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Susceptibility of *Nilaparvata lugens* (Stal.) to a botanical insecticide formulation of Neem (*Azadirachta indica* A. Juss) and Citronella Oil (*Cymbopogon nardus* L. Rendle) mixture

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Abstract

Nilaparvata lugens (Stal.) is one of the main insect pests of rice plant in Indonesia. The farmer usually using synthetic insecticide to control this pest insect. However, unwise of using insecticides cause many negative impact such as resistance, resurgence, environmental contamination, and healthy problem. These were encouraged to search and development of natural resources origin from plant for insect pest control. Neem (*Azadirachta indica*) and citronella (*Cymbopogon nardus*) have insecticidal activity and safety for the environment. This study aimed to compare the susceptibility of *N. lugens* from Cipunagara (field population) and Banyuwangi (standard population) to the insecticide formulation made from neem and citronella oil mixture (50:15). The experiments used leaf-stem dipping and root dipping methods. *N. lugens* instar 4th were exposed to the treated rice plant for two days and after that they were provided with rice plants without treatment until ten days. The results showed that the LC_{50} value in the root dipping method was lower than that in the leaf-stem dipping method. In this test, the toxicity of neem and citronella oil mixture formulation was higher in the root dipping method and was effective in controlling *N. lugens* in both populations with the LC_{95} values were 2.09%-2.30%. The Resistance Ratio (RR) of *N. lugens* from Cipunagara population and from Banyuwangi population were < 1 in both leaf-stem dipping and root dipping tests, respectively. Therefore, *N. lugens* from Cipunagara population is still susceptable to neem and citronella oil mixture formulation.

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Introduction

Brown planthopper (*Nilaparvata lugens* Stal.) is one of the main insect pests of rice. In Indonesia, *N. lugens* attack rice plants in both rainy and dry season (Baehaki, 2012). *N. lugens* feeding activity can cause plant death or reduce crop yields (Watanabe and Kitagawa, 2000). Infested rice plants turn yellow and dry quickly or known as Hopperburn symptom (Baehaki and Widiarta, 2011).

Cipunagara District (Subang, West Java, Indonesia) is one of the centers of rice cultivation. In period April-June 2017, *N. lugens* and rice grassy stunt virus (RGSV) attack in Cipunagara area was 585 ha of 6,537 ha (8.9%) (ICRR, 2017). Farmers use synthetic insecticides to control *N. lugens*.

Excessive and continuous use of synthetic insecticides has negative effects such as death of natural enemies, residue in crops, and insect resistance to the applied insecticides. To address this problem, environmentally friendly insecticides are required. Botanical insecticides have a broad spectrum of target insects (Suryaningsih and Hadisoeganda, 2007), and their mixtures are expected to increase the effectiveness and delay resistance (Arisanti and Dono, 2015; Cloyd, 2010). Neem insecticide (*Azadirachta indica* A. Juss (Meliaceae)) can delay insect development, disrupt molting process, and inhibit ovarian development (Pathak and Tiwari, 2017). Citronella functions as an insect repellent (Ganjewala, 2009). The mixture of neem with citronella oil from *Cymbopogon nardus* L. Rendle (Poaceae) is expected to reduce the amount of insecticides and increase effectiveness (Cloyd, 2010).

Insecticides can be applied by spraying on leaves, application to soil, injection, and other methods, depending on the characteristics of the insecticides, insects, and plants. The susceptibility of *N. lugens* populations from Cipunagara (Subang, West Java, Indonesia) compared with Banyuwangi (Banyuwangi, East Java, Indonesia) to the botanical insecticide formulation from the mixture of neem and citronella oil. *N. lugens* from the population of Banyuwangi as a susceptible standard with rearing without exposure to insecticides. The susceptibility of both populations of *N. lugens* to insecticides was tested using leaf-stem dipping method and root dipping method.

Materials and methods

Test insects

The tests used two different groups of *N. lugens*. They are *N. lugens* from Banyuwangi and Cipunagara population, respectively. *N. lugens* of Banyuwangi population were obtained from the Indonesian Center for Rice Research (ICRR) and they had been reared in a greenhouse for 35 generations at the time of the test (*N. lugens* reared without synthetic insecticide exposure and obtained *N. lugens* susceptible standards.). The population of *N. lugens* from Cipunagara (taken from the field in July 2018) came from Padamulya Village, Cipunagara District, Subang Regency, West Java, Indonesia. Tests were carried out on *N. lugens* 2nd generation.

Test insecticides

Botanical insecticides used are made from 50% neem seed oil and 15% citronella oil in the Emulsifiable Concentrate formulation. Five series of insecticide concentration and a control were evaluated for their effect on *N. Lugens* with 60 test insects per treatment. Botanical insecticide concentrations were prepared by dilution with distilled water containing the sticker and spreader Agrictic ® (alkyl aryl polyglycol ether 400g/l) at a concentration of 0.5ml/liter. The control was only treated with distilled water solvent containing Agristic®. Preliminary tests were conducted to obtain insecticide concentrations that resulted in the mortality of test insects of $0 < X < 100\%$.

Application of insecticides

The experiment used leaf-stem dipping method (residual contact test) and root dipping method. The leaf-stem dipping method refers to Surahmat *et al.* (2016) and based on the IRAC test method number 005. Rice seeds planted in a greenhouse for 10-12 days are cleaned with water to remove soil from their roots. Two sheets of wet tissue covering the roots of ten rice plant. The test plants were inserted into a plastic tube (5cm diameter, 5.5cm height).

Then, 1% (w/v) Jelly-water solution at the tempertaure of 37°C was poured into a tube to cover the roots and then left at room temperature to harden the jelly. After that, the leaves and stem of rice seedling was dipped for 10 seconds in botanical insecticides solution (Fig. 1A), dried for 15 minutes, and put into a cage. Then ten *N. lugens* instar 4th were released in the cage (diameter of 5cm and a height of 20cm) (Fig. 1B).

The root dipping method was conducted by dipped the roots of ten rice plant (10-12 days after seedling and clean from soil) into insecticides. Ten test plants were inserted into a plastic tube (5cm diameter, 5.5cm height) and the roots dipped to 25ml of botanical insecticide solution (Fig. 1C). Then ten *N. lugens* instar 4 were then released in the cage (diameter of 5cm and a height of 20cm).

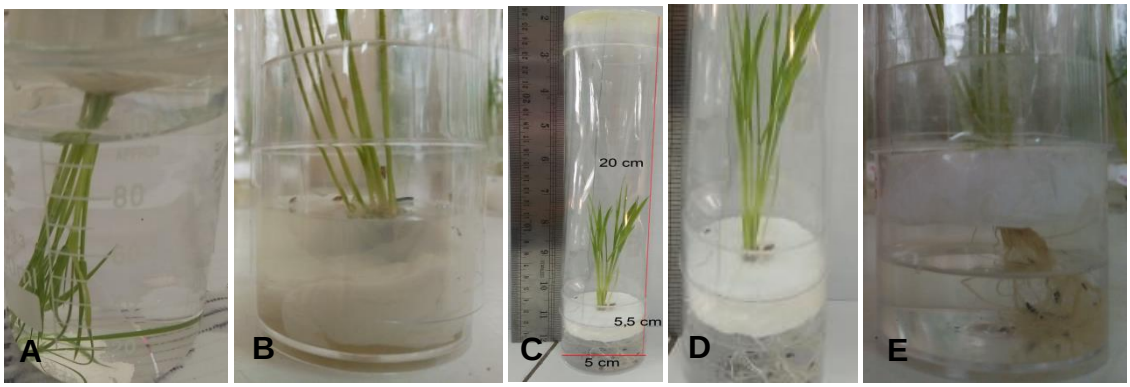

Fig. 1. Test the leaf-stem dipping method (A, B) and root dipping method (C, D, E).

The observation of mortality *N. lugens* after insecticide application was 10 days. Two days the tests was doing by given the plants with insecticides treatment, and gradually every two days the plants were replaced with an untreated one. The data were analyzed using the Polo Plus Version 1.0 application program to obtained LC_{50} and LC_{95} values. Measuring the sensitivity of insects to insecticides using resistance ratio (RR). RR= LC_{50} Cipunagara (field population)/LC_{50} Banyuwangi (standard population). If RR < 1 means the insects are from field populations are sensitive to botanical insecticides (Dono *et al.*, 2010).

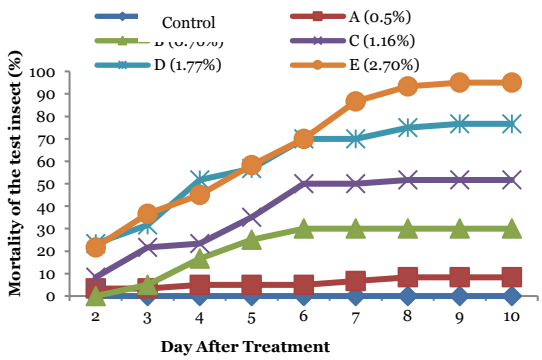

Fig. 2. Mortality of *N. lugens* of Banyuwangi population after treatment with Neem and Citronella mixture (50:15) formulation using leaf-stem dipping method.

Result

The lowest and highest concentrations needed to cause the death of *N. lugens* of Cipunagara and Banyuwangi populations in the leaf-stem dipping method were 0.5% and 2.7%, respectively. Figs. 2 and 3 show that insecticides work slowly in causing the death of test insects. At the second day to five days after application were still had an increase in mortality and stand constant after sixth day period.

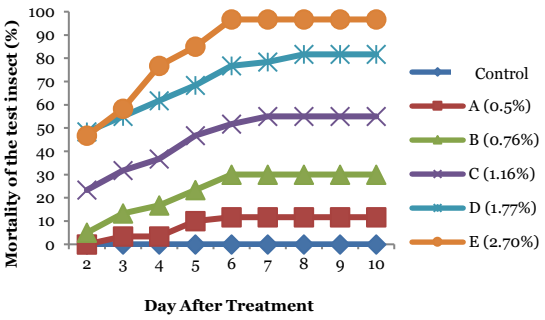

Fig. 3. Mortality of *N. lugens* of Cipunagara population after treatment with Neem and Citronella mixture (50:15) formulation using leaf-stem dipping method.

The lowest and highest concentrations in the root dipping method (0.3% and 2.5%) were lower than those in the leaf-stem dipping method (Figs. 4 and 5).

In this study, the formulation of neem and citronella mixture effectively caused the death of *N. lugens* from the two populations without causing phytotoxic effects to the rice plant. The concentrations needed to cause the mortality of 0 < x < 100% in both populations were quiet the same. This indicates that the two populations have similar susceptibility against a mixture of neem with citronella oils.

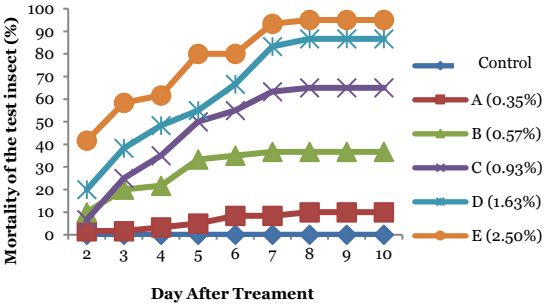

Fig. 4. Mortality of *N. lugens* of Banyuwangi population after treatment with Neem and Citronella mixture (50:15) formulation using root dipping method.

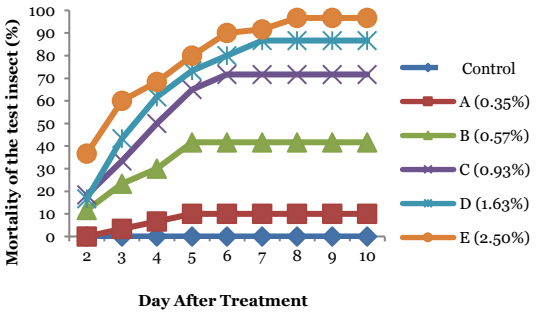

Fig. 5. Mortality of *N. lugens* of Cipunagara population after treatment with Neem and Citronella mixture (50:15) formulation using root dipping method.

Based on the results of probit analysis, the toxicities of the formulation of neem and citronella oil mixture using root dipping method (LC_{50} 0.75% and 0.70%; LC_{95} 2.30% and 2.09%) were higher than those using the leaf-stem dipping method (LC_{50} 1.10% and 1.03%; LC_{95} 2.91% and 2.67%). *N. lugens* from Cipunagara population was still susceptible to the mixture of neem and citronella with a resistance ratio of < 1 (Table 1).

Table 1. Resistance ratio (RR) of *N. lugens* origin from Banyuwangi (standard population) and Cipunagara (field population) to the mixture of neem and citronella oils (50:15) formulation.

Treatment Method	Population	a±SE	b±SE	LC_{50} (%)	$CI_{95\%}$	LC_{95} (%)	$CI_{95\%}$	RR
Leaf-stem Dipping Method	Banyuwangi (Standard)	-0.16 ± 0.09	3.89 ± 38	1.10	0.99-1.22	2.91	2.43-3.76	0.94
	Cipunagara (Field)	-0.06 ± 0.093	3.99 ± 0.39	1.03	0.93-1.14	2.67	2.25-3.41	
Root Dipping Method	Banyuwangi (standard)	0.42 ± 0.09	3.40 ± 0.33	0.75	0.67-0.85	2.30	1.89-3.03	0.93
	Cipunagara (Field)	0.53 ± 0.10	3.48 ± 0.35	0.70	0.62-0.79	2.09	1.73-2.73	

Description:

Probit analysis the correlation of the concentration of mixture of insecticide and mortality of test insect was analyzed at ten days after treatment

a : Intercept

b : Slope

SE : Standard Error

LC : Lethal concentration (%)

CI : Confidence interval

RR : Resistance Ratio (LC_{50} field population/LC_{50} standard population)

Discussion

The tests of neem and citronella oil mixture formulation against *N. lugens* found that the root dipping method caused a higher mortality than the leaf-stem dipping method. Javed *et al.* (2007) describe that roots absorb metabolites from neem and translocate them to other parts of the plant's body. The tomato root can absorb the phenol component of neem insecticide applied (Alam *et al.*, 1980). Senthil-Nathan *et al.* (2007) explained that neem extracts and its derivatives work together as systemic insecticides, where they were absorbed into the plant, carried into

the plant tissues, and eventually ingested by the insects when they feed. The application of azadirachtin with application to roots or injection of stems benefited in efficiency and persistence, can inhibit the development of *Mizus persicae* and decreases the number of generation (Birgucu *et al*., 2018; Pavela *et al.* 2013). The efficiency of an application by spraying is lower because the weather and UV light cause insecticides to be easily degrading (Nasiruddin and Mordue (Luntz), 1993).

In insects with haustellate mouth types such as *N. lugens*, Weathersbee and McKensie (2005) explain that insects digested azadirachtin when feeding on treated phloem tissue. Neem is more toxic when it works in the digestive process than in contact (Akol *et al*., 2002). However, the results of another study Kumar *et al*. (2005) showed that the application of the neem to the leaves is more effective to the test insects than the application to soil. The application of neem cake to the soil was found to have an antifeedant effects to *Nephotetix virescens* (Saxena *et al*., 1987).

This study found malformations in *N. lugens* such as in the formation of wings and imperfect body parts as well as failure to moult. The application of neem was previously reported to cause malformations in test insects (Zanuncio *et al.,* 2016). Mordue (Luntz) and Nisbet (2000) explained that azadirachtin administration before hormone release caused the release of blocked ecdysteroids and the death of insects before moulting or after changing of instar. Azadirachtin affects post-transcriptional regulation and proteins involved in the cytoskeleton, transcription and translation, hormone regulation, and energy metabolism (Wang *et al*., 2014). Malformations in treated insects may be related to the imperfection of moulting due to the influence of azadirachtin which acts as an insect growth regulator (Zanuncio *et al*., 2016).

This study showes that *N. lugens* from the Cipunagara population are still susceptible to mixtures of neem and citronella oils with the resistance ratio of less than one. Therefore, this mixture can be used as an alternative for resistance management of *N. lugens* to synthetic insecticides. Mordue and Nisbet (2000) stated previously that neem has an important role in the management of resistance. Neem application has also been shown to reduce the level of detoxification enzymes (due to blockages in protein synthesis), and useful for management insects of resistant strains (Lowery and Smirle, 2000). The use of a mixture of active compounds is also intended to minimize the occurrence of resistance (Koul *et al*., 2004).

Conclusion

Based on this study, it can be concluded that *N. lugens* of Cipunagara population (Subang, West Java, Indonesia) is still susceptible to the mixture of neem and citronella oils in comparation to *N. lugens* population of Banyuwangi (Banyuwangi, East Java, Indonesia) with the RR value of < 1. The application of insecticide using root dipping method (LC_{50}=1,10% and 1,03%; LC_{95}=2,91% and 2,67%) was more toxic than the leaf-stem dipping method (LC_{50}= 0.75% and 0.70%; LC_{95}=2.30% and 2.09%).

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