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Baseline Susceptibility of Brown Planthopper, *Nilaparvata lugens* (Stål) to Mesoionic Insecticide Triflumezopyrim of Some Rice Areas in West and Central Java of Indonesia

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Original Research Article

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Abstract: Issues inability of neonicotinoid and others insecticides to control of brown planthopper (BPH), *Nilaparvata lugens* (Stål) was suspected due to BPH had been resistant to those insecticides. To response this issues was carried out the research to evaluate rice BPH from 6 districts of West and Central Java against mesoionic triflumezopyrim (TFM) and neonicotinoid imidacloprid (IMI). The research used topical application method with probit analysis by POLO-PC. The result showed that the probits analysis of BPH to TFM and IMI were accepted to determined LD_{50}, fiducial limit, and accurate for making probit regression model base on natural response, t-ratio, g-value and heterogeneity. Responce BPH from Subang was highly susceptible category to TFM with LD_{50} was 0.103 ng/g body weight and resistance ratio ($RR_{calculate}$) was 0.15-fold. BPH from Indramayu, Klaten, and Pati tend to decreased susceptibility to TFM with LD_{50} were 1.46-1.840 ng/g body weight and $RR_{calculate}$ were 2.15-2.70-fold. BPH from Pemalang was decreased susceptibility category to TFM with LD_{50} was 2.687 ng/g body weight with $RR_{calculate}$ was 3.95-fold. BPH from Karawang was low resistance category to TFM with LD_{50} was 3.730 ng/g and $RR_{calculate}$ was 5.48 fold. BPH from Indramayu and Pemalang were highly susceptible to IMI with the LD_{50} were 91.450 and 101.346 ng/g body weight, whereas the $RR_{calculate}$ were 0.83 and 0.92-fold respectively. BPH from Karawang, Subang, and Klaten tend to susceptible category to IMI with LD_{50} were 149.293-195.638 ng/g body weight and $RR_{calculate}$ were 1.35-1.77-fold. BPH from Pati tend to decreased susceptibility to IMI with LD_{50} was 313.243 ng/g body weight and $RR_{calculate}$ was 2.84-fold. TFM provide a low LD_{50} and this insecticide can be used as complementary insecticide in the field and to solve the problem BPH that had been resistant to other insecticides by a sequential release method in IPM strategies.

Keywords: Brown planthopper, insecticides, resistance, rice.

INTRODUCTION

Rice is the most important food crop in Indonesia because it is a staple food that is difficult to replace. Farmers in rice cultivation are always exposed to some insects pest that are always present in almost every season. The pests that often appear in large quantities are brown planthopper, yellow stem borer and rice black bug, in addition there are still some minor pests in the low population. The rice brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) is the major pests that destructive to rice plantation as an importance economic value among rice pests. BPH have various habit in terms of bio-ecology and behavior, among of them development pattern follows a biological clock depend on environment cue that matched well in both rainy and dry season. Untill 1994, the BPH was only found in the rainy season, but after that year, BPH contributes to attack the rice crop in the rainy and dry season especially when rain continues into the dry season due to the La Nina phenomenon.

Control to BPH use insecticides previously effective, then will become resistant due to continuously use of insecticides with the same mode of action. BPH resistance to insecticides may be defined as a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest [1].

Both nymphs and adults of BPH damage rice directly by removing nutrients in cell sap and indirectly by transmitting rice pathogens of rice ragged stunt virus (RRSV) and rice grassy stunt virus (RGSV). BPH is an insect that have a high genetic plasticity to adapt to new released rice variety and various environments in a relatively in short time. This character urges to be easier and faster to form a new biotype. Baehaki [2] reported a chronology of the BPH explosion in Indonesia occurred since 1971 due to broken resistant of rice varieties and BPH changed in to biotype 1. In 1975, to overcome the BPH biotype 1 outbreak had been introduced IR26 variety that carrying resistant Bph1 gene, but in 1976 occurred BPH outbreak due to the BPH population had changed from biotype 1 in to biotype 2. In 1980, to overcome the BPH biotype 2 had been introduced IR42 variety (bph2), but in 1981 occurred BPH outbreak in Simalungun, North Sumatra and some other areas, because the BPH population had changed from biotype 2 in to biotype 3. To overcome the BPH biotype 3, in 1983 had been introduced IR56 (Bph3) and in 1986 was introduced IR64 ($Bph1^{+}$). Alam and Cohen (1998) [3] reported a total of seven QTLs associated with resistance were identified, located on 6 of the 12 rice chromosomes of IR64. Baehaki and Iswanto [4] reported in 2006 that resistance gene for BPH population in IR64 had broken down due to BPH population changed to biotype 4. Actually in the 1991 also released IR74 (Bph3), but didn't distribute to large rice areas, because the taste under IR64.

The outbreak rhythm of BPH in Indonesia occurred every 12 years, there are in 1986, 1998, and 2010 that had destroyed rice plantation about 61,255; 115,484 and 137,768 ha respectively. Although the BPH had the rhythm of outbreak, but the little damage throughout the years always happen [5]. BPH control with neonicotinoids (acetamiprid, dinotefuran, imidacloprid, and thiamethoxam), phenylpyrazole (fipronil), and BPMC have been widely used in Indonesia and also in many other countries.

Imidacloprid (IMI) has been an important tool for controlling BPH in China from the early 1990's to 2005 [6] and was a key insecticide in suppressing the BPH population [7]. Farmers applied IMI every season to prevent BPH outbreak in many rice growing areas, therefore the other insecticides with different modes of action were seldom used because of their relatively lower efficacy against BPH. Unfortunately, high resistance development to IMI in BPH may be closely associated with the long application history of the chemical. In 2003, however, the development of insecticide resistance against neonicotinoids (mainly IMI) in BPH was first observed in Thailand and then has been found in other neighboring countries such as Vietnam, India and China [8].

Resistance levels BPH populations in Nanning (Guangxi), Haiyan (Zhejiang), and Nanjing and Tongzhou (Jiangsu) field move from 200 in 2005 to 799-fold in 2007, but in laboratory population, the resistance ratio had increased from 200 to 1,298-fold after 23 generations [9]. In the other hand BPH of field populations had developed moderate to high level of resistance to IMI enhanced in the same growing season if the insecticide was sprayed over and over [10].

To avoid these problems, alternative MOA insecticides must be developed for use against BPH to better manage the development of resistance to the old ones. For Indonesia, information about the BPH resistance to new mesoionics insecticide of triflumezopyrim (TFM) and neonicotinoids are very little, almost no data for it. TFM is a new hopper insecticide with low impact on non-target organisms including pollinators [11]. The Entomological Society of Indonesia Bandung Branch and DuPont Crop Protection cooperated for a research to determined insecticide susceptibility of BPH that was collected from West and Central Java. The objective of the study to determine BPH baseline susceptibility to new insecticide that will help to monitor the future susceptibility shifts of Indonesian BPH population for insecticide resistance management programs.

MATERIALS AND METHODS

BPH Collection

The BPH were collected from 2 provinces comprised of 6 districts from West Java in Karawang, Subang, and Indramayu and from Central Java in Pemalang, Klaten, and Pati. Insects were collected during the rice season when they were most prevalent. GPS coordinates of locations of BPH collection including altitude, time of collection, and rice variety was recorded. The BPH standard comparison was Biotype 3 that was maintained on IR42 since 1994 in the Laboratory of Indonesian Center for Rice Research (ICRR), West Java, Indonesia**.**

About 50 healthy adults BPH brachypterous (short-winged) females or about 100 nymphs are collected from each site. BPH are collected from the field using a sweep net, and transferred immediately to plastic container of 2.5-5.0 liter capacity with rice seedlings. A circular window covered by nylon mesh was installed in the plastic container.

Rearing BPH

In the screen house, BPH from all sites and different rice varieties were reared separately on the Pelita I/1susceptible rice variety before using for treatments. The adult males and females BPH at 1:1 ratio from plastic container were transferred into the oviposition (egg-laying) of circular mylar cages with 30 days old rice plant.

After a period of 1 month of rearing one generation, adult BPH were removed from the oviposition cage and were transferred to other 5 circular mylar cages with 30 day old rice plants. Each cage was infested by 20 pairs of BPH and 3 days after infestation all of adult hoppers were removed. The eggs that were laid in rice in the plastic container were reared up to adult BPH brachypterous females emerging. The adult BPH brachypterous from generation one or more were used for bioassays.

Topical application

A stock solution of TFM and IMI using pure acetone (reagent grade) was made, 100 and 1000 ppm respectively. Tests Solutions were carried out using 6 concentrations for TFM: 10, 2, 0.4, 0.08, 0.016 ppm, and 0 (untreated) and for IMI: 500, 100, 20, 4, 0.8 ppm, and 0 (untreated).

The 1-2 days-old BPH brachypterous females were collected from the culture cages using an aspirator. They were confined into a vial with a wire-mesh screen. Ten (10) insects were collected per vial and anaesthetized with carbon dioxide (CO_2) for 10-15 seconds to facilitate handling during treatment. The anaesthetized insects were transferred on a watch glass wrapped with gauze secured by a rubber band. Insecticide was applied topically on the dorsal surface of the thorax with a hand Hamilton Repeating Dispenser microapplicator plus a 10-µL microsyringe.

The 0.0002 mL (0.2 µL) of each solution was applied to the dorsal thorax of each of 10 adult females. Treated insects were placed into a vial testing unit containing rice seedlings so they can recover and feed. Three vials of insects was used for each treatment (30 females/treatment). For the control treatments, 0.0002 mL (0.2 µL) of pure acetone was applied to the dorsal thorax of each of 10 adult females previously anaesthetized with carbon dioxide. The control treatment used 3 vials of insects (30 adult females). The vial testing units with treated insects were placed in a controlled room with temperature of 24 ^{o}C and 12 hours of light. Mortality was determined on 48 h after treatment for all insecticides.

Another set was established to determined the average body weight for 30 insects of the overall tested populations. Baehaki *et al*., [5] give a guide that the following data was collected before and after treatment of each bioassay: (a) weight of 30 insects from each batch of insects used for testing - this information will be used to calculate the dose delivered; (b) the dose in ppm concentration of the insecticide must be convert to dose (ng/g); (c) the total number of insects treated in each replication; (d) total number of insects treated in all replications; (e) total number of dead insects observed in 48 h after application of treatments and all replication; (f) the number of insects dead in each treatment (chemical + concentration); and (g) % mortality = (total number of dead insects/ total number of insect treated) x 100%.

Statistical analysis

Lethal dose LD_{50}, fiducial limits (lower and upper), 95% confidence interval, intercept, slope, natural response, heterogeneity, and the g-value of each origin of BPH to insecticide will be obtained from probits analysis by Polo-PC Program developed by LeOra software probits method [12]. Control mortality was corrected by using Abbott's formula [13] for each probits analysis. In probits analysis, the ppm was converted to dose (ng)/body weight (g) namely Dose (ng/g) = [(Dose (ppm)*amount applied (µL)/1,000)/weight of insect (g)]*1,000 [14]. The mortality in probits was taken from transformation table of percentages mortality to probits [15]. The resistance ratio (RR) is calculated at the LD_{50} level as follows:

$$RR_{calculate} = \frac{LD_{50} \text{ of BPH field population}}{LD_{50} \text{ of BPH biotype 3 as susceptibility laboratory population}}$$

$RR_{calculate}$ compared to standardization RR were described by WHO (1980) in Lai *et al.* [16] as follows: susceptible ($RR_{standard}$ = 1), decreased susceptibility ($RR_{standard}$ = 3-5), low resistance ($RR_{standard}$ = 5-10), moderately resistance ($RR_{standard}$ = 10-40), highly resistance ($RR_{standard}$ = 40-160), and very highly resistance ($RR_{standard}$ > 160). In the other hand the value $RR_{standard}$ <1 can be described as a highly susceptibility and the value 1> $RR_{standard}$ <3 as unstable susceptibility of insects [5]. The unstable susceptibility of insects can be divided to $RR_{standard}$ = > 1- 2 as tend susceptible and value 2 > $RR_{standard}$ <3 as tend decreased susceptibility category

The rated RR insecticides indicate how many times the dose of insecticide used to kill 50% of BPH resistant. In the other hand, the resistance level less than 4 show the use of insecticides is still effective, but if the resistance level more than 4 disadvantageous because too much insecticide should be given to the crop [17].

Regression equation probit mortality (Y) as dependent variable and the log concentration TFM or IMI (X) as independent variable is expressed as Y = a_1+b_1 log X, where a_1 as intercept and b_1 as a slope. The accurately of regression equation is calculated with t-ratio (slope/SE) must more than 1.96.

RESULTS AND DISCUSSIONS

Information of origin BPH

The BPH was collected from the BPH outbreak area of West Java (Karawang, Subang, Indramayu) and Central Java (Pemalang, Klaten and

Pati) (Figure 1). In the other hand BPH Biotype 3 from screen house Sukamandi Research Station. The village, sub district, and district of all sites along of North Coastal of West Java and Central Java, except Klaten district is in middle area of Central Java. The latitude, longitude and altitude of each location in Table 1.

BPH population were gotten from IR42, Ciherang, Mekongga, Inpari 16, because those varieties were damaged by BPH, especially on the three early mentioned that released at years of 1980, 2000, and 2004 respectively showed heavy damaged.

Fig-1: Location map of Brown planthopper origin (blue circle) of West and Central Java. Indonesia

Table-1: Location characters of origin brown planthopper samples of West and Central Java

Province	District	Village and sub District	Latitude/longitude/Altitude	Rice variety and time collected
West Java	Karawang	Tempuran-Purnajaya	06.20976° S, 107.47210° E, 16 m Alt	Ciherang. September, 2014
	Subang	Sukamandi Jaya-Ciasem	06.35238° S, 107.65063° E, 44 m Alt	Inpari 16, August, 2014
	Indramayu	Kandang Haur-Karang Sinom	06.39048° S, 108.09794° E, 16 m Alt	IR42. September 2014
Central Java	Pemalang	Kebon Dalam-Pemalang	06.92027° S, 109.39662° E, 20 m Alt	Ciherang, September, 2014
	Klaten	Ngaran-Polan Hardjo	07.62423° S, 110.65615° E, 193 m Alt	Mekongga, November, 2014
	Pati	Kalidoro-Pati	06.74993° S, 111.04795 ° E, 15 m Alt	Ciherang, January, 2015

Bioassay BPH to TFM

All probits analysis BPH resistance to TFM provides a natural response that naturally mortality in control smaller than 0.2 (= 20%). This shows that calculation of LD_{50} can be accepted as the basis for calculation of insecticides resistance. IRAC (2009) [18] reported to discard data if control shows mortality reading above 20%.

Responce BPH from Subang was highly susceptible category to TFM with LD_{50} was 0.103 ng/g body weight and resistance ratio (RR) was 0.15-fold, it mean the $RR_{calculate}$ LD_{50} BPH field/LD_{50} BPH laboratory <1 include to highly susceptible category. Reaction BPH from Subang to TFM was low LD_{50} significant compared BPH from Indramayu, Klaten, and Pati, and highly significant to BPH from Karawang (Table 2). LD_{50} of TFM to BPH from Indramayu, Klaten, and Pati were 1.46, 1.840, and 1.664 ng/g respectively. The $RR_{calculate}$ BPH from Indramayu, Klaten, and Pati were 2.15, 2.70, and 2.44-fold near to $RR_{standard}$=3-5, it mean the BPH from Indramayu, Klaten, and Pati tend to decreased susceptibility to TFM.

BPH from Pemalang was decreased susceptiblility category to TFM with LD_{50} was 2.687 ng/g body weight with $RR_{calculate}$ was 3.95-fold. BPH from Karawang to TFM with LD_{50} was 3.730 ng/g and it $RR_{calculate}$ was 5.48 fold, it mean $RR_{calculate}$ in the range $RR_{standard}$ = 5-10 include to category low resistance.

Table-2: The LD_{50} values (ng/g) of brown planthopper population to triflumezopyrim (TFM)

Origin of BPH Population	LD_{50} (ng/g)	Fiducial Limit (lower to upper) (ng/g)	Intercept	Slope ± SE	t-ratio	Natural response	Htg	g-value	$RR_{cal.}$
Biotipe 3	0.681	0.028 -2.594	0.1016	0.608 ± 0.154	3.948	0.134± 0.062	0.03	0.247	1.00
Karawang	3.730	0.973 -8.515	-0.5261	0.952± 0.191	4.984	0.141± 0.066	0.42	0.155	5.48
Subang	0.103	0.000 -0.706	0.6204	0.629 ± 0.208	3.024	0.167± 0.068	0.20	0.418	0.15
Indramayu	1.464	0.155 -4.428	-0.1083	0.690± 0.160	4.313	0.137± 0.064	0.68	0.208	2.15
Pemalang	2.687	0.067 - 11.990	-0.1780	0.412 ± 0.123	3.350	0.099± 0.054	0.15	0.341	3.95
Klaten	1.840	0.054 - 7.769	-0.1201	0.448 ± 0.125	3.584	0.099± 0.054	0.20	0.301	2.70
Pati	1.664	0.326 - 4.143	-0.1722	0.800± 0.167	7.766	0.103± 0.056	0.53	0.168	2.44

Remarks:The t-ratio of slope/SE >1.96 that show significantly of regression. Htg= Heterogeneity was <1, this indicate that data bioassay was fit for model probit regression to BPH. The value LD_{50} and confidence interval limit of 95% was acceptable as effective dose if g < 0.4. Natural response/ mortality acceptable if less than 0.2 (<20%), $RR_{cal.}=RR_{calculate}$.

In case the t-ratio which were calculated from the slope/SE was greater than 1.96, show that the regression was significant, this indicate the insecticide treatment has effect to BPH. The regressions equation between probit mortality (Y) of the BPH biotype 3, BPH origin of Karawang, Subang, Indramayu, Pemalang, Pati and Klaten with log concentration TFM (X), show that the regression was significant and this indicate the treatments has effect to BPH from all sites.

In the other hand when viewed from a heterogeneity shows that the regression equation between probits mortality (Y) of BPH biotype 3, BPH origin of Karawang, Subang, Indramayu, Pemalang, Pati and Klaten with log concentration TFM (X) give heterogeneity less than 1, this indicate that the bioassay data was fit for probits regression models to BPH from all sites (Table 2).

Another vital aspect of quantal data analysis there were tested the hypotheses of BPH Biotype 3 from laboratory and all site of West Java and Central Java to TFM in the sameness of the slopes and intercepts of the each regression lines were rejected. This indicates the lines were significantly different (Figure 2).

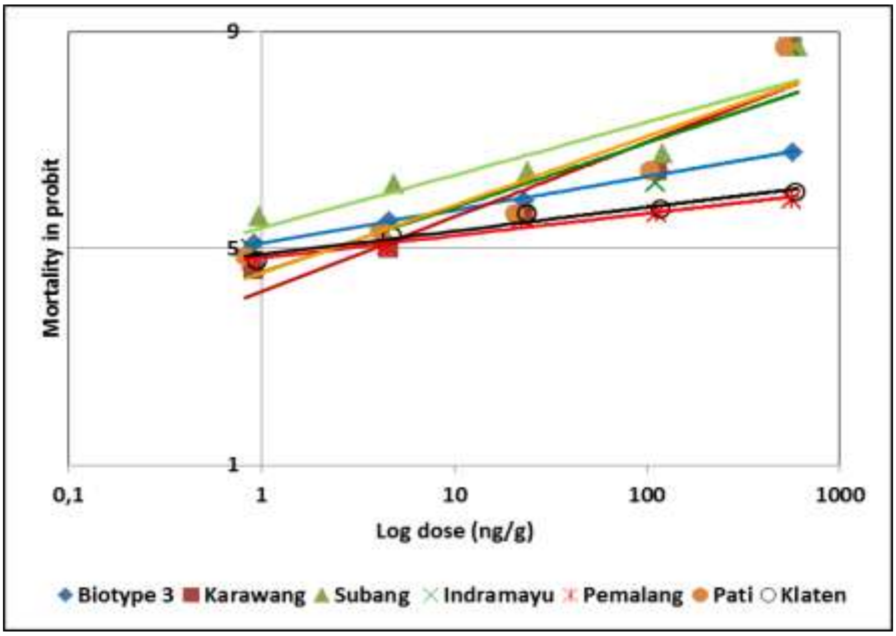

Fig-2: Relationship between log dose (ng/g) triflumezopyrim and mortality probits of BPH

Bioassay of BPH to IMI

All probits analysis BPH resistance to IMI provides a natural response that naturally mortality in control smaller than 0.2 (= 20%). This showed that calculation of LD_{50} of IMI to BPH can be accepted as the basis for calculation of insecticides resistance.

The lowest LD_{50} of IMI to BPH from Indramayu was 91.450 ng/g with the $RR_{calculate}$ compared to Biotype 3 standard was 0.83-fold, followed by BPH from Pemalang with LD_{50} was 101.346 ng/g and $RR_{calculate}$ was 0.92-fold included to BPH highly susceptibility to IMI. LD_{50} of IMI to BPH from Karawang, Subang, Klaten, and Pati were 195.638, 149.293, 162.994, and 313.243 ng/g respectively. The $RR_{calculate}$ of BPH from Karawang, Subang, and Klaten were 1.77, 1.35, and 1.48 respectively near to $RR_{standar}$ = 1, it mean the BPH from Karawang, Subang, and Klaten tend to susceptible category. The RR $_{calculate}$ of BPH from Pati was 2.84-fold near to $RR_{standar}$ =3-5, it mean the BPH from Pati tend to decreased susceptibility category (Table 3).

Table-3: The LD_{50} values (ng/g) of brown planthopper population to imidacloprid (IMI)

Origin of BPH Population	LD_{50} (ng/g)	Fiducial Limit (lower to upper) (ng/g)	Intercept	Slope ± SE	t-ratio	Natural response	Htg	g-value	RR $_{cal.}$
Biotipe 3	110.277	10.726 -355.907	-1.1544	0.565 ± 0.135	4.185	0.100± 0.055	0.06	0.218	1.00
Karawang	195.638	28.283 -576.608	-1.3797	0.606 ± 0.139	4.359	0.103± 0.057	0.79	0.202	1.77
Subang	149.293	6.589 - 616.477	-1.1460	0.529 ± 0.143	3.699	0.198± 0.072	0.78	0.285	1.35
Indramayu	91.450	0.123 - 572.803	-0.7032	0.360± 0.127	2.835	0.134± 0.063	0.18	0.479	0.83
Pemalang	101.346	2.453 - 438.820	-0.8797	0.440± 0.127	3.465	0.101± 0.055	0.21	0.322	0.92
Klaten	162.994	7.602 -645.805	-1.0793	0.492 ± 0.136	3.618	0.137± 0.064	0.59	0.294	1.48
Pati	313.243	48.094 - 974.951	-1.3733	0.549± 0.130	5.545	0.099± 0.054	0.04	0.215	2.84

Remarks: The t-ratio of slope/SE >1.96 that show significantly of regression. Htg= Heterogeneity was <1, this indicate that data bioassay was fit for model probit regression to BPH. The value LD_{50} and confidence interval limit of 95% was acceptable as effective dose if g < 0.4. Natural response/ mortality acceptable if less than 0.2 (<20%), RR $_{cal.}$=$RR_{calculate}$.

In case the t-ratio which is calculated from the slope / SE is greater than 1.96, show that the regression was significant, so this indicate the insecticide treatment has effect to BPH. Therefore, the regressions equation between probits mortality (Y) of the BPH biotype 3, BPH origin of Karawang, Subang, Indramayu, Pemalang, Pati and Klaten with log concentration IMI (X), show that the regression was significant and this indicate the treatments has effect to BPH from all sites.

In the other hand when viewed from a heterogeneity shows that the regression equation between probit mortality (Y) of BPH biotype 3, BPH origin of Karawang, Subang, Indramayu, Pemalang, Pati and Klaten with log concentration IMI (X) give heterogeneity less than 1, this indicate that the bioassay data was fit for probits regression models to BPH from all sites (Table 3).

Another vital aspect of quantal data analysis there were tested the hypotheses of BPH Biotype 3 from laboratory and all site of West Java and Central Java to IMI in the sameness of the slopes and intercepts of the each regression lines were rejected. This indicates the lines were significantly different (Figure 3).

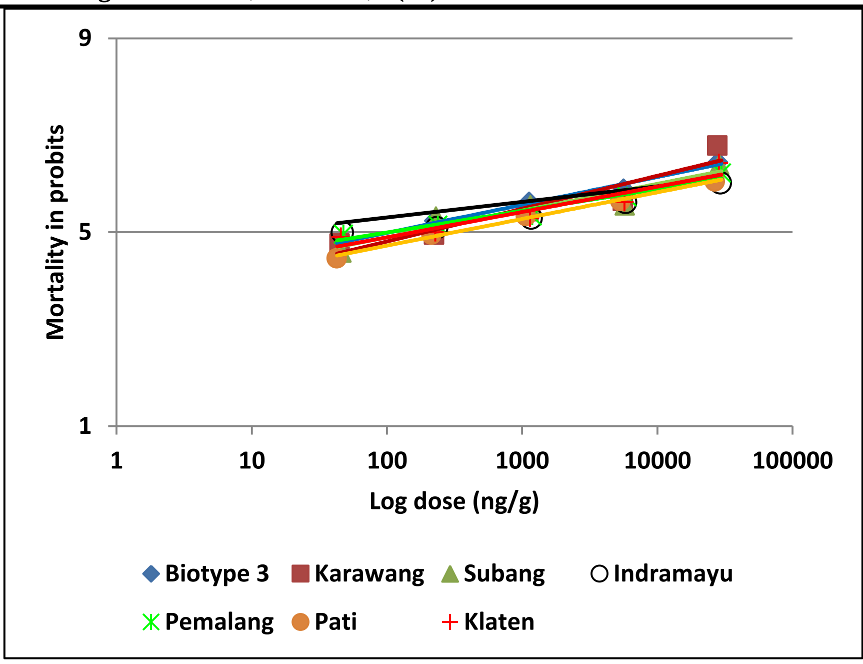

Fig-3: Relationship between log dose (ng/g) imidacloprid and mortality probits of BPH

All data bioassay of BPH susceptibility to IMI almost look like with data Baehaki *et al*. [5] that reported LD_{50} of IMI to BPH from East Java (Ngawi, Lamongan, and Banyuwangi) was 96.608-216.00 ng/g body weight with RR was 0.88-1.96-fold. Matsumura *et al*. (2008) [19] reported the LD_{50} values of IMI for the BPH populations collected from some location in Japan were 4.3-16.4 µg/g (=4,300-16,400 ng/g), China (Rongcheng, Fuqing, and Fujian) was 11.0 µg/g (= 11,000 ng/g), Taiwan were 6.9-12.8 µg/g (=6,900-12,800 ng/g) and Vietnam were 5.9-24.2 µg/g (=5,900-24,200 ng/g), and from the Philippines were 0.18–0.35 µg/g (= 180-350 ng/g). This fact showed that BPH in Japan, China, Vietnam, and Taiwan had very high resistance to IMI than BPH in Indonesia (91.45-313.243 ng/g). In the other hand BPH in the Philippines almost similar reaction with the Indonesian BPH with LD_{50} very close. LD_{50} of IMI to BPH in Java-Indonesia was the lowest (91.450-313.243 ng/g) and did not resistant, this is due to the used of IMI in Indonesia didn't fully replaced the other insecticides.

In India the BPH from Soraba and Mandya populations were more susceptible to insecticides compared to Gangavati and Kathalagere populations, especially the RR to IMI varied were 0.53- 13.50-fold [20]. IMI is a good insecticide, because although BPH in China had high resistance to IMI, but the RR to IMI sharply declined with the succession of rearing generations without insecticides from 359.94-fold at F_1 to 6.50-fold at F_{14} compared with the susceptible strain [21]. In the other hand if the BPH maintenance in IMI had been able rapidly increase to high resistance. The resistance ratio of IMI decreased faster at F_4 to F_8, and it became stable at F_{10} to F_{14}. LD_{50} in F_1 was 11.158 µg/g and LD_{50} in F_{14} was 0.202 µg/g. It is clearly that incidence of insecticide resistance due to the frequent use of certain insecticides. Wang *et al*. [22] reported that the BPH had been able to develop 1424-fold resistance to IMI in the laboratory after the insect was selected with IMI for 26 generations. In the field populations had developed variable resistance levels to neonicotinoids, with a high resistance level to IMI (RR: 135.3–301.3-fold), a medium resistance level to imidaclothiz (RR: 35–41.2-fold), a low resistance level to thiamethoxam (up to 9.9-fold) and no resistance to dinotefuran, nitenpyram and thiacloprid (RR < 3-fold). Further examinations indicated that a field population had developed medium resistance level to fipronil (up to 10.5-fold), and some field populations had evolved a low resistance level to buprofezin.

TFM and IMI has been classified to mode of actions (MoA) of main group 4 and primary site of action nicotinic acetylcholine receptor (nAChR) competitive modulators. The insecticide action is bind to the acetylcholine site on nAChRs, causing a range of symptoms from hyper-excitation to lethargy and paralysis. Acetylcholine is the major excitatory neurotransmitter in the insect central nervous system [1]. In the chemical sub-group, IMI include to 4A as neonicotinoids together with acetamiprid, clothianidin, dinotefuran, nitenpyram, thiacloprid, and thiamethoxam. The sub-group 4B as nicotine (nicotine), 4C as sulfoximines (sulfoxaflor) and 4D butenolides (flupyradifurone). In the news of sub-group was 4E as mesoionic insecticide TFM. The Mesoionic insecticides TFM act via inhibition of the orthosteric binding site of the nAChR despite previous beliefs that such action would translate to poor insect control [23]. Further more Cordova *et al*.(2016) [23] describe the physiological and biochemical action of TFM and related mesoionic insecticides on native and expressed nAChRs. TFM competes for the same binding site as imidacloprid. However, voltage-clamp studies conducted with neurons from the American cockroach, *P. americana*, and *Xenopus oocytes* expressing the Drosophila-chicken chimeric nAChR, Da2/cb2, show that, unlike neonicotinoid, TFM inhibits rather than activates nAChRs. This unique class of mesoionic chemistry targets the nAChR, inducing a physiological action which is distinct from that of neonicotinoids [11]. Singh *et al.* [24] reported that DuPont Crop Protection is commercializing Triflumezopyrim (Pyraxalt™), a fast-acting, xylem-mobile compound with a unique mode of action that provides excellent control of Nilaparvata and Sogatella pest species while still sustaining the rice ecology. Pyraxalt™ will be a powerful tool for rice growers since it will help reduce the total number of sprays to control hoppers, keep virus transmission in check, and contribute to maximizing rice yield potential.

The TFM and IMI did not cross-resistance, although these compounds are believed to have the same target site, current evidence indicates that the risk of metabolic cross-resistance between subgroups is low [1]. Imidacloprid resistant hoppers did not show cross resistance to all the neonicotinoid insecticides and high level of imidacloprid resistance in BPH was very unstable [10]. In the other hand the resistant strain selected with imidacloprid showed substantial cross-resistance to imidaclothiz, thiacloprid, and acetamiprid, and slight levels of cross-resistance to dinotefuran and thiamethoxam, but no obvious cross-resistance to nitenpyram, buprofezin, and fipronil [9]. This difference is depends on the location of BPH and the history of insecticides use in the area.

In Indonesia the TFM insecticides show that can be used to solve the problem of BPH that had resistance against other insecticides and the other hand released TFM as a complement to the diversity (mosaic) of insecticide in the field. In the fact that LD_{50} of IMI on a accurately bioassay using topical method did not show the decreased sensitivity of BPH with RR <3 [5], therefore the distribution of TMF to the field is a sequential released method of insecticides as vigilance in order the insecticide in the field did not driven into high resistance. The objective of sequential release method first as releasing new insecticide before the old one goes high resistant and second to provide some alternative insecticide the field.

Sequential released method of insecticides in IPM strategies must be attention to pests resistance due to frequent contact with insecticides, and also must be attention to the properties of bio-ecological and pests behavior that will accelerate resistance. Characters of pests that will accelerate insecticide resistance among others had growth rate in the r-strategic pests with exponential curve, quickly multiply, short of life cycles and be able to use food sources well before other insects competed, the pattern of development pest follow biological clock, long dispersal and quickly distribution to wider area to find a new habitat before the old habitat unuseful, be able to weaken the work of insecticides that are considered potent before, be able to break down the resistance rice varieties and changes to new biotypes. Rice pests that have the ability of the above properties are rice planthoppers, especially BPH.

The relative potency toxicity values of TFM compares to IMI of BPH from Karawang, Subang, Indramayu, Pemalang, Klaten, Pati were 0.32, 9.0, 0.39, 0.23, 0.55, and 1.16 folded respectively. TFM is a good toxicity to BPH in Subang and Pati, but to BPH in Karawang, Indramayu, Pemalang, Klaten the TFM low toxicity compared to IMI. In the further TFM insecticide can be used to solve the problem resistance of BPH against other insecticides

CONCLUSIONS

All probits analysis BPH resistance to TFM and IMI provides a natural response that naturally mortality smaller than 0.2 (= 20%). This showed that calculation of LD_{50} of TFM and IMI to BPH can be accepted as the basis for calculation of insecticides resistance. In case the t-ratio was greater than 1.96, show that the insecticide can be used to solve the BPH problem that was resistance against to the other insecticides.

TFM as a new insecticide showed a low LD_{50} to BPH, therefore in regression was significant, heterogeneity less than 1, this indicate that bioassay data was fit for model probit regression to BPH treatment. The null hypothesis of equality tests the sameness of the slopes and intercepts of the regression lines were rejected for BPH from all sites, this indicate the regression lines were significantly.

Responce BPH from Subang was highly susceptible category to TFM with LD_{50} was 0.103 ng/g body weight and resistance ratio ($RR_{calculate}$) was 0.15-fold, it mean the $RR_{calculate}$ LD_{50} BPH field/LD_{50} BPH laboratory <1 include to highly susceptible category. Response BPH from Indramayu, Klaten, and Pati to TFM resulted the LD_{50} between 1.46-1.840 ng/g body weight with $RR_{calculate}$ were 2.15-2.70-fold tend to decreased susceptibility category. BPH from Pemalang was decreased susceptiblility category to TFM with LD_{50} was 2.687 ng/g body weight and $RR_{calculate}$ was 3.95-fold. BPH from Karawang was low resistance category to TFM with LD_{50} was 3.730 ng/g and $RR_{calculate}$ = 5.48 fold.

BPH from Indramayu and Pemalang were highly susceptible to IMI with the LD_{50} were 91.450 and 101.346 ng/g body weight, whereas the $RR_{calculate}$ = 0.83 and 0.92-fold respectively. BPH from Karawang, Subang, and Klaten tend to susceptible category to IMI with LD_{50} were 149.293-195.638 ng/g body weight and $RR_{calculate}$ were 1.35-1.77-fold. BPH from Pati tend to decreased susceptibility to IMI with LD_{50} was 313.243 ng/g body weight and $RR_{calculate}$ was 2.84-fold.

The IMI is still effective to BPH from all sites and TFM provide a low LD_{50} and this insecticide can be used as complementary insecticide in the field and to solve the problem BPH that had been resistance to other insecticides by a sequential release method in IPM strategies.

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