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Resistance Monitoring of *Nilaparvata lugens* Stall against Pymetrozine Insecticide with Determination of Diagnostic Concentrations

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Abstract. *Nilaparvata lugens* Stall is one of the main insect pests on rice crops. Intensive control of this pests using insecticides has resulted in the development of insect resistance. This study aimed to find out the level of resistance of the *N. lugens* population to pymetrozine insecticides by determining the diagnostic concertations. *N. lugens* was collected from five endemic areas in Central Java Province from October 2019 to June 2020. The data from the bioassay test were analyzed with probit analysis to obtain the LC_{50} value. The results of the sensitivity test showed that the Kajen population has the highest RF value (2.47), while the Karanganyar population which has the lowest RF value (1) was the most sensitive population. The determined diagnostic concentration was LC_{95} = 25.52 ppm with the lowest concentration limit of 7.67 ppm and the highest of 30.05 ppm. To conclude, the determined diagnostic concentration (LC_{95} = 25.52 ppm) is effective for detecting the susceptibililty of *N. lugens* population. This finding would be beneficial for monitoring resistance of *N. lugens* population against pymetrozine insecticides in the field.

Key words: diagnostic concentration; *N. lugens*; pymetrozine; resistance

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INTRODUCTION

Rice is the staple food of Indonesian society. However, the attack of *Nilaparvata lugens* Stall (brown planthopper) can cause huge losses in all rice producing countries (Bottrell & Schoenly, 2012), especially in Asia (Khoa et al., 2018). These pests can attack rice plants from various stages in the nursery until just before harvest and cause light damage to the *puso* (crop failure). If this condition was occured over time, the rice plants will dry up (hopperburn) and eventually die (Rizal et al., 2017).

In early 2014, in Central Java Province, *N. lugens* attacked widely and almost occurred in every rice planting center. Due to high rainfall in most of Central Java in January and February 2014, there has been an increase in the population of *N. lugens* in the 2013/2014 planting season rice cultivation. Until early March 2014, in various areas in Central Java (22 districts) with an area of 4,651 ha had been attacked and 46 ha of them experienced puso (Murtiati et al., 2015).

Based on data from BPTPH Central Java, in 2014, N.lugens pests attacked paddy fields covering an area of 42057 ha then decreased in 2015 and 2016 (8500 - 9000 ha), but in 2017 there was an explosion of *N. lugens* pests, reaching 20336 ha and causing grass stunts and hollow dwarfs. Based on data from the Directorate of Food Crops, the total area of the provinces of West Java, Central Java, East Java, and South Sulawesi which attacked by *N. lugens*, from January to July 2017 reached 67,749 ha (Minarni et al., 2018).

The use of contact insecticides has begun to be reduced because it encourages resistance and resurgence of *N. lugens* that results in increasing pest population faster than before spraying. The intensive use of the same active ingredients and mixing pesticides are often applied by farmers in controlling *N. lugens* (Heong et al., 2015). *N. lugens* attacks occur frequently in rice-producing areas with high use of insecticides so that they are more resistant to insecticides used by farmers (Baehaki et al., 2016). According to Sutrisno (2014), the *N. lugens* population in the field was known to be resistant to BPMC, carbofuran, MIPC, and imidacloprid. This underlies the use of systemic insecticides, one of which is the active ingredient pymetrozine. In 2014, Indonesian farmers started to use the active ingredient pymetrozine so that it has not been known the resistance of *N. lugens*.

Pymetrozine is a new insecticide that being used in Indonesia recently. So far, pymetrozine does not

have a knockdown effect and acts as an inhibitor for eating (Surahmat et al., 2016). The active ingredient of the pimetrozine insecticide belongs to group 9B and represents pyridine azomethine derivates (IRAC, 2015). The active ingredients of diotefuran and pymetrozine are systemic and are still effective because they have not been circulating for a long time so it is suspected that there has not been resistance/resurgence. Pymetrozine has the common name (E) -4,5-dihydro-6-methyl-4- (3 pyridylmethyleneamino) -1,2,4-triazin-3 (2H) - which is an insecticide that acts to inhibit feeding. Pymetrozine has low acute toxicity by the oral (mouth), dermal (skin), and inhalation routes (Hock, 2016).

Pymetrozine is a systemic poison. Its action does not directly kill the pest. This insecticide works as a poison that is sprayed and sticks to the plant and is then absorbed into the plant tissue through the leaves or roots. According to Johana et al. (2016), the inhibition component of feeding contained in plants can be detected by insects through the sensory system or due to stimulation through the central nerve of insects that regulate the feeding process. The high inhibition of this feeding activity can give a synergistic effect where the activity of eating will slow down or reduce the amount of food eaten by test insects that can weaken the condition of the body of the insect and if the test insects eat the leaves of the treatment containing toxins then the insect will experience a faster death.

The inability of insecticides in controlling pest is one of the causes of *N. lugens*' explosion which cause resistance and resurgence. Resistance causes mutations through biochemical, physiological, and insecticidal processes. Resistance is one of the evolutionary phenomena due to a selection process that lasts for several generations in pests that are always treated with insecticides with the same active ingredients (Setiawan et al., 2017).

The resistance of *N. lugens* to insecticides was measured by LD_{50} (lethal dose 50%) when using the topical method or LC_{50} (lethal concentration 50%) with the dip method (IRAC, 2012). Bioassay is widely used in the determination of resistance or as an increase in the LC_{50} or LC_{90}, which is able to kill 50 or 90% of the test insects. According to Baehaki et al. (2016), the increase in resistance is expressed as the ratio either LC or LD of resistant insect populations compared to sensitive populations. Bioassay is the most widely used method because it directly provides information related to the mortality of the test organism by toxic substances (Rifai et al., 2016).

Bioassay can also be performed for calculating LC_{50} or LD_{50} values or for calculating the frequency of resistant and susceptible individuals in a population using diagnostic concentrations or doses. LC or LD values are often used as a standard for sensitive populations because in reality it is difficult to find insect populations that are still sensitive to insecticides (Utami, 2018). The resistance level of an insect population in an area to a certain insecticide is indicated by the value of the resistance factor (RF) or resistance ratio (RR) (Wu et al., 2018). The RF value shows the level of resistance of the insect population in an area to a certain insecticide. The higher the FR value of the insect population, the higher the resistance level of the population.

Insect resistance monitoring to insecticides is usually carried out by testing 4-5 concentration series resulting in 10-90% mortality. The mortality data obtained were used to estimate the LC_{50} and LC_{90} values. The mortality data obtained were used to estimate the LC_{50} value and the probit value was then linked to one another. An alternative mortality concentration test can be used to detect the resistance level. This diagnostic concentration can also be detected in the presence of inherited resistance. According to Tarwotjo & Rahadian (2017), the involvement of genes or several genes in hereditary resistance affects the level of resistance characteristics in the population.

Determination of diagnostic concentrations is preceded by testing the sensitivity of a population of insects to certain insecticides. The sensitivity testing is closely related to the way determining diagnostic concentration that is strongly influenced by the conditions of the insect population being tested. The objective of this research was to find out the level of resistance of the *N. lugens* population against pymetrozine insecticides by determing diagnostic concertations. The benefit of monitoring N. Lugens' resistance to the insecticide pymetrozine with a more rapid diagnostic determination to measure low frequency or recently emerging resistance events. This method can also accept resistance in different areas so that it can inhibit the development of resistance.

METHODS

Research on the resistance test of *N. lugens* to pymetrozine insecticide was carried out at IP2TP Ungaran BPTP in Semarang Regency, Central Java, by collecting *N. lugens* populations from the fields specifically from rice production centers and endemic areas. The research was conducted from October 2019 to June 2020.

N. lugens Rearing

The population of *N. lugens* was collected from endemic areas in Central Java Province, namely Sukoharjo, Karanganyar (Pekalongan), Mungkid (Magelang), Kajen (Pekalongan), and Sawangan (Magelang) Districts. The population of *N. lugens* from each area was propagated by rearing by planting rice plants aged 30-35 days which were planted in buckets, and maintained in a hood in the IP2TP Ungaran greenhouse. The tested insects for determining insecticide sensitivity were taken between instars two, three, and four.

Sensitivity test of *N. lugens* populations to pymetrozine

Each field population was tested for its sensitivity to the insecticide pymetrozine. Preliminary testing used 10 series of concentrations that can kill 16-84% of the predetermined test insects. These concentrations were then used in sensitivity testing to estimate LC50 values.

Sensitivity Test

The method of insecticide sensitivity testing to the tested insects was the feed treatment method based on the method number of 005 (IRAC, 2012) using six recommended insecticide concentration levels which were expected to result in the death of the tested insects ranging from 0% <x <100%. Insecticide treatment was based on the six concentration series and control (without insecticide). The insecticide for each dose was dissolved in distilled water in a plastic cup. Rice plants during the vegetative period around the age of 20-30 days were immersed in each series of insecticide concentrations and for the control the rice was immersed in water for 10 seconds.

The rice plants that have been dyed were drained and dried, then put into a test glass as much as one rice stem (previously labeled according to the concentration given). Each treatment container was filled with 10 nymphs of 2nd and 3rd instar of *N. lugens* and each treatment was repeated three times so that for one concentration there were 30 indiviuals used. Mortality observations of *N. lugens* were carried out 48 hours after the treatment. In the calculation, mortality was the mortality rate of 30 *N. lugens*. Data analysis was performed using probit analysis with the procedure to obtain the LC_{50} value followed by the fiducial limit (FL) value.

Data Analysis

The data obtained from the bioassay were analyzed using Probit analysis to obtain the LC_{50} value. Data analysis was performed when the control mortality was <20% (Busvine, 2011). The level of resistance of a population to other populations was compared by calculating the resistance factor (RF) using the following formula:

$$RF = \frac{\text{LC50 of the tested insects}}{\text{LC50 from sensitive insects}}$$

An insect population has been resistant to certain insecticides if the population has a RF value of ≥ 4. A resistance level of <4 indicates that the use of insecticide is still economically beneficial, but if the resistance level> 4 is less profitable because too much insecticide must be applied to cultivated plants.

Diagnostic concentration determination

Determination of diagnostic concentration was based on the results of testing the sensitivity of an insect population to a particular insecticide. The data obtained from the results of probit analysis can be seen from the resistance status. Diagnostic concentration is determined as valid concentration if the value of X^2 count < X^2 table (at $\alpha = 0.05$ with degrees of freedom = 1). The calculated value of X^2 is determined by the following formula:

$$\chi^2 = \frac{(observed - expected)^2}{expected}$$

RESULTS AND DISCUSSION

Determination of LC50 *N. lugens* Field Population against Pymetrozine Insecticide

The mortality of *Nilaparvata lugens* after 48 hours of pymetrozin exposure at several locations is shown in Figure 1. Based on Figure 1, the mortality percentage of *N. lugens* varies from different locations. The highest percentage (92%) of *N. lugens* mortality with a concentration of 72 ppm (the highest test concentration) was found in Karanganyar. Meanwhile, the lowest mortality was 68%, found in the Kajen area. These results indicate that the population of *N. lugens* from Karanganyar is the most sensitive to pymetrozin. According to Surahmat et al. (2016), the insecticide pymetrozine does not have a knockdown effect, but works quickly to control sucking insects by inhibiting eating.

Based on Table 1, the LC_{50} pymetrozine on the population of *N. lugens* is different for each location. LC_{50} is a concentration that can kill the test organism as much as 50% of the population in a certain period. The LC_{50} value obtained reflects the toxicity of the material to the test animals. According to Imam (2013), the greater the LC_{50} number, the smaller the toxicity and vice versa.

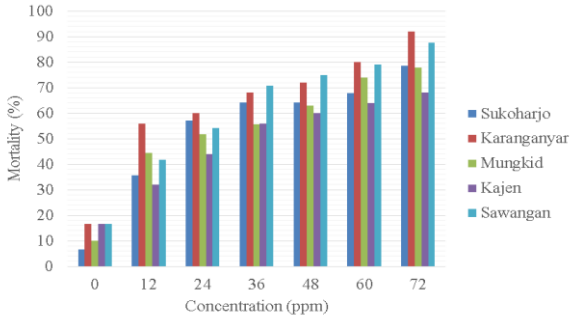

Figure 1. Graph of response of the five populations of *N. lugens* to pymetrozine

The results of the sensitivity test of the five tested populations showed that the Karanganyar population was still sensitive to the insecticide pymetrozine. It was evidenced from the test results that there was a mortality of > 90% at a concentration of 72 ppm (Figure 1). These differences in resistance levels in geographically different populations may reflect natural variation between populations. According to Rashid et al. (2017), population abundance and insect pest dynamics is dependent on ecological fitness parameters, such as survival rate.

Table 1. Probit analysis results of pymetrozine testing on mortality of *N. lugens* at several locations

Locations	LC50 (ppm)	Fiducial Limit	Slope ± S.E	RF (Resistance Factor)
Sukoharjo	20.92	11.24-15.16	1.272 ± 0.369	1.74
Karanganyar	11.99	7.670-13.9	1.237 ± 0.382	1
Mungkid	19.16	10.50-15.14	1.151 ± 0.367	1.60
Kajen	29.66	13.09-16.35	1.201 ± 0.368	2.47
Sawangan	17.71	10.74-14.22	1.650 ± 0.383	1.48

Based on the slope value, the one with the lowest slope was at the Mungkid location. A low slope value indicates that the level of heterogeneity is high. According to Sari et al. (2018), heterogeneous populations show a diversity of population responses to insecticides and if the population is subjected to continuous selection pressure with the same or different insecticides but still with the same active ingredient, it will potentially become a resistant population.

The sensitivity level of the *N. lugens* population from the five test locations was based on the calculation of the resistance factor (RF) or RR (relative risk). The resistance factor was obtained from the comparison between the LC_{50} of the tested insects and the LC_{50} of sensitive insects or the lowest LC_{50} value. The results of Table 1 show that the Karanganyar LC_{50} value is used as a comparison to determine the resistance factor of the *N. lugens* population from the five locations tested using pymetrozine insecticide.

According to Kerns et al. (1998) in Wibisono et al. (2007), resistance levels can be classified as follows:

Table 2. RF/RR value

RF / RR Value	Meaning
1	Non-resistant
2-24	Low
25-99	Moderate
100-199	High
>200	Very high

Based on this classification (Table 2), the tested population can be grouped into non-resistant population (Karanganyar), approaching low resistance (Sawangan, Sukoharjo and Mungkid population) because the RR value is 1< x< 2, and population with low resistance levels (Kajen). Siegfried et al. (2005) suggested that these differences in resistance levels among geographically different populations are likely a response to natural variation rather than variation due to selection pressure for prior insecticide exposure.

Resistance of *N. lugens* of Field Population to Pymetrozine Insecticide

Table 1 shows that Karanganyar (Pekalongan) population as a sensitive population because the value of LC_{50} is 11.99 ppm with a confidence interval of 7.67 and 13.90. The small LC_{50} value indicates great toxicity and has a high sensitivity. The regression equation of Karanganyar population between the mortality probit (Y) and the log concentration (X) is Y = 3.6657 + 1.2374 logX. The slope of the probit regression equation was 1.237 with the t-ratio (slope / SE) of 1.237 / 0.382 = 3.23> 1.96. The t-ratio value shows accurate regression and significantly different result, which indicates that pymetrozine treatment has an effect on *N. lugens*.

The resistance test of *N. lugens* in Mungkid and Sawangan populations located in Magelang Regency, through probit analysis, obtained that the LC_{50} was 19.16 ppm and 17.71 (Table 1) with almost close proximity of confidence intervals between 10-15. Both have resistance factor (RF) values which are

also close to 1.48 and 1.6 and have a low level of resistance and the use of pymetrozine insecticide has an effect on *N. lugens* because it is greater than 1.96.

The resistance level of the *N. lugens* population was known by comparing the LC_{50} of the tested insects with the LC_{50} of the sensitive insects. The use of pymetrozine insecticide against *N. lugens* in the entire population is beneficial because the RF values were <4. Pymetrozine is a systemic insecticide that has only been used in 2014 to control *N. lugens*, and has implemented IPM principles in operation. Operational factors are the more dominant factor in determining the speed at which resistance arises. Insecticide resistance is different from insecticide tolerance. The insecticide tolerance is the natural ability to withstand insecticide action, and is not the result of genetic changes caused by the insecticide selection pressure (Dang et al., 2017). The impact of pest resistance to insecticides is a) pests become immune, because insecticides with recommended doses cannot control, b) use of insecticides at recommended doses will cause pest explosions, c) insecticide doses for pest control must be increased from recommended doses according to the level of resistance, and d) pollution occurs due to excessive use of insecticides due to increased doses. Insecticide resistance can occur, mainly due to the abuse or the overuse. In the field, cross-resistance can occur when the insect pests have resistance to one insecticide and provide resistance to other insecticides that were recently used.

Determination of diagnostic concentrations

The results of the sensitivity test of five *N. lugens* populations tested to pymetrozine were used for diagnostic concentration determination. The population used in determining the diagnostic concentration is the population whose sensitivity test results have ^{2} count < ^{2} table. The insect populations tested from five locations were still sensitive so that the diagnostic concentration was determined by comparing ^{2} of the calculated sensitivity test results and ^{2} table (Table 3).

The LC_{95} determination of Kajen population exceeds the recommended concentration of 60 ppm of the active ingredient so that it cannot be used as a diagnostic concentration. If the diagnostic concentration exceeds 60 ppm there will be a 100% mortality, so it will not be sensitive to small changes in the frequency of resistance.

The population sensitivity test results from Kajen location cannot be used for determining the diagnostic concentration because it produces an LC_{95} of 69.44 ppm. This result is too high when seen from the results of the sensitivity test, because the concentration of 25.52 ppm has caused mortality of > 90%. The results of the sensitivity test showed that the five populations tested were still sensitive to pymetrozine so that the X^2 count < X^2 table could be used for determining the diagnostic concentration. The diagnostic concentration determined was LC_{95} 25.52 ppm with the lowest concentration limit of 7.67 ppm and the highest was 30.05 ppm. Based on the results of the sensitivity test, all test populations were still sensitive at a concentration of 25.52 ppm with a mortality of > 90%.

Table 3. Chi-square and X table values of pymetrozine insecticide at several locations

Locations	LC_{95} (ppm)	Fiducial Limit	Chi-Square	X table
Sukoharjo	41.07	24.17-28.09	0.950	9.49
Karanganyar	25.52	20.95-27.18	3.169	9.49
Mungkid	51.54	24.80-29.44	0.821	9.49
Kajen	69.44	26.78-30.05	0.078	9.49
Sawangan	17.59	20.71-24.19	0.680	9.49

Pymetrozine is systemic so that *N. lugens* is a penetration resistance where resistant insects absorb toxins slower than susceptible insects. Penetration resistance occurs when the insect's outer cuticle develops a barrier that slows down the absorption of the chemical into its body. This can protect the insects from various insecticides. Resistance mechanisms occur in several types of insecticides, such as organochlorines, organophosphates, carbamates and pyrethroids. The insects only stop eating if they discover a certain insecticide or leave the area where spraying occurs. According to Tarwotjo (2018), enzyme activity varies from population to population because of different detoxification systems and is associated with increased enzyme expression. This also determines the resistance of *N. lugens* to the insecticide pymetrozine.

The resistance of *N. lugens* in the five populations to insecticides was different from one another. The *N. lugens* of the Karanganyar population was somewhat sensitive to pymetrozine, while the *N. lugens* of the Kajen population reacted resistant to pymetrozine insecticides. The resistance of *N. lugens* population in areas in Central Java Province was different, the same thing happened in India that the resistance of *N. lugens* in Karnataka (Gangavati, Kathalagere, Kollegala, Soraba, and Mandya) was different from one another (Basanth et al., 2013). The difference in *N. lugens* resistance to an insecticide is not only

between regions, but also between China, India, Indonesia, Malaysia, Thailand, and Vietnam (Gorman et al, 2008)

In general, there is a tendency that *N. lugens* population in one area is more susceptible to insecticides than in other areas. Even the *N. lugens* population is resistant to some of the older generation insecticides but their resistance is low to the new generation insecticides. Pymetrozine is a new generation insecticide because it has been circulating in Indonesia since 2014 and is systemic in nature. *N. lugens* have already developed resistance against thiamethoxam in China, and this population is also resistant to imidacloprid (Wu et al, 2018). This is because farmers do not often use thiamethoxam as well as there is no cross-resistance from imidacloprid (Wen et al, 2009). The determined diagnostic concentration of pymetrozine insecticides would be useful for detecting the new generation insecticides which is usually difficult to be detected using the other methods, thus at the end it will be useful for implementing an effective insect pests monitoring system in rice and other crops.

CONCLUSION

The resistance of *N. lugens* populations to pymetrozine insecticides in several areas in Central Java Province are still sensitive to pymetrozine insecticides with an LC_{50} between 11.99 - 29.66 ppm. The Kajen population has the highest RF value, while Karanganyar population has the lowest RF value which means as the most sensitive population. The diagnostic concentration of LC_{90} = 25.52 ppm is suitable for monitoring the sensitivity of the field population of *N. lugens* in other locations.

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