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Suppression of joint predators (*Pardosa pseudoannulata* and *Verania lineata*) against *Nilaparvata lugens* in conditions exposed to MIPC insecticide

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Abstract. MIPC is one of the insecticides widely used by farmers to control brown planthopper or BPH (*Nilaparvata lugens*) in Padang City, Indonesia. Excessive use of insecticides can cause BPH to become resistant, followed by the death of natural enemies. This study aimed to determine the suppression of joint predators (*Pardosa pseudoannulata* and *Verania lineata*) against *Nilaparvata lugens* under MIPC Insecticide Exposure Conditions. This study used a Completely Randomized Design (CRD) factorial with two factors and five replications. The first factor was the dose of MIPC insecticide (0.75 g/l, 1.5 g/l, 2.25 g/l, and without application) and the second factor was the time of application (During BPH infestation and joint predators, before BPH infestation and joint predators, after WBC infestation and before joint predator infestation). The parameter observed were predation, body weight, and mortality. The results showed no interaction between dose and time of application on joint predator predation. Increasing the MIPC dose decreased the predation. Joint predators could suppress BPH populations of 40.6-43.6 individuals (78-81%) per day, but MIPC application and the presence of joint predators together reduced their predation rates. It is better to apply MIPC insecticide before or after the presence of joint predators to avoid negative impacts on joint predators.

Keywords: Brown planthopper, doses, interaction, ladybug beetle, time, wolf spider

1. Introduction

The brown planthopper or BPH (*Nilaparvata lugens* Stal, Hemiptera: Delphacidae), is one of the main pests that damage rice around the world [1, 2, 3], including Indonesia. By sucking the plant's phloem sap, which often contains nutrients and photosynthetic products, it assaults rice plants. Malnourished rice plants develop stunted growth, yellowing, and withering leaves, and eventually the plant dries up or is called as hopper burn. Since the 1980s, *N. lugens* attacks have been reported in Indonesia, and their effects have been worse every year [4]. West Sumatra is one of the provinces in Indonesia that is also affected by the BPH attack. The attacks have started to show an increase since 2015. The highest attack occurred in 2020, reaching 1,103.56 ha [5]. In the same year, the area of BPH attacks in Indonesia reached 48.105 ha [6]. The overlapping generations occurred between nymph and adult (brachypteran and macropteran, male and female), which shows that BPH has adapted to rice fields for a long time [7].

There are several natural enemies as the predator of BPH found in rice fields, including *Pardosa pseudoannulata* (Araneae: Lycosidae) [2, 8-16] and *Verania lineata* (Coleoptera: Coccinellidae) [13, 15]. *P. pseudoannulata* is a generalist predator that catches the most abundant prey closest to it [17-19] and consumes it without regard for time [20]. The effectiveness of *P. pseudoannulata* in single predation has been widely reported [21-25]. Five to twenty N.lugens can be consumed by *P. pseudoannulata* each day [22, 25-27], while *V. lineata* can consume 1-11 BPH per day [25,41].

P. pseudoannulata and *V.lineata* togetherly have a high potential to suppress BPH populations in rice fields by increasing the predation rate when the availability of prey increases. The ability of both predators to control the BPH population has been tested in the laboratory. *P. pseudoannulata* can suppress BPH from the lower stem, while *V. lineata* can suppress it from the top. The joint presence of *P. pseudoannulata* and *V. lineata* can increase competition between them but can still suppress the BPH population by up to 80% [16, 25]. A joint predator with a composition of one *P. pseudoannulata* and three *V. lineata* is classified as safe because they prey on up to 89.6% of BPH [17].

Applying excessive insecticides in the rice field can cause insect resistance, followed by the mortality of natural enemies [33]. One insecticide classified as safe for controlling BPH is buprofezin [28] which is an insect growth regulator insecticide, but not the farmer's choice because it has no knockdown effect like carbamate. The carbamate chosen by farmers is MIPC (Methyl Isopropyl Carbamate) or BPMC (Butyl Phenil Metil Carbamate), which are classified as contact and stomach poison. MIPC can inhibit the activity of the AChE (acetylcholinesterase) enzyme in the nervous system of insects [29]. The resistance level of BPH to carbamate insecticide is lower than that of organophosphates, eight times for MIPC and ten times for BPMC [30].

Applying BPMC insecticide to control BPH in Payakumbuh District, West Sumatra Province, is still economically profitable because the resistance ratio is low, with a value of less than 4. This is indicated by the application of BPMC as recommended, causing the mortality of BPH nymph to reach 73 % [31]. The natural enemies' presence in a rice field that is applied with BPMC is lower than those that are not, such as the low population of spiders [33]. Furthermore, BPH in Padang City, West Sumatra Province, is still relatively susceptible to MIPC with a low resistance ratio. i.e., 0.2-0.6 for five days of observation [32]. That the time of Buprofezin application has more effect on the predation rate of joint predators than the dose. The predation rate of joint predators (*Pardosa pseudoannulata* and *Verania lineata*) on BPH is higher when buprofezin is applied before the presence of joint predators, with a predation rate of 64.4%. Buprofezin application with different doses did not affect the predation rate of the two predators [34]. The predation rate of join predators, when exposed to MIPC, has never been carried out. This study was conducted to determine the suppression of joint Predators (*Pardosa pseudoannulata* and *Verania lineata*) against *Nilaparvata lugens* under MIPC insecticide exposure conditions.

2. Material and Methods

2.1 Predator collection

P. pseudoannulata and *V. lineata* were gathered from rice fields in Pauh District, Padang, West Sumatera, and chosen according to the uniformity of body size. To avoid cannibalism, each predator was individually placed in a plastic cup and fed five *N. lugens* nymphs. Each time the prey was consumed by predator, the amount of prey was increased. All predators were starved for 24 hours after a week of collection and maintenance in the laboratory. For all treatments and replications, 60 individuals of *P. pseudoannulata*,180 individuals of *V. lineata* sp were used.

2.2 N. lugens rearing

N. lugens has been reared in the laboratory on the IR 42 rice variety since February 2021. The seeds are soaked for 24 hours, then air-dried for one hour before being put into a culture jar (capacity = 25 liters) with 2 mm of water. The water level was kept at the level that allowed the seeds to be completely covered. Ten pairs of *N. lugens* adults were placed in a culture jar five to seven days after planting. The first nymph instar appeared seven to ten days later. A total of 3.000 seconds to third instar nymphs were used in this study.

2.3 MIPC Preparation
The MIPC insecticide used was Mipcinta 50 WP, where the recommended dose was 1.5 g/l, sub-lethal dose was 0.75 g/l, and 1.5 recommended dose was 2.25 g/l. The insecticide according to the treatment was dissolved with water in a beaker glass with a volume of 1000 ml. The insecticide solution was stirred until well blended. The amount of insecticide needed was 3 ml per replication so that 120 ml of MIPC was needed in one spraying. Insecticide application according to treatment was carried out when the plants were 14 days after sowing.

2.4 Treatment
The study was conducted at the Laboratory of Insect Bioecology and Integrated Pest Management, Faculty of Agriculture, Universitas Andalas, from July-September 2022. The study used a Completely Randomized Design (RCD) factorial in two factors. The first factor was the difference in the dose of MIPC insecticide (0.75 g/l, 1.5 g/l, 2.25 g/l, and control). The second factor was the difference in application time of MIPC insecticide (During BPH and joint predator presence, before BPH and joint predator presence, after BPH presence but before joint predator presence). Each treatment was repeated 5 times, so that 60 experimental units were needed.

For each replication, two 360 mL plastic cups were provided. A heated nail with a diameter of approximately 2 mm was used to perforate the bottom of one cup. Three seven-day-old rice seedlings were inserted through a hole into the cup, with the roots positioned outside the cup. The second cup was filled to a height of 2 mm with water and placed on top of the first cup to serve as root development media. Then, the nymphs of *N. lugens* were placed in the cup in accordance with the treatment. Joint predators that had been starved for 24 hours were weighed using analytical scales with a precision of four decimals before being used in the treatment. The joint predators for each treatment were a combination of *P. pseudoannulata* and *V. lineata* with a composition of 1:3. The BPH used as prey was 50 individuals in 2-3 instar per replication.

2.5 Variables observed

2.5.1 *BPH mortality by MIPC insecticide (%).*he BPH mortality was counted for 1x24 hours and then calculated using the formula:

$$M = \frac{a}{b} x\ 100 \quad (1)$$

M = Mortality (%)
a = Number of BPH dead
b = Number of BPH provided

The characteristics of BPH that died because of being preyed on by *P. pseudoannulata* were the distribution of BPH skin on the bottom of cup. The characteristics of BPH that died because of being preyed on by *V. lineata* was the reduced number or presence of BPH body parts, such as wings or heads. While the characteristics of BPH that die as a result of being exposed to insecticide were to die with body parts that were still perfect.

2.5.2 *Predation rates.* The number of *N. lugens* consumed by joint predators was calculated by counting the number of *N. lugens* consumed three times over a period of 24 hours. The percentage of predation was then calculated using the formula:

$$P = \frac{n}{N} x\ 100 \quad (2)$$

P = Predation
n = Number of *N.lugens* consumed
N = Number of *N.lugens* provided

*2.5.3 Predator mortality (%).*The two predators' mortality was counted for 1x24 hours and then calculated using the formula:

$$M = \frac{nm}{Np} x\ 100 \quad (3)$$

M = Mortality (%)
Nm = Number of predators dead
Np = Number of predators provided

2.5.4 *Bodyweight gain (g).* Each predator's bodyweight was determined by weighing it with four-decimal precision using analytical scales. The difference between the bodyweight on 1st to 3rd days compared to the bodyweight before treatment was used to calculate the bodyweight gain.

2.6 Data analysis
Predation, predator mortality, BPH mortality and body weight growth data were analyzed using Statistix 8 software and the ANOVA factorial and LSD tests at a 5% significance level.

3. Result and discussion

3.1 BPH mortality by MIPC insecticide (%)
BPH mortality for one day ranged from 0 – 52.8%. There was an interaction effect between different doses and application times on BPH mortality due to insecticide (P=0.0013). The highest mortality was found when applying MIPC insecticide at a dose of 2.25 g/l during or after the presence of joint predators (51.2 and 52.8%). The lowest BPH mortality was found when applying MIPC insecticide before the presence of joint predators (Table 1).

Table 1. Effect of different doses and application time of MIPC insecticide on the mortality of brown planthopper (*Nilaparvata lugens*) (%).

Dose (g/l)	Application time			Average
	A	B	C	
0.75	23.00 cd	13.00 e	23.60 cd	20.00
1.50	38.40 b	18.00 de	25.20 c	27.20
2.25	51.20 a	17.00 de	52.80 a	40.30
Control	0 f	0 f	0 f	0
Average	28.10	12.00	25.40	(+)

Notes: A. During BPH and joint predator presence
B. Before BPH and joint predator presence
C. After BPH presence but before joint predator presence
*The number followed by different small letter are significantly different according to LSD test at 5% significance level

3.2 Predation rates
The predation rate of joint predators for one day ranged from 14.60 – 43.80 individuals, from 50 individuals provided. There was no interaction effect between the different doses and application times on the predation (P=0.1253). However, the different doses and application times significantly affected the predation rate (P_{dose}=0.0000) (P_{time}=0.0000). The higher the dose, the lower the predation rate. Furthermore, the application of MIPC insecticide before and after the presence of joint predators can

maintain an individual predation rate of 28.25-29.80; this predation rate was higher than the application of MIPC togetherly with the presence of joint predators (Table 2).

Table 2. Effect of different doses and application time of MIPC insecticide on the predation rate of joint predators (*Pardosa pseudoannulata* and *Verania lineata*) on brown planthopper (*Nilaparvata lugens*) (individual/day).

Dose (g/l)	Application time			Average
	A	B	C	
0,75	20.40	29.80	27.20	25.66 b
1,50	14.60	26.40	22.80	21.26 c
2,25	10.20	22.40	19.20	17.26 d
Control	40.60	40.60	43.80	41.66 a
Average	21.45 b	29.80 a	28.25 a	(-)

Notes: A. During BPH and joint predator presence
B. Before BPH and joint predator presence
C. After BPH presence but before joint predator presence
* The number followed by different small letter are significantly different according to LSD test at 5% significance level

The predation rate of joint predators for one day ranged from 20.40-87.60%, from 50 individuals provided. There was no interaction effect between the different doses and application time on the predation (P=0.2833). However, the different doses and application times significantly affected the predation rate (P_{dose}=0.0000) (P_{time}=0.0000). The higher the dose, the lower the predation rate. Furthermore, the application of MIPC insecticide before and after the presence of joint predators can maintain an individual predation rate of 56.50-59.60; this predation rate was higher than the application of MIPC insecticide togetherly with the presence of joint predators (Table 3).

Table 3. Effect of different doses and application time of MIPC insecticide on the predation percentage of joint predators (*Pardosa pseudoannulata* and *Verania lineata*) on brown planthopper (*Nilaparvata lugens*) (%).

Dose (g/l)	Application Time*			Average
	A	B	C	
0.75	25.20	59.60	54.40	46.40 b
1.50	29.20	52.80	45.60	42.53 b
2.25	20.40	44.80	38.40	34.53 c
Control	81.20	81.20	87.60	83.33 a
Average	39.00 b	59.60 a	56.50 a	(-)

Notes: A. During BPH and joint predator presence
B. Before BPH and joint predator presence
C. After BPH presence but before joint predator presence
* The number followed by different small letter are significantly different according to LSD test at 5% significance level

3.3 Predator mortality (%)

The mortality of *P. pseudoannulata* for one day ranged from 0 – 80%. There was no interaction effect between the different doses and application times on the mortality (P=0.4921). However, the MIPC insecticide application significantly affected the mortality (P_{dose}=0.0090), but it did not happen to application times (P_{time}=0.2635). Applying MIPC insecticide caused mortality of *P. pseudoannulata* although the different doses did not affect the mortality rate (Table 4).

Table 4. Effect of different doses and application time of MIPC insecticide on the mortality of *Pardosa pseudoannulata* on brown planthopper (*Nilaparvata lugens*) (%).

Dose (g/l)	Application Time			Average
	A	B	C	
0.75	20.0	40.0	40.0	33.3 a
1.50	80.0	20.0	60.0	53.3 a
2.25	60.0	20.0	60.0	46.6 a
Control	0	0	0	0 b
Average	40.0 a	20.0 a	40.0 a	(-)

Notes: A. During BPH and joint predator presence
B. Before BPH and joint predator presence
C. After BPH presence but before joint predator presence
*The number followed by different small letter are significantly different according to LSD test at 5% significance level

The mortality of *V.lineata* for one day ranged from 0 – 73.3%. There was no interaction effect between the different doses and application time on the mortality (P=0.3579). However. the MIPC insecticide application and application times significantly affected the mortality (P_{dose}=0.0000; P_{time}=0.0006). Applying MIPC insecticide caused mortality of *V.lineata* although the different doses did not affect the mortality rate. The application of MIPC insecticide before and after the presence of joint predators can decrease the mortality of *V.lineata*; this mortality was lower than the application of MIPC insecticide togetherly with the presence of the predator (Table 5).

Table 5. Effect of different doses and application time of MIPC insecticide on the mortality of *Verania lineata* on brown planthopper (*Nilaparvata lugens*) (%).

Dose (g/l)	Application Time			Average
	A	B	C	
0.75	66.60	6.66	40.00	37.70 a
1.50	73.30	33.30	53.30	53.30 a
2.25	73.30	20.00	40.00	44.40 a
Control	0	0	0	0 b
Average	53.30 a	14.90 b	33.30 a	(-)

Notes: A. During BPH and joint predator presence
B. Before BPH and joint predator presence
C. After BPH presence but before joint predator presence
* The number followed by different small letter are significantly different according to LSD test at 5% significance level

3.4 Bodyweight gain (g)

Body weight of *P. pseudoannulata* for one day ranged from 0.0010 – 0.0100 g. There was no interaction effect between different doses and application times on body weight (P=0.5089). The application times did not affect on body weight. Applying MIPC insecticide decreased the body weight but different doses did not affect on body weight of *P. pseudoannulata* (Table 6).

Body weight of *V.lineata* for one day ranged from 0.0018 – 0.0100 g. There was no interaction effect between different doses and application times on body weight (P=0.1847). the MIPC insecticide application and application times significantly affected the mortality (P_{dose}= 0.0000; P_{time}=0.0010). Applying MIPC insecticide decreased the body weight but different doses did not affect on body weight of *V.lineata*. The application times did not affect on body weight. Applying MIPC insecticide during the presence of joint predators can decrease the body weight of *V.lineata* (Table 7).

Table 6. Effect of different doses and application time of MIPC insecticide on the body weight (g) of *Pardosa pseudoannulata* on brown planthopper (*Nilaparvata lugens*).

Dose (g/l)	Application time			Average
	A	B	C	
0.75	0.0056	0.0023	0.0027	0.0035 b
1.50	0.0013	0.0062	0.0039	0.0038 b
2.25	0.0011	0.0035	0.0010	0.0018 b
Control	0.0066	0.0100	0.0083	0.0083 a
Average	0.0036 a	0.0055 a	0.0040 a	(-)

Notes: A. During BPH and joint predator presence
B. Before BPH and joint predator presence
C. After BPH presence but before joint predator presence
*The number followed by different small letter are significantly different according to LSD test at 5% significance level

Table 7. Effect of different doses and application time of MIPC insecticide on the body weight (g) of *Verania lineata* on brown planthopper (*Nilaparvata lugens*).

Dose (g/l)	Application time			Average
	A	B	C	
0.75	0.0041	0.0054	0.0034	0.0043 b
1.50	0.0015	0.0070	0.0045	0.0043 b
2.25	0.0018	0.0062	0.0032	0.0037 b
Control	0.0066	0.0100	0.0100	0.0088 a
Average	0.0035 b	0.0071 a	0.0053 a	(-)

Notes: A. During BPH and joint predator presence
B. Before BPH and joint predator presence
C. After BPH presence but before joint predator presence
*The number followed by different small letter are significantly different according to LSD test at 5% significance level

Applying MIPC insecticide directly has not only an impact on the BPH population but also on joint predators. Based on this study, BPH mortality due to MIPC application ranged from 0 – 52.8% for one day. The highest mortality was found when applying MIPC insecticide at the highest dose, during or after the presence of joint predators (51.2 and 52.8%) (Table 1). Meanwhile, the predation rate of joint predators ranged from 14.60 – 43.80 individuals or 20.40-87.60% of the 50 individuals provided. The higher the dose, the lower the predation rate. Applying MIPC insecticide togetherly with the presence of joint predators reduced the predation rate (Table 2, Table 3), causing the mortality *of P. pseudoannulata* to reach 80% and the mortality of *V.lineata* to reach 73.3% (Table 4, Table 5), compared to the application was carried out before or after their presence. That happens because predators are more sensitive to direct exposure to insecticides. The mortality of natural enemies is significant if insecticides are not applied selectively in terms of time and method [42]. The mortality of natural enemies can occur due to direct or indirect contamination through contaminated pesticides. The spider's soft body and active on the soil surface make this arthropod prone to exposure to synthetic insecticides, which can cause death [43], so this is one of the factors for the high mortality of *P. pseudoannulata*. Predators are more active in moving so that insecticide residues stick to plant surfaces compared to spiders [16, 35, 36]. The predator's predation rate is influenced by the speed at which the predator finds its prey and the self-defense exercised by its prey [39]. The number of prey predators is influenced by the rate of the predator in finding prey and the time to consume it [40].

Insecticides from the carbamate group (anticholinesterase carbamate) constitute contact and stomach poisons. MIPC can inhibit and bind to the action of the acetylcholine esterase (AChE) enzyme. The

AChE enzyme in the insect's body functions to hydrolyze acetylcholine, and if this enzyme is bound, it will cause the accumulation and increase of the AChE enzyme. Acetylcholine enzyme sends nerve impulses to insects' central nervous system. This results in nerve impulses being stimulated continuously, causing symptoms of tremors or uncontrolled movement of insect pests and death [32, 37, 38].

Carbamate insecticides can enter the insect's body if the insect makes direct contact with the insecticide or the insect walks on the surface of a plant that already contains the insecticide. Insecticides enter the insect's body through the cuticle and are transported to the part of the insect's body where the insecticide is active so that it can cause the death of the insect. However, if an insect eats the plant, the poison can also enter the insect's body through the digestive tract, which can cause the insect's death [44].

Applying MIPC insecticide also decreased the body weight of *P. pseudoannulata* and *V.lineata* (Table 6, Table 7). The predation rate on prey will be directly proportional to the predator's body weight. This tended to be influenced by insecticides, one of which is reducing the appetite of predators after prey is exposed to insecticides, even though the predation process still occurs [25]. The predator's body weight increases when the predation rate increases.

4. Conclusion

There was no interaction between the different doses and application times on joint predator predation. Increasing the dose of MIPC insecticide decreased the joint predator's predation. Joint predators could suppress BPH populations of 40.6-43.6 individuals (78-81%) per day. The application of MIPC insecticide and the presence of joint predators togetherly also reduced their predation rate. The highest dose of MIPC insecticide can reduce the predation rate of joint predators. It is better to apply MIPC insecticide before or after the presence of joint predators to avoid negative impacts on joint predators.

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