recommended dosage of the DANE botanical pesticide brand. Pest control using 80 grams per liter of dry leaf extracts is also more efficacious [28].

The use of neem plants as botanical insecticides offers several invaluable advantages. [29] One of these is the ability of their active compounds to degrade in nature, resulting in relatively minimal residues quickly. This benefits the environment and reduces the risk of residue exposure to humans and livestock [30]. Additionally, neem plants have selective properties that allow them to target insects without harming other organisms, such as vertebrates. This helps maintain the safety and health of humans and pets. Another advantage is their ability to control insects without causing significant resistance, as their active compounds are diverse [31].

In addition to being an insecticide, neem plants possess various other properties beneficial in pest control, such as fungicidal, virucidal, nematicidal, bactericidal, miticidal, acaricidal, and rodenticidal effects. The fact that azadirachtin, the main compound in neem, can be mixed and synergized with other biopesticides adds to its flexibility and effectiveness in sustainable agricultural practices [32].

In the past ten years, entomologists have discovered that neem plant extracts affect over 200 species of insects, nematodes, fungi, bacteria, and even viruses. In these studies, various pests important to agriculture and households were tested. Examples include six species of cockroaches, *Aedes aegypti* mosquitoes, Mexican bean beetles, Colorado potato beetles, grasshoppers, and tobacco hornworms [34]. This is in line with the research, which found that neem leaf extract can inhibit fungal growth in vitro, and Agr I is more potent in controlling Colletotrichum spp at a concentration of 10%.

Conclusion

From the results of this research, it can be concluded that wet and dry neem leaf extracts are equally effective in killing larvae. The ideal dose of neem extract for pest control is 80 grams per liter. This extract performs equally well in both wet and dry forms. Neem leaf extract at a dose of 20 grams per liter, whether damp or dry, has proven more effective against pests than the recommended dose of the botanical pesticide brand Dane. An 80-gram dry neem leaf extract dose is also superior to Dane botanical pesticide even when the latter is applied at three times its recommended dose. Synthetic pesticides used at their recommended doses show impressive results; they can reduce pest populations by up to 80% in less than 50 minutes and eliminate the entire pest population in under 50 minutes.

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