Bioactivity of Neem Seed Oil mixed with Pyroligneous Acid from Rice Husks against *Spodoptera litura*

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Abstract. Biopesticides are environmentally friendly solutions used for pest control management. This is a feature of Neem (*Azadirachta indica*) seed oil and tar, which provides a synergistic effect on the bioactivity of pyroligneous acid, and both are known to have bioactive compounds. Therefore, the purpose of this study was to evaluate the effect of neem seed oil and tar on pyroligneous acid in conventional emulsion form, and their efficacy on the polyphagous insect *Spodoptera litura*. Neem seed oil was added at concentrations of 10, 20, 30, and 40%, while the concentration of tar was 0.5, 1.0, and 2.0%. The conventional emulsion formed was then characterized using a digital microscope. The addition of neem seed oil and tar were able to increase the antifeedant activity of pyroligneous acid by 63.6% while both neem oil and tar by 72.6%. The 2.0% tar formulation (N4PT2) showed the highest antifeedant activity against *S.litura* (97.9%) and had the smallest droplets size ranges (2.90 - 24.16 µm). The addition of tar tends to reduce the droplet size of neem oil and increase antifeedant activity.

1. Introduction

The development of pesticides that are effective, environmentally friendly, and safe for natural enemies is a challenge for researchers because humans need healthy food [1]. In its application, 80 percent of the pesticides do not reach the target insects and are disposed of in the soil and waters, so the active compound of pesticides will affect the life of biota in soil and water [2]. Pesticide residues can also be found in agricultural products. This problem requires an alternative solution by using environmentally friendly bioactive. Neem seed oil and pyroligneous acid can be alternative options because of their potency and anti-insecticidal properties. Pyroligneous acid is a pyrolysis product of lignocellulosic materials, while neem oil is an extract from the neem plant (*Azadirachta indica* A. Juss).

Pyrolineous acid is a product of the decomposition of hemicellulose, cellulose, and lignin in a pyrolysis reactor. It has anti-insecticidal, disinfectant, herbicide, antioxidant and antibacterial properties. The product can be produced from the carbonation process of lignocellulosic material waste [3]. The process will be very profitable because it increases the product's added value. The potential for lignocellulosic waste from agricultural products is relatively abundant. Therefore, the utilization
of agricultural waste such as rice husks and straw is expected as alternative raw materials of pyroligneous acid. Pyroligneous acid's effectiveness is increased when combined with neem oil components [4].

In contrast, Neem is a plant known to have bioactive that act as bioinsecticides. The active compound from the neem plant includes azadirachtin, meliacin, salanin, valassin, tignic acid, 2-3 dehydrosalanol gedunin, nimbin, nimolicinol, odoratone, azadironolide, isoazadironolide, naheедин, dan mahmoodin. In neem seed oil, 5-methyl-2-butanic acid is the component responsible for the specific odor, while nimbidin provides the main bitter taste extracted from neem oil [5]. According to Schmutterer [6], most compounds such as salannin, salanol, salannolacetate, 3-deacetyl salannin, azadiradion, 14-epoxyazaradion, gedunin, nimbiren, and deacetylnimbinen has antifeedant activity. Neem oil bioactive compounds function as a growth inhibitor, antifeedant, insect morality, and insect pests repellent [2]. Research on neem plants has been widely conducted and has shown high effectiveness against many insect pests in agriculture. Neem has the potential of the right pesticide for food crops [7].

An emulsion is one form of formulation that is advantageous and easy to use. Emulsion usually uses water as a dispersant. In this research, an emulsion will be made consisting of neem oil as an oil phase and pyroligneous acid as the dispersing phase. Pyroligneous acid is the result of the condensation of the pyrolysis process of lignocellulosic materials. The main components in pyroligneous acid are organic acid and phenol derivatives. Therefore, the purpose of this study was to evaluate the effect of neem seed oil and tar on pyroligneous acid from rice husks in conventional emulsion form and their efficacy on the polyphagous insect Spodoptera litura.

2. Materials and methods

2.1. Materials.

Neem seeds are obtained from East Java and rice husks are purchased from Bogor, West Java, Indonesia. Commercial surfactants sodium dodecylbenzene sulfonate and ethoxylate alkyl phenol were purchased from PT Indochem. Spodoptera litura is obtained from agricultural crops, Bogor, Indonesia.

2.2 Methods

2.2.1. Pyroligneous acid preparation and characteristic. In the initial stage, the pyrolysis process of rice husks was carried out using a pyrolysis reactor. The process refers to the research of Prianto et.al. [8]. The container containing the sample was put into the pyrolysis reactor. The pyrolysis process is carried out at a temperature of 100 - 400 °C. The crude liquid was precipitated and filtered to separate the pyroligneous acid and tar. The titration method analyzed the acid content, and the phenolic content of pyroligneous acid was analyzed by the Folin-Ciocalteu method (8).

2.2.2. Neem seed oil extraction. Neem oil production was obtained by pressing methods. Before pressing the seeds, they were dried and separated from impurities. The clean seeds were then pressed with a screw press. The oil obtained was filtered to obtain pure neem oil.

2.2.3. GC-MS analysis. GC-MS analysis using Capiler Column Type Phase Rtx-5MS; 60 m; 0.25 mmID with a column temperature of 50 °C. The analysis was carried out at an oven temperature of 280/10 minutes, an interface temperature of 280 °C while the injector temperature was 250 °C, a carrier gas of helium with a pressure of 101 kPa, a flow rate of 0.85 µl / minute, and an injection volume of 1 µl.

2.2.4. Formulation Preparation. A Half mL of surfactant was added to two mL of neem oil while stirring with a sonicator bath (low energy) for 5 minutes (a). Then, pyroligneous acid was added gradually to
the mixture (a) in the proportion of 80, 70, 60, and 50%. The formulation incorporating tar was made in a 40% neem oil composition with a tar composition of 0.5, 1.0, and 2.0%.

The formulation of neem oil and pyroligneous acid mixture were made with a fixed surfactant (10%). The ratio of neem oil and pyroligneous acid was 1:8 (N1P8), 2:7 (N2P7), 3:6 (N3P6), and 4:5 (N4P5).

To determine the tar's effect on antifeedant properties, the ratio of neem oil: pyroligneous acid: tar as follows 4:4,95:0.5 (N4PT0.5), 4:4,9:1.0 (N4PT1), and 4:4,8:2.0 (N4PT2) was made. The droplet size was performed under a digital microscope (keyence) with a magnification of 100-5000x. The microscope is equipped with software to measure the size of the droplets.

2.2.5. Antifeedant activity. *Spodoptera litura* insect colonies were maintained at room temperature of 24 ± 1 °C, relative humidity > 72%. It was fed taro leaves every day. Feeding inhibitory activity in the formulation of neem extract and wood vinegar was carried out using the choice feeding test. Taro leaves (3.0 cm2) are dipped in the extract mixture and air-dried. Then the treatment and control leaves and one *S.litura* (fourth instar) were put in the test container and observed after 24 hours. The test was carried out with seven replications for each treatment. The antifeedant activity can be calculated by the following formula: antifeedant activity (%) = [(C-T)/(C+T)]×100, where C: control leaf consumed and T: treated leaf consumed.

3. Results and discussion

The production of pyroligneous acid uses a pyrolysis reactor equipped with temperature control. The pyrolysis process was carried out within five hours at a temperature of 100-400 °C. According to Desai and Park [9] and Ma et al. [10], the pyrolysis process depends on the raw material's character, the manufacturing process and can take 4-7 hours. The process produced a pyroligneous acid of 34.5%. In the pyrolysis, there is a decomposition of hemicellulose, cellulose, and lignin. It begins at a temperature of 100-120 °C in large quantities, and the color tends to be more transparent. The increased temperature results in a darker pyroligneous acid color because the lignin begins to decompose into tar.

3.1. Physical Properties of pyroligneous acid and Neem Seed Oil.

The pH value of pyroligneous acid obtained was 3.6. This acidity level is influenced by organic acids and phenols’ levels because phenol has acidic properties, which influence its aromatic rings. Also, the water content of raw materials can affect the pH of liquid smoke. According to Yatagai et al. [11], pyroligneous acid pH ranges from 1.5 - 3.7. The pH value of neem seed oil was 5.05. It was caused by the content of fatty acids and secondary metabolite compounds contained in neem seed oil.

| No. | Property of pyroligneous acid | Value |
|-----|-------------------------------|-------|
| 1   | pH                            | 3.6   |
| 2   | Color                         | Brown |
| 3   | Specific gravity              | 1.0088|
| 4   | Odor                          | Pleasant-smoky aroma |
| 5   | Titratable acidity            | 0.1 % |
| 6   | Phenol content                | 0.91% |

According to Bhargava and Madhav [12]; Prianto et al. (2), there are 13 types of fatty acids in the crude extract of neem seed oil, while the chloroform fraction is reported to attract 14 types of fatty acids. The specific gravity of pyroligneous acid was 1.0088, higher than neem seed oil of 0.9390. Measurement of
neem seed oil viscosity with a Brookfield viscometer was 95 cps with spindle number 1 (one) and a speed of 12 rpm. Azadirachtin compound is unstable in strongly acidic and alkaline solutions, but it is most stable in pH between 4 and 6 [13]. Therefore, the pH value of the pyroligneous acid, neem oil, and their mixture should be in the range of 4-6 to maintain their bioactivity.

### Table 2. Neem oil characteristic

| Specific gravity | Viscosity (cps) | pH   |
|------------------|----------------|------|
| 0.939            | 95             | 5.05 |

3.2. GCMS analysis of pyroligneous acid (PA).

GC-MS analysis showed that pyroligneous acid from rice husk had 12 components (Table 3). Acetic acid is the largest component in pyroligneous acid (8.47%), 2 (3H)-Furanone, dihydro- (3.17), and phenol (3.14%). The content of organic acids results from the decomposition of cellulose and hemicellulose, while lignin is decomposed into phenols and their derivatives. Maruf et al. [14] stated that the cellulose contents in rice husks are cellulose 32.67, hemicellulose 31.68, and lignin 18.81, ash 11.88, and silica (% from Ash) 91.09%.

### Table 3. GCMS analysis

| No. | Ret. Time (min) | Compound                | Similarity (%) | Molecular formula | Concentration (%) |
|-----|-----------------|-------------------------|----------------|-------------------|-------------------|
| 1   | 3.909           | Acetic acid             | 91             | C₂H₄O₂            | 8.47              |
| 2   | 4.507           | 2- butenoic acid        | 95             | C₄H₈O₂            | 0.62              |
| 3   | 5.233           | 2 (3H)-Furanone, dihydro- | 95         | C₆H₈O₃            | 3.17              |
| 4   | 5.617           | Phenol                  | 94             | C₆H₁₂O         | 3.14              |
| 5   | 6.386           | Pentanoic acid          | 87             | C₅H₁₀O₂          | 2.08              |
| 6   | 6.856           | Methoxy phenol          | 94             | C₇H₁₀O₂          | 2.29              |
| 7   | 7.411           | 2,5-dimethyl phenol     | 89             | C₇H₁₀O         | 1.03              |
| 8   | 7.603           | 4-ethyl phenol          | 94             | C₇H₁₀O₂         | 1.58              |
| 9   | 9.097           | 4-ethyl-2-methoxy phenol| 83             | C₈H₁₂O₂         | 2.35              |
| 10  | 9.951           | 2,6- dimethoxy phenol   | 96             | C₈H₁₀O₃         | 1.90              |
| 11  | 11.125          | 4 - methyl-syringol     | 93             | C₈H₁₀O₃         | 1.62              |
| 12  | 12.236          | 1-(4-methylthiophenyl)-2-propanone | 80   | C₆H₁₀O₂   | 2.19              |

The chemical components in pyroligneous acid of rice husk are five types of acids and six types of phenols. The total organic acids were 12.08%, while the identified phenols and derivatives were 12.29%. The high lignin content makes the resulting pyroligneous acid have a high phenol content. According to Prianto et al. [3], acetic acid in pyroligneous acid has antifeedant activity against S. Exigua, while phenol, p-cresol, and creosol and furfural components show antimicrobe activity. Phenols and their derivatives are components with high anti-insecticidal properties [11]. Pyroligneous acid contained a low phenol
will not be effective against bacteria [15]. On the other hand, the cellulose content of rice husks influences the acetic acid level of their pyroligneous acid. The breakdown of cellulose into acetic acid occurs in two steps. The first is the hydrolysis reaction of cellulose to glucose, followed by pyrolysis to acids, water, furans, and phenols. Materials with a high cellulose content will also produce a high total acid [16].

Table 4. The droplet size of neem and pyroligneous acid formulation.

| Formulation                          | Droplet size (µm) |
|--------------------------------------|-------------------|
| Neem 40%, PA 50%, and surfactan 10%  | >125.00           |
| Neem 30%, PA 60%, and surfactan 10%  | 7.20 – 14.35      |
| Neem 20%, PA 70%, and surfactan 10%  | 4.64 – 40.76      |
| Neem 10%, PA 80%, and surfactan 10%  | 1.63 – 25.03      |

3.3. Droplets size of emulsion.

The measuring of the droplet size of neem oil dispersed in pyroligneous acid showed that the smaller the neem oil concentration, the smaller the droplet's size. The smallest droplet size (1.63 - 25.03 µm) was obtained in the combination of 10% neem oil, 10% surfactant, and 80% pyroligneous acid (Table 4). The figure distribution of neem oil droplets in pyroligneous acid rice husk can be seen in Figure 1.

![Figure 1](image-url)

Figure 1. a. formulation of neem oil (40%), PA (50%), and surfactan (10%), b. formulation of neem oil (30%) PA (60%), and surfactan (10%), c. formulation of neem oil (20%) PA (70%), and surfactan (10%), and d. formulation of neem oil (10%), PA (80%), and surfactan (10%).
Tar is a byproduct in the pyrolysis process of lignocellulosic material, where tar is the result of the decomposition of lignin contained in rice husks. Tar is thought to affect the effectiveness and properties of the emulsion system. Therefore, to determine tar's effect, the emulsion properties were tested by adding tar to the formulation with the largest droplet (N4P5). Tar was made in three different concentrations; there were 0.5, 1.0, and 2.0%. Table 2 showed that the droplet size of neem oil was dispersed in pyroligneous acid with several tar concentrations. The addition of tar was found to reduce the droplet size of dispersed neem oil. Increasing the tar concentration from 0.5 to 2% can reduce the droplet size from 125 µm to the range 2.90 - 24.16 µm.

Table 5. The droplets size of neem, pyroligneous acid (PA) and tar formulation.

| Formulation                  | Droplet size (µm) |
|------------------------------|-------------------|
| Neem 40%, PA 49.5%, surfactan 10% and tar 0.5% | 3.46 – 17.48      |
| Neem 40%, PA 49%, surfactan 10% and tar 1%      | 3.86 – 6.94       |
| Neem 40%, PA 48%, surfactan 10% and tar 2%      | 2.90 – 24.16      |

Observation of the emulsion system using a digital microscope Keyence (magnification 500-1000x) showed that neem oil was in the form of spherical droplets. Figure 2 shows the distribution and shape of neem oil droplets and tar mixture on pyroligneous acid rice husks. These droplets are coated with a surfactant that surrounds the neem oil droplets; this is in accordance with Atun et al.[17], the emulsifier forming a thick line that protects the oil.

Figure 2. a. formulation of neem oil (40%), PA (49.5%), surfactan (10%) and tar (0.5%), b. formulation of neem oil (40%), PA (49%), surfactan (10%) and tar (1.0%), c. formulation of neem oil (40%), PA (48%), surfactan (10%) and tar (2.0%).
3.4. Bioactivity of formulation.

The antifeedant activity test was carried out using *Spodoptera litura* larvae on third instar (Figure 3). The treatment consisted of combining a mixture of neem oil, pyroligneous acid, and surfactant. The antifeedant activity of formulation (without tar) N4P5 shows the highest activity (92.8%), while the lowest activity is in the N1P8 formula (60.5%). This phenomenon showed that the antifeedant activity was more influenced by neem oil content than pyroligneous acid. According to Koul et al. [18] that neem oil contains azadirachtin, research results show that azadirachtin is a strong antifeedant.

The bioactivity of the formulation with the influence of tar shows that the highest antifeedant activity (97.9%) was obtained for N4PT2 formulations with a composition of 40% neem oil, 10% surfactant, 48% pyroligneous acid, and 2% tar. Antifeedant activity at 1% (N4PT1) and 0.5% tar concentrations (N4PT0.5) was not significantly different. Their antifeedant activity was 97.7% and 97.2%, respectively. So it can be concluded that the addition of 0.5% tar is optimal in increasing the antifeedant activity of *S. litura* larvae. The mixture of birch tar and pyroligneous acid exhibits a repellent effect against snails [19]. Whereas Thong and Feng [20] states that tar salts at an application rate of 338e675 g/ha control infestation as effectively as synthetic aphidicides.

![Figure 3. Antifeedant activity of neem, PA and tar formulation](image)

4. Conclusion

Neem oil at a concentration of 10% had the smallest dispersed particle size. The addition of tar tends to reduce the size of neem oil and increase antifeedant activity. The formula with 10% neem oil and 40% neem oil with tar provides the smallest droplet size diversity. The highest antifeedant activity (97.9 %) was obtained in the formulation of N4PT2.

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