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Bioassay of Botanical Insecticide Formulas Against *Nilaparvata lugens* Stahl (Hemiptera: Delphacidae)

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Abstract. The *Nilaparvata lugens* Stahl (Hemiptera: Delphacidae) is one of the main pests in the rice field (*Oryza sativa* L.). Recently the farmer still used botanical insecticide to control *N. lugens*. However, the application of synthetic insecticides generates a lot of negative effects on humans and other useful insects. One of the alternative control strategies that can reduce the negative impact of chemical insecticides is using botanical pesticides from clove oil. This study aims to compare the effectiveness of botanical insecticide formulas with the active ingredient eugenol using different solvents. The study was conducted by testing the toxicity of 12 botanical insecticide formulas with the active ingredient of clove oil with different concentrations of emulsifier, surfactant, and solvent. Each formula was tested for toxicity against 10 adults of *N. lugens*. The experiment was carried out by spraying the test solution onto the one-month rice plants grown in plastic pots with 15 cm diameter and 20 cm height, covered with mica plastic covered with gauze at the top. Applications were carried out at a concentration of 5 cc/l by spraying the test solution onto the stems of the rice plants where the planthoppers perched. The results showed that the most effective and promising formula to be developed is a formula containing 500 ml of clove oil, 450 ml of carosene, and 50 ml of Silwet HS 312. Besides, it also has the lowest production cost of approximately US\$ 7.17 per liter.

INTRODUCTION

Brown planthopper *Nilaparvata lugens* Stahl (Hemiptera: Delphacidae) is a significant insect of rice in three regions of Asia, i.e. South, East, and Southeast [1]. This pest causes plant damage by sucking the liquid from inside the plant. Therefore, the leaves of the rice plant will be yellow and wilting [2]. Recently, some farmers still depend on the use of synthetic insecticides, however continuous application of synthetic insecticides generates various impacts such as poisoning, residues in rice, soil, and water, and killing useful insects like natural enemies and pollinator insects [3,4].

One of the alternative control strategies that can reduce the negative impact of chemical insecticide is by using botanical insecticide which contains the active plant secondary compounds [5]. The use of plant secondary compounds as active insecticides is based on their function for plants as repellents, attractants, pest killers, and insect-feeding inhibitors, which are naturally used for pest protection. More than 1,500 plants are currently reported to produce secondary compounds that have the potential to be developed as active ingredients in botanical insecticides [6]. These plants are classified as Meliaceae, Annonaceae, Asteraceae, Piperaceae, and Rutaceae [7].

The use of plant materials as active ingredients in botanical insecticides is expected to be able to replace the use of synthetic insecticides, allowing synthetic chemical residues in various agricultural products, which are known to have negative effects on nature and surrounding life, to be as low as possible. Eugenol, found in clove oil (*Syzygium aromaticum* L.) is one of the secondary plant compounds that can be used as an active ingredient in botanical insecticides. Eugenol has been shown to be resistant to warehouse pests *Tribolium castaneum* (Herbst.) [8]. This is because eugenol is a member of the allylbenzene class of chemical compounds that prevent the insect due to the smell and can disturb the respiratory of the insect [9].

Carosene and isopropyl alcohol (IPA) are known as the solvent, which is carosene can be able to use as an environmentally friendly fuel. On the other hand, Isopropyl alcohol (IPA) is a popular name for chemical compounds with the molecular formula C_3H_8O or C_3H_7OH . This compound is flammable compound and colorless, with a strong

odor. This compound is the simplest secondary alcohol IPA is a tertiary alcohol that can be used as an antiseptic, cleaning, dispersant pigment, and wet wipe, and the biggest use of IPA is as a solvent [10]. A lot of botanical insecticides contain essential oil, however, some of their effectiveness can be able to kill the pest in the field, this is because the liquid of those botanical insecticides does not have the right solvent to optimize the work of these botanical insecticides. Therefore, this study aims to compare the effectiveness of botanical insecticide formulas with the active ingredient eugenol using different solvents.

METHODOLOGY



FIGURE 1. Mass propagation of brown planthopper on rice plants grown in pots

The study was carried out at the greenhouse of the Indonesian Spices and Medicinal Crops Research Institute (ISMCRI). Clove oil is a plant secondary compound containing eugenol that is used as an active ingredient in botanical pesticides. Carosene, isopropyl alcohol (IPA), and Silwet HS 312 are good solvents that can be used in pesticide formulations. The second generation of brown planthopper, *Nilaparvata lugens* Stahl, was used as a test insect and was cultured in a greenhouse (Fig. 1.). Botanical insecticides are formulated by combining all the ingredients used in various compositions (Tables 1 and 2). The mixture is then thoroughly mixed to ensure that all the ingredients are completely dissolved. The tested concentration was 5 cc/l obtained by dissolving 5 cc of botanical insecticide formula in 1 liter of water.

TABLE 1. Composition of botanical insecticide formula with IPA solvent

Number	Clove Oil (ml)	Isopropyl alcohol/ IPA (ml)	Teepol (ml)	Silwet HS 312 (ml)
F1	200	750	0	50
F2	200	700	100	0
F3	200	650	100	50
F4	200	750	0	50
F5	200	700	100	0
F6	200	650	100	50

TABLE 2. Composition of botanical insecticide formula with carosene solvent

Number	Clove Oil (ml)	Carosene (ml)	Tepol (ml)	Silwet HS 312 (ml)
F7	500	450	0	50
F8	500	400	100	0
F9	500	350	100	50
F10	500	450	0	50
F11	500	400	100	0
F12	500	350	100	50

The bioassay was carried out based on the method developed by the Pesticide Commission [11] with minor modifications. The study used a completely randomized design with 12 treatments and three replications. Each treatment was tested on 10 adult female insects reared on the Ciherang variety in 15 cm diameter and 20 cm tall pots (Fig. 2). Spraying 5 ml of the test solution onto the stems of rice plants at a height of 10 cm above the soil surface was used to administer the treatment. The death of the test insects was observed at 1, 2, and 3 days after treatment.

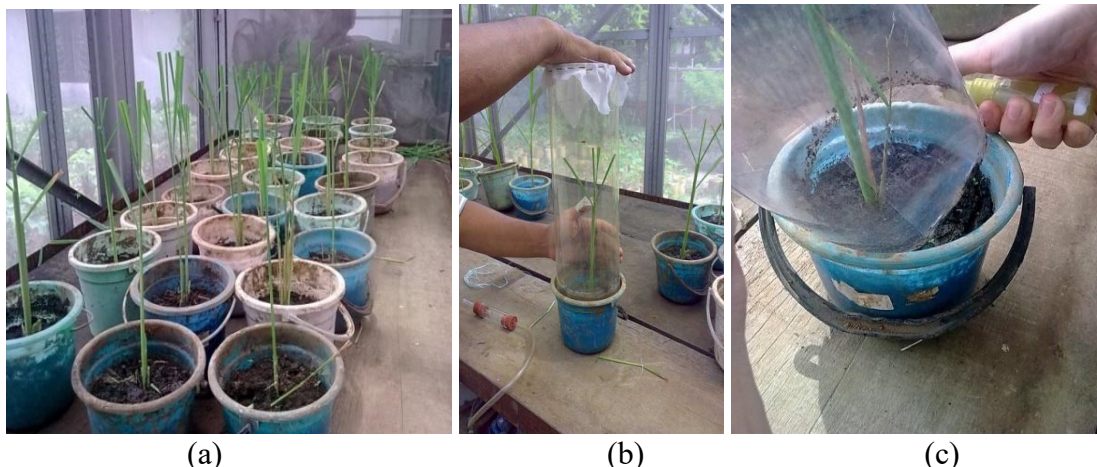


FIGURE 2. Rice plants aged 1 month (a), rice plants covered in plastic (b), and application of botanical insecticides to rice plants that have been inoculated with 10 adults of brown planthopper (c)

RESULTS AND DISCUSSION

Mortality of the Brown Plant Leafhopper (*Nilaparvata lugens*)

The results of the experiment revealed that the botanical insecticide formulas could kill the tested insects at all concentrations tested (Table 3 and 4). In the first hour, Formula F4 had the same mortality rate of the test insects as the F5 treatment, which was 60%. Meanwhile, 72 hours after application, the mortality rate of both formulas increased to 80%. The treatment of botanical insecticide applications with isopropyl alcohol solvent (F6) 72 hours after application resulted in the highest mortality, 96.33 percent (Tabel 3.). The treatment demonstrated that using a high concentration of isopropyl alcohol was not always effective in increasing the mortality of the test insects. The presence of Tepol and Silwet HS 312 suggests the same thing. However, the addition of the mixture influences the quality of the formula, making it appear more homogeneous.

TABLE 3. Mortality of brown planthopper after application of botanical insecticide formulas with IPA solvent

Formula	The average of mortality (%)			
	1 HAT*	24 HAT	48 HAT	72 HAT
F1	30.00a	36.67a	46.67a	56.67a
F2	26.67a	40.00a	43.33a	50.00a
F3	36.67c	46.67b	56.67b	56.67a
F4	60.00d	76.67c	76.67c	80.00b
F5	60.00d	70.00d	73.33c	80.00b
F6	66.67e	86.67e	90.00d	93.33c

*HAT: Hours after treatment

Values followed by the same letter and the same column are not significantly different.

The higher the toxicity, the more ingredients are mixed in the essential oil formulation as a botanical insecticide [12]. Isopropyl alcohol contains a hydrophilic group that attracts water molecules, allowing it to dissolve polar water molecules. Furthermore, these compounds contain an alkyl group -CH₂ with a short carbon chain, resulting in a very high polarity level [13]. Furthermore, because the formula contains Silwet HS 212, a surfactant that causes the formula

to spread evenly across the entire surface of the insect, the F6 formula easily enters the body of the test insect, resulting in higher insect mortality than other treatments.

One hour after the application of a botanical insecticide with Carosene solvent, the mortality rate of *N. lugens* ranged from 43.33 to 80 percent. The mortality rate of the tested insects increased to 60-100 percent 72 hours after application. Formulas F10 and F11 produced the best results, allowing all of the tested insects to be killed (Table 4).

TABLE 4. Brown planthopper mortality after application of botanical insecticide formulas with carosene solvent

Formula	The average mortality (%)			
	1 HAT*	24 HAT	48 HAT	72 HAT
F7	53.33a	63.33a	66.67a	70.00a
F8	43.33b	53.33b	53.33b	60.00b
F9	56.67a	66.67a	70.00a	73.33a
F10	80.00c	96.67c	100.00c	100.00c
F11	70.00d	90.00c	100.00c	100.00c
F12	60.00e	76.67d	93.33c	93.33c

*HAT: Hours after treatment
Values followed by the same letter and the same column are not significantly different

Carosene was found to be effective in killing test insects when used as a solvent in botanical insecticide formulas. This indicates that this material is a natural chemical compound that is widely used as a solvent for synthetic pesticides that were previously widely sold in the market. As a result, formulations of botanical insecticides containing carosene can increase their effectiveness in killing target pests. Local farmers in Nigeria use carosene emulsion concentrate as an insecticide as well as a fungicide to protect Nigerian crops from pests [15]. IPA and Silwet HS have also been studied previously and can be used as emulsifiers and adjuvants in the formulation of vegetable pesticides [14,16]. Pesticides containing these two chemicals can be seen to be evenly mixed and easily emulsified in water.

The botanical insecticide formula containing the active compound eugenol which has been formulated was found to be effective in killing brown planthoppers [13]. This formula can control these pest attacks on rice cultivation in paddy fields, as well as increase crop production by up to 25%. This proves that even though botanical insecticides are proven to control pest attacks. However, because the eugenol compound is volatile, its effectiveness on the second day has significantly decreased. Therefore, its use in plantations should be carried out on a scheduled basis, do not wait for pest attacks to increase, and then control can be carried out.

Production Cost of Formula

Based on the production costs of various formulas of botanical insecticides, the composition of botanical insecticide formulas based on cloves mixed with isopropyl alcohol/IPA has different production costs. Formula F6 is the most effective formula to kill *N. lugens*. The production cost is quite affordable, only US\$ 4.93 where 1 US\$ is equivalent to Rp. 14,500. (Table 5). Despite having lower production costs of US\$ 4.48, the formulas F1 and F4 have lower mortality rates than the F6 formula.

Production costs in the formulation of botanical pesticides are critical so that the efficiency level of making botanical pesticides can be calculated and botanical pesticides can be used by the farmer at a reasonable price. Because of the reliance on chemical pesticides, the cost of using them is high because the success of the production process is frequently dependent on chemical pesticides.

TABLE 5. Costs of producing botanical insecticides tested with IPA

Code of Formula	Clove oil (US\$)	Isopropyl alcohol/ IPA (US\$)	Teepol (US\$)	Silwet HS 312 (US\$)	Total costs (US\$)
F1	2.41	1.55	-	0.52	4.48
F2	2.41	1.45	0.66	-	4.52
F3	2.41	1.34	0.66	0.52	4.93
F4	2.41	1.55	-	0.52	4.48
F5	2.41	1.45	0.66	-	4.52
F6	2.41	1.34	0.66	0.52	4.93

The use of botanical pesticides still has advantages over the use of chemical pesticides, such as a 50 percent difference in the cost of production factors such as raw materials, labor costs, and factory overhead [17]. The cost of manufacturing botanical pesticide formulas with formula numbers F70 F12 is higher than the cost of manufacturing formula numbers F1 to F7. This is due to the presence of more active insecticide ingredients, specifically clove oil, in formulas F7 to F2. The results showed that the higher the concentration of clove oil used, the greater the mortality of the test insects.

TABLE 6. Costs of producing botanical insecticides tested with carosene

Code of Formula	Clove Oil (US\$)	Carosene (US\$)	Teepol (US\$)	Silwet HS 312 (US\$)	Total costs (US\$)
F7	6.03	0.62	-	0.52	7.17
F8	6.03	0.55	0.55	-	7.14
F9	6.03	0.48	0.55	0.52	7.59
F10	6.03	0.62	-	0.52	7.17
F11	6.03	0.55	0.55	-	7.14
F12	6.03	0.48	0.55	0.52	7.59

CONCLUSION

The combination of emulsifier, surfactant, and solvent affects the toxicity of the tested insecticides. The most effective formula and the least expensive to produce were those containing 500, 450, and 50 ml of clove oil, carosene, and Silwet HS 312, respectively with a production cost of only about US\$ 7.17 per liter. The results of this study can be used as a basis for further research to test its effectiveness against other major pests of rice so that when applied to crops its potential can be identified more clearly.

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