

RELATIVE FITNESS AND FEEDING CAPACITY OF IMIDACLOPRID RESISTANT *Nilaparvata lugens*

KEBUGARAN RELATIF DAN KEMAMPUAN MAKAN *Nilaparvata lugens* RESISTEN TERHADAP IMIDAKLOPRID

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ABSTRACT

Imidacloprid is a neonicotinoid insecticide that is recommended for controlling *Nilaparvata lugens*. In Asian countries, such as, China, Vietnam, India, and Thailand, imidacloprid has caused resistance to *N. lugens*. Imidacloprid has also caused resistance to *N. lugens* based on some previous studies in Indonesia. The aim of this study was to determine the fitness and feeding capacity of imidacloprid-resistant *N. lugens*. The population of *N. lugens* used in this study had a resistance level of 50.64 times compared to the susceptible population. When the resistant and susceptible population of *N. lugens* did not receive any exposure to imidacloprid, the susceptible population had better fitness than the resistance one. However, the fitness of the resistant population increased when this population was resistance which sublethal concentration (LC₅₀ & LC₂₀) of imidacloprid. The increase fitness of this resistant population most likely related to the increase in feeding capacity of the resistant population when they were treated which sublethal imidacloprid. These findings suggest that the field population of *N. lugens* that have developed resistance would increase the probability of outbreak if they were sprayed with imidacloprid.

Keywords: feeding capacity, imidacloprid, *Nilaparvata lugens*, relative fitness, resistance

INTISARI

Imidakloprid adalah insektisida neonicotinoid yang direkomendasikan untuk mengendalikan *Nilaparvata lugens*. Di negara Asia, seperti, China, Vietnam, India, dan Thailand, imidakloprid telah menyebabkan resistensi terhadap *N. lugens*. Di Indonesia, berdasarkan beberapa penelitian sebelumnya dilaporkan imidakloprid juga menyebabkan resistensi terhadap *N. lugens*. Tujuan penelitian ini untuk mengetahui kebugaran relatif dan kemampuan makan *N. lugens* resisten terhadap imidakloprid. Populasi *N. lugens* yang digunakan dalam penelitian ini mempunyai tingkat resistensi 50,64 kali dibandingkan dengan populasi peka. *N. lugens* populasi resisten dan peka apabila tidak dipapar dengan imidakloprid, populasi peka mempunyai kebugaran lebih baik dari pada resisten, namun kebugaran dari *N. lugens* populasi resisten meningkat ketika populasi tersebut dipapar dengan imidakloprid konsentrasi subletal (LC₅₀ & LC₂₀). Peningkatan kebugaran populasi *N. lugens* resisten diduga terkait dengan peningkatan kemampuan makan akibat dipapar lagi dengan imidakloprid subletal. Hasil penelitian ini menunjukkan bahwa ledakan *N. lugens* akan terjadi, apabila populasi *N. lugens* yang telah resisten terhadap imidakloprid dipapar lagi dengan imidakloprid.

Kata kunci: imidakloprid, kebugaran relatif, kemampuan makan, *Nilaparvata lugens*, resisten

INTRODUCTION

The brown planthopper, *Nilaparvata lugens* Stal (Hemiptera: Delphacidae), is a major pest of rice, which infests rice planted in many countries like Australia, Bangladesh, Bhutan, Myanmar, Cambodia, China, Fiji, India, Indonesia, Japan, North and South Korea, Laos, Malaysia, Nepal, Pakistan, Papua New Guinea, the Philippines, Sri Lanka, Taiwan, Thailand, and Vietnam (Chen *et al.*, 2012; Liu *et al.*, 2012). The loss caused by *N. lugens* infestation was around 2.7 million tons in China in 2005, 0.7 million tons in Vietnam in 2007, 1.1 million tons in Thailand between

2009 to 2011, and 0.8 million tons in Indonesia in 2011 (Botrell & Schoenly, 2012). The insect is a serious threat to worlds' rice sustainability by affecting to the instability of rice production, monetary loss, and harvest failure (Catindig *et al.*, 2009).

Insect pest resistance to insecticides is a major issue in many countries including Indonesia since it is directly related to the success in insect pest control. *N. lugens* resistance has been reported occurring in many rice producing countries like China, Thailand, India, and Vietnam (Catindig *et al.*, 2009; Liu *et al.*, 2012). In China, the resistance to buprofezin reached

28.8 times. In Thailand, the resistance to fenobucarb ranged between 3–5.6 times. In India the resistant level to thiamethoxam and clothianidin were 10.7 and 4.9 times, respectively (Catindig *et al.*, 2009). *N. lugens* resistance cases were reported in many regions in Java especially to diazinon, carbaryl, carbofuran, carbamate, and fentoat (Sutrisno, 1989). Melhanah *et al.* (2002) found that *N. lugens* developed resistance to fipronil with resistance level of 59.5 times compared to the susceptible population after four generations of laboratory selections. These studies show the capability of *N. lugens* to develop resistance to many different insecticides.

Imidacloprid belongs to neonicotinoid class which was released in 1991. It is sold US \$ 1.560.000.000 annually or almost 17% of the global marketed insecticides (Jeschke & Nauen, 2005; Elbert *et al.*, 2008). Neonicotinoid is consisted of at least seven main chemicals with market share of 25% of the global insecticides (Bass *et al.*, 2015). The effectivity of this insecticide intensified its use which eventually created the resistance in *N. lugens*. During 2005–2006 in China, the resistance of *N. lugens* to imidacloprid raised to 79.1–81.1 times compared to susceptible *N. lugens* (Wang *et al.*, 2008; Catindig *et al.*, 2009). Resistance to imidacloprid was found in *N. lugens* collected from some locations in Central and East Java (Y.A. Trisyono, 2012, personal communication).

The exposure of insects to sublethal dose or concentration of insecticide is one factor causing resistance. Sublethal dose affects pest biology (Wang *et al.*, 2008) such as the length of insects' stadium. The length of larval and adult stages could be longer, however the developmental and consumption rates decreased (Herbert & Harper, 1987). The reduction of eggs viability, oviposition period and prolonged larval stadium were related to the development of resistance in *Leptinotarsa decemlineata* selected with *Bacillus thuringiensis* CryIII δ endotoxin (Trisyono & Whalon, 1997). Liu *et al.* (2012) found that unselected population developed faster to the next generation, while selected population endured higher fitness cost covering the significant reduction of larval longevity, adult eclosion, the rate of copulation, fecundity and hatching rate, and relative fitness of resistant *N. lugens* on the next generation.

Insects' fitness components like fecundity is related to insects' feeding capacity and insects' food use, which are the main component in the life of an organism (Leather, 1995). Ratna (2011) reported that resistant *N. lugens* showed different feeding capacity compared to the susceptible one. Chelliah and

Heinrichs (1984) found that reproduction rate of *N. lugens* increased when they were fed with rice sprayed with deltamethrin, methyl parathion, cypermethrin and fenvalerat. They suspect this condition was the consequence of insecticide residue or its metabolites, which induced chemical changes within the sprayed host plants. The rise of reproduction rates in *N. lugens* may be caused by the increase of consumption. This study was aimed to determine the fitness and feeding capacity of imidacloprid resistant *N. lugens*. The results of this study can be used as the bases to develop management tactics to delay or combat resistance to imidacloprid in *N. lugens*.

MATERIALS AND METHODS

Mass rearing of Nilaparvata lugens

N. lugens population used in this study was collected in 2010 from the District Klaten. The Laboratory susceptible population was used as a comparison. The laboratory population has been reared in Pesticide Toxicology Laboratory (PLT) of the Faculty of Agriculture, Universitas Gadjah Mada (FA-UGM) since 1986 without any exposure to insecticides and no additional field population. These population were reared using an establish laboratory rearing procedures. One hundred g of sorted Cisadane seeds were carefully cleaned and soaked for 24 h. The seeds were germinated in a plastic jar (diameter = 30 cm, height = 32 cm), and covered with gauze. Seven d-old seedlings were used as the oviposition media and the diet of *N. lugens*.

Resistant adults of *N. lugens* (75–100 pairs) were moved to rice seedlings for oviposition. After 10 d or after eggs hatching, *N. lugens* nymphs were moved to a new jar previously filled with fresh rice seedlings. Used seedlings were placed above these fresh ones in an upside down position, held by wires attached to the side of the jar to allow the newly hatched nymphs to move to the fresh seedlings. The replacement of the seedlings was conducted every 6–7 d until *N. lugens* reached adult stage. Newly formed adults were directly transferred into a new jar containing with fresh 7 d old seedlings to develop the next generation.

Insecticide

The insecticide used was imidacloprid (Confidor 5 WP; PT Bayer Indonesia). Imidacloprid is a wide spectrum neonicotinoid, systemic, worked by contact and stomach poisoning and this chemical is recommended to control plant pests including *N. lugens* (Elbert *et al.*, 2008).

The Resistance of *Nilaparvata lugens*

The resistance of *N. lugens* to imidacloprid after the five generations of selection was 50.64 times compared to the susceptible population (Londingkene *et al.*, 2016, in press). LC₅₀ and LC₂₀ of imidacloprid to resistant *N. lugens* was 33.93 and 1.18 ppm, respectively. These sublethal concentrations were used in the following studies.

Study 1. Relative Fitness (preoviposition, oviposition, fecundity, egg hatching percentage, adult longevity, and life cycle) of *Nilaparvata lugens*

The fitness of *N. lugens* was studied using CRD with four treatments and 20 replications. The treatments were resistant *N. lugens* without exposure to imidacloprid (unexposed), resistant *N. lugens* exposed to 33.93 ppm (LC₅₀) and 1.18 ppm (LC₂₀) of imidacloprid, and susceptible *N. lugens* without exposure to imidacloprid. There were three applications administered to three consecutive generations. The insects were fed with the 6 d old seedlings previously dipped thoroughly in insecticide solution or water.

Preoviposition and oviposition experiment was conducted by placing a pair of newly formed adults into a test tube filled with 5-d old rice seedling. The seedling was replaced and examined daily to ensure the oviposition. The examination of preoviposition period was stopped when the first egg was laid, and right after the observation of the oviposition period was started.

Fecundity was examined by placing a pair of newly molted adults (1-d old) into a plastic cup (d = 10 cm, h = 11 cm) filled with 10 rice seedlings of 5-d old. The seedlings were replaced every four days. The number of eggs laid was examined by dissecting the leaves sheath toward the stem to count eggs mass under the dissecting microscope. The egg hatching percentage or eggs' viability was counted (Heinrichs *et al.*, 1981).

The longevity of female adults was determined by placing a newly formed female adult into a test tube filled with a 5 d old seedling. The seedling was replaced every three days. The observation was ended when the insect died. Nymphal stages were determined by placing a newly hatched nymph into plastic cup having rice seedlings. Subsequently, the second instar that emerged from the first instar was placed into a new plastic cup. Procedure for observing the following stages were similar until the nymphs reached adults. If *N. lugens* dead during the test, they were substituted with *N. lugens* of the same age.

Study 2. Feeding capacity of *Nilaparvata lugens*

Feeding capacity of *N. lugens* was determined with ninhydrin method (Heinrichs *et al.*, 1981) with modifications. The study was aimed to compare the feeding capacity of the imidacloprid exposed resistant *N. lugens* to the unexposed resistant ones. Three times exposure were made at 28, 53, and 78 days after planting (DAP), and each was replicated 10 times. Trays with the size of 50×50 cm were used for planting the seeds. The 14 DAP seedlings were removed from the trays and planted in the pots (d = 12 cm, h = 12 cm) and allowed to grow until 28, 53, and 78 DAP. The rice hill of 28 DAP were cut to leave only one stem in the pot. Every pot was enclosed with a plastic cup (d = 12 cm, h = 25 cm) equipped with a hole and cotton on its top to free the upper part of the stem, while keeping *N. lugens* inside the cup. The base of the cup was layered with plastic plate to allow the placement of filter paper and to prevent the moisture around the soil to enter the cup. A square opening of 4×4 cm was placed on the side of the cup and enclosed with gauze to allow air circulation. The surface of the stem was brushed with imidacloprid (33.93 and 1.18 ppm) or with water for the control (the unexposed resistant *N. lugens* and susceptible one). A Whatman filter paper (No 42, d = 12 cm) was placed around the lower stem. On every treatment five brachypteran female *N. lugens* adults (≤ 1 d old) were placed. These insects were previously starved for ± 3 h. After 24 h, the filter paper was retrieved, sorted according to the treatments and sprayed with 0.1% ninhydrin in acetone. The papers were then picked up with a pair of forceps and dried in oven for 5 min, at 100°C. Feeding capacity of *N. lugens* was observed by measuring the spots of amino acid (mm²) left by the brachypteran females. The spots were the honey dew excretion of *N. lugens* containing amino acid which became purple after reacted with ninhydrin. The spots were measured with *Leaf Area Meter* (Heinrichs *et al.*, 1981).

Calculation

The preoviposition period was calculated from the formation of female adult until its first oviposition. The oviposition period was determined from the first egg was laid until no more eggs were deposited. Fecundity was counted as the number of deposited eggs and unhatched ones by a female. Eggs viability represented the ratio between the number of new nymphs and deposited eggs. The life cycle of *N. lugens* counted since eggs were placed to the adults. The longevity of female adults was observed since the emergence of female adult to its death.

Data Analysis

LSD on 5% level was used to analyse every variable of fitness and feeding capacity. The significance test was performed when anova showed significant difference among treatments. The statistical analysis was conducted using Statistix 8.0.

RESULTS AND DISCUSSION

The Fitness of *Nilaparvata lugens*

The application frequency of imidacloprid did not affect the fitness (preoviposition, oviposition, fecundity, female age, eggs viability, and life cycle) of *N. lugens*. However, there was a significant difference between resistant *N. lugens* (control), exposed resistant *N. lugens*, and susceptible *N. lugens* (Table 1.).

The application of imidacloprid affected all fitness variables including preoviposition, oviposition, female age, fecundity, and eggs viability of *N. lugens*. Fecundity of resistant *N. lugens* exposed to imidacloprid was significantly higher compared to the unexposed resistant one for all applications (Table 1). This suggests that imidacloprid increases the fecundity of resistant females. Chelliah and Heinrichs (1984) explained that reproduction rate of *N. lugens* raised when they were fed with rice plants sprayed with deltamethrin, methylparathion, cypermethrin, and fenvalerate. Furthermore, the increase of *N. lugens* reproduction was also enhanced by the increase of nutrition consumption capacity due to the sublethal treatment of insecticide. Basit *et al.* (2012) said that insect resistant to insecticide influences other fitness component such as longevity, development, fecundity, and fertility. According to Moriarty (1969), the sublethal dose of insecticide affected insect population by altering their capacity to live, reproduce, and the genetic materials of the survival generation. The application of triazophos and fenvalerate on sublethal concentration increased the reproduction of female *N. lugens* (Bao *et al.*, 2009). The increase of reproductive capacity happens through the stimulation of fecundity (Yin *et al.*, 2008). Cutler *et al.* (2009) reported that the sublethal concentration of imidacloprid and azadirachtin stimulated the reproduction of *Myzuz persicae*. Wang *et al.* (2005) informed that imidacloprid applied to first instar larvae of *Tryporyza incertulas* increased the fecundity of its adult, due to the increase of juvenile III hormone on female adult. Both

studies showed a lesser fitness than the susceptible population of *N. lugens*.

In general, the application of imidacloprid to the resistant *N. lugens* prolonged the period of egg and nymph stages. Both resistant *N. lugens* (exposed and non exposed to imidacloprid) had longer egg and nymph period compared to the susceptible *N. lugens* (Table 2). This might be due to the lesser fitness level of resistant *N. lugens* compared to susceptible *N. lugens* which was better in fitness. Liu and Han (2006) found that the fitness of imidacloprid resistant *N. lugens* can drop to the initial state if the use of imidacloprid is stopped. Abbas *et al.* (2012) reported that the fitness of imidacloprid resistant *Spodoptera litura* was far below the susceptible one, in terms of the length of larvae, pupae and adult stadia. Klot and Ghanim (2012) mentioned that the population of unselected insects could develop very fast, while the fitness of selected population was very low in terms of larval longevity, the number of molted adults, and the rate of copulation, fecundity, and hatching rate. All of these were significantly lower which caused a bigger fitness cost.

Feeding capacity of *Nilaparvata lugens*

The frequency of 1st, 2nd, and 3rd application of imidacloprid did not affect feeding capacity of *N. lugens*. However, its feeding capacity was affected by the kind of treatments used (Table 3). Feeding capacity of imidacloprid exposed *N. lugens* (LC₅₀ dan LC₂₀) was higher than to the unexposed resistant *N. lugens*, but the feeding capacity of these two populations was less than the susceptible population. Elsa *et al.* (2003) suggested that feeding capacity was an important factor that influenced *N. lugens* fecundity which determined whether pest resurgence will happen or not. Kumar and Champan (1984) reported that application of LC₅₀ like permethrin and fenvalerate decreased the habit and feeding activity. Increasing the feeding capacity of the resistant population when they were treated with imidacloprid might have contributed to the increase of their fecundity as indicated in the previous linkings/findings: Together with the fact the field population of *N. lugens* has developed resistance and continuations use of imidacloprid would result in increase probability of outbreak.

Table 1. The effect of imidacloprid to the fitness of *Nilaparvata lugens*

Application	<i>Nilaparvata lugens</i> population	Imidacloprid (ppm)	Preoviposition (d)	Oviposition (d)	Female age (d)	Fecundity (eggs/female)	Eggs viability (%)
1	Resistant	-	2.17 c	7.15 c	7.05 c	105.00 c	86.3 c
	Resistant	LC50 (33.93)	1.88 c	7.50 b	8.90 b	127.75 b	95.7 b
	Resistant	LC20 (1.18)	2.29 b	7.05 c	8.80 b	131.40 b	94.3 b
	Susceptible	-	2.93 a	8.70 a	11.65 a	159.75 a	98.3 a
2	Resistant	-	2.11 b	7.10 b	7.50 c	109.20 c	85.7 c
	Resistant	LC50 (33.93)	1.97 c	7.00 b	8.85 b	149.40 b	92.3 b
	Resistant	LC20 (1.18)	2.18 b	7.25 b	8.65 b	144.15 b	94.6 b
	Susceptible	-	3.04 a	9.15 a	11.10 a	167.05 a	98.4 a
3	Resistant	-	2.37 b	7.85 c	7.70 c	108.40 c	83.3 c
	Resistant	LC50 (33.93)	2.22 b	8.05 b	8.80 b	141.05 b	91.7 b
	Resistant	LC20 (1.18)	2.09 c	8.14 b	8.65 b	138.40 b	92.3 b
	Susceptible	-	3.42 a	9.80 a	11.35 a	147.20 a	98.7 a

Note: A pair of brachypterous *Nilaparvata lugens* was released two days after imidacloprid application. Means in each column for each application followed by similar letter is not significantly different (LSD; $\alpha = 0.05$). d = day

Table 2. The effect of imidacloprid to eggs and nymphs of *Nilaparvata lugens*

<i>Nilaparvata lugens</i> population	Imidacloprid (ppm)	Eggs (d)	1st instar (d)	2nd instar (d)	3rd instar (d)	4th instar (d)	5th instar (d)
1st application							
Resistant	-	12.15 b	2.80 bc	2.65 b	2.85 a	2.65 b	2.95 a
Resistant	LC50 (33.93)	13.50 a	3.40 a	3.45 a	3.10 a	3.20 a	3.30 a
Resistant	LC20 (1.18)	12.95 b	3.22 a	2.90 b	3.00 a	3.00 a	3.15 a
Susceptible	-	11.20 c	2.40 c	2.15 c	2.30 b	2.45 b	2.35 b
2nd application							
Resistant	-	12.10 b	2.80 b	2.65 c	2.85 b	2.70 b	2.95 b
Resistant	LC50 (33.93)	13.65 a	3.55 a	3.50 a	3.40 a	3.45 a	3.45 a
Resistant	LC20 (1.18)	13.10 a	3.25 a	3.05 b	3.25 a	3.25 a	3.30 ab
Susceptible	-	9.80 c	2.30 c	2.40 c	2.25 c	2.30 c	2.25 c
3rd application							
Resistant	-	12.15 b	2.80 b	2.90 b	3.05 b	2.75 b	3.05 b
Resistant	LC50 (33.93)	13.70 a	3.40 a	3.45 a	3.45 a	3.55 a	3.50 a
Resistant	LC20 (1.18)	13.15 a	3.00 b	3.00 b	3.30 ab	3.20 a	3.25 ab
Susceptible	-	9.90 c	2.25 c	2.25 c	2.20 c	2.35 c	2.25 c

Note: The average on the column with similar letter is not significantly different on 5% LSD for its application

Table 3. The effect of imidacloprid on feeding capacity of female brachypteran of *Nilaparvata lugens*

<i>Nilaparvata lugens</i> population	Imidacloprid (ppm)	Amino acid area (mm ²)/5females		
		28 DAP	53 DAP	78 DAP
Resistant	-	7.0 c	7.5 c	7.2 c
Resistant	LC50 (33.93)	9.0 b	11.5 b	10.0 b
Resistant	LC20 (1.18)	9.0 b	9.5 b	10.5 b
Susceptible	-	14.0 a	17.0 a	16.0 a

Note: Means in each column followed by similar letter is not significantly different at 5% level (LSD).

CONCLUSION

Although the fitness and feeding capacity of imidacloprid-resistant *N. lugens* were less than the susceptible population, application of imidacloprid at sublethal concentration to the resistant population increased their fitness and feeding capacity. These effects could contribute to the outbreak of *N. lugens*. In other words, the probability of outbreak is higher when imidacloprid is applied to field population of *N. lugens*.

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