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Resistance of whitebacked planthopper, *Sogatella furcifera* (Horvarth) against mesoionic insecticide triflumezopyrim using topical application method

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Abstract
The Whitebacked planthopper (WBPH), *Sogatella furcifera* (Horvarth) originally was k-strategic insect and well-developed into a low r-strategic that caused damage to rice crops. Related to that matter, WBPH control by insecticides sometimes become ineffective on the recommend dose, such situation is suspected that pest had been resistant to insecticides. To response this issues, was carried out the research in the 2014 to evaluate WBPH from West Java province (Karawang, Subang, and Indramayu) of Indonesia against triflumezopyrim, imidacloprid, and cyazypyr. The research used topical application method with probits analysis by POLO-PC. The results showed that the response WBPH from Karawang to imidacloprid tend to susceptible category with LD_{50} was 128.158 ng/g and $RR_{calculate}$ = 1.25. Response WBPH from Subang and Indramayu to imidacloprid was highly susceptible with LD_{50} were 78.446 and 76.193 ng/g and $RR_{calculate}$ were 0.77 and 0.74-fold respectively. Response WBPH from Karawang, Subang and Indramayu to triflumezopyrim tend to decreased susceptibility category with LD_{50} were 2.311, 2.152, 1.791 ng/g and $RR_{calculate}$ were 2.94, 2.74, 2.28-fold respectively. Response WBPH from Karawang, Subang and Indramayu to cyazypyr tend to decreased susceptibility category with LD_{50} were 236.149, 194.808, 207.606 ng/g and $RR_{calculate}$ were 2.94, 2.42, 2.58-fold respectively. Triflumezopyrim was fantastic insecticide to provide a low LD_{50} and didn't induce resistance to WBPH almost of all sites. This insecticides can be used to solve the problem resistance of WBPH against to the other insecticides.

Keywords: lethal dose, insecticides resistance, Whitebacked planthopper, rice

1. Introduction
The whitebacked planthopper (WBPH), *Sogatella furcifera* (Horvarth) (Hemiptera: Delphacidae) as a second destroyer hopper on rice after brown planthopper (BPH), *Nilaparvata lugens* (Stål) focused in East Asia, Southeast Asia, China, and Indochina. w

In the field at the normal environmental conditions the WBPH come early at the rice tillering stage compared to BPH which came later around premordia phase. Baehaki and Rifki, (2002) [1] reported that both hoppers development occurred competition that winning by BPH as a strong competitor. Development of BPH and WBPH in certain comparison on IR64 resistant rice variety of pairs adult BPH: WBPH = 1:1, 2:1, 4:1, and 2:2-pairs had been won by BPH as the absolute competitor. On the Muncul susceptible rice variety the absolute competitor was BPH only at the comparison BPH: WBPH = 4:1-pairs adult. In the IR64 variety, WBPH has absolute competitor only at the comparison BPH: WBPH = 1:4-pairs, but in Muncul variety the WBPH didn't out the absolute winner at all comparison, although the composition of BPH: WBPH = 1:4 pairs. The mutually competition of BPH and WBPH on IR64 at comparison 1:2 and 4:4-pairs, and on Muncul the mutually competition of BPH and WBP at 1:1, 1:2, 1:4, 2:1, and 2:2-pairs [1].

WBPH outbreak in China, Vietnam, and Thailand are driven by the expansion of hybrid rice crop areas, and also in Indonesia the WBPH outbreak emergent on hybrid rice. Baehaki and Mejaya (2012) [2] reported that WBPH outbreak on hybrid rice plantation and the composition of WBPH was higher compare BPH, whereas the WBPH population the winner competition than BPH. In the other hand BPH population at the center outbreak of hybrid was the lowest and increasing according with increasing distance from center of the outbreak [2].

WBPH development in Indonesia has reached the level of pests that live throughout the rice season and reach up to 3-4 generations per each season. On the La Nina of dry Season 1998, the population WBPH on Muncul rice variety was 5,841 WBPH/30 hills, while abundance of BPH population was 17,140 BPH/30 hills. In the 2009 the WBPH attack hybrid rice SL-8 covering 500 ha at the seed center Perum Sang Hyang Seri in Sukamandi-Subang and attack hybrid rice Intani-2 in Kuningan district of West Java covering of 5 hectares. Outbreak WBPH in China was 5.1 million ha in 2002, with 8.5 million ha being the greatest observed damage in 2006. In 2007 about 1.5 million ha of damage covered only one province. In Malaysia, the lowest area damage was 541 ha in 2001, with 1,256 ha being the largest area damage in 1999, there were no available data from 2003 to 2007. Thailand provided only limited data on the damage caused by WBPH

(14,905 ha in 1999 and 1 hectare in 2001) [3].

Outbreaks of the WBPH damaged rice plants during their early stages, and the damage was aggravated by the pandemic of southern rice black-streaked dwarf virus (SRBSDV), which is transmitted by WBPH [4]. Thus the insect and the disease caused heavy yield losses of rice throughout China, northern Vietnam, and Japan [5, 6]. Suppression of the WBPH as a virus vector frequent by insecticides applications of the earlier stages of rice [7] was the only method to prevent virus disease epidemics, especially when a virus-resistant cultivar was lacking. The use of insecticide sprays have become the main method for controlling planthoppers in most regions of China [8].

Fewer research investigations have been conducted on insecticide resistance in WBPH than on BPH [9, 10]. Resistance 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 species. Cross-resistance occurs when resistance to one insecticide confers resistance to another insecticide, even where the insect has not been exposed to the latter product (http://www.irac-online.org/about/resistance/)

Insecticide selection pressure reportedly is the major force driving the evolution of resistance in [11, 12] and also insecticide as the main force driving to WBPH to the evolution population build up, as well Matsumura *et al*., (2008) [13] reported that almost all the WBPH populations from Japan, Taiwan, China, Vietnam and the Philippines had extremely large LD_{50} values (19.7–239 μg/g or more) for fipronil, except for several populations from the Philippines and China.

To overcome continuous outbreak of WBPH is required policies to limited the expansion of hybrid rice areas, providing horizontal resistant varieties and pattern of rice varieties distribution that designed base on local biotypes or malignancy of WBPH. The development of WBPH need to watch especially if the initial population is quite high in the crop is expected to occur explosion. Monitoring WBPH development is needed to determine the initial population in the crop is expected to occur explosion and determined base line to insecticides as a standard to measurement the level of resistance ratio.

2. Materials and Methods

2.1 Locations of WBPH Collection

The WBPH were collected from West Java of Karawang, Subang, and Indramayu districts. Insects was collected during the rice season when they are most prevalent in highest populations. Recorded coordinate of locations (longitude and latitude) by GPS, altitude above of sea level, time of collection, and rice variety. The WBPH standard from laboratory of Indonesian Center for Rice Research (ICRR), West Java, Indonesia that maintenance on IR26 rice variety since 2004.

About 50 healthy unparasitized adult macrpopterous (long-winged) females or about 100 nymphs are collected from each site. BPH are collected from the field using a sweep net and the fresh planthoppers are transferred immediately to tape ware of 2.5-5.0 liter in volume with rice seedlings. The tape ware with window circular and covered by nylon mesh.

2.2 Rearing methods

In the screen house the WBPH are reared in susceptible variety Pelita I/1. The adult males and females WBPH (at 1:1 ratio) from tape ware are transferred into the oviposition (egg-laying) of circular mylar cages with 30 days old rice plant.

After one month reared and finished one generation, adult hoppers are removed from the oviposition cage and was transferred to other 5 circular mylar cages with 30 days old rice plant. Each cage infested by 20 pairs of hopper. Three days after infestation, all of adult hopper removed and eggs that laid in rice stem were maintained up to adult WBPH macrpopterous females emergence. The adult WBPH macropterous from generation one or more were used for bioassays.

2.3 Topical application

Make a stock solution of triflumezopyrim, imidacloprid, and cyazypyr in pure acetone (reagent grade or better) as much 100, 1000 and 1000 ppm respectively. Tests solution were carried out on 6 concentrations of triflumezopyrim, namely 10, 2, 0.4, 0.08, 0.016, and 0 ppm, imidacloprid were 500, 100, 20, 4, 0.8 and 0 ppm, and cyazypyr with 250, 50, 10, 2, 0.4, and 0 ppm.

The WBPH macropterous females within 1-2 days-old, are collected from the culture cages using an aspirator. They are confined into a vial with a wire-mesh screen. Ten insects are collected per vial and anaesthetized with carbon dioxide (CO_2) for 10-15 seconds to facilitate handling during treatment. The anaesthetized insects are 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.

Apply 0.0002 mL (0.2 μL) of each solution to the dorsal thorax of each of 10 adult females. Placed the treated insects into a vial testing unit containing rice seedlings so they can recover and/or feed. Treat 3 vials of insects for each treatment (30 females/treatment). The control treatment apply 0.0002 mL (0.2 μL) of pure acetone to the dorsal thorax of each of 10 adult females previously anaesthetized with carbon dioxide. The control treatment also with 3 vials of insects (30 adult females). Mortality was determined on 48 h after treatment for all insecticides. The vial testing unit with treated insects are placed in a controlled room with temperature of 24ºC and 12 hours of light. In the another activity was determined the average body weight for 30 insects of the overall tested populations.

Baehaki *et al*., (2016) [14] give a guide that the following data must be collected before and after treatment in each bioassay: (a) the weight of 30 insects from each batch of insects used for testing. This weight 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 and moribund (considered as dead) in each treatment (chemical + concentration), and (g) % mortality = (total number of dead insects/ total number of insect treated) x100%.

2.4 Statistical analysis

The LD50-value, 95% confidence interval, and slope of regression line were calculated by the LeOra software [15]. Control mortality was corrected by using Abbott's formula [16] for each probits analysis. In probits analysis, convert the ppm to dose (ng)/body weight (g) namely Dose (ng/g) = [(Dose (ppm)*amount applied (µL)/1,000)/weight of insect (g)]*1,000[17]. The resistance ratio (RR) is calculated at the LD_{50} level as follows:

$$RR_{calculate} = \frac{\text{LD50 of WBPH field population}}{\text{LD50 of WBPH of laboratory population}}$$

$RR_{calculate}$ compared to standardization RR were described by WHO (1980) in RRs [18] as follows: susceptible ($RR_{standard}$ = 1), decreased susceptibility ($RR_{standard}$ = 3-5), low resistance ($RR_{standard}$ = 5-10), moderate resistance ($RR_{standard}$ = 10-40), high resistance ($RR_{standard}$ = 40-160), and very high resistance ($RR_{standard}$ > 160). In the other hand the value RRstandard <1 can be described as a highly susceptibility and the value 1> $RR_{standard}$ <3 as unstable susceptibility of insects [14]. 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 [19].

3. Results and Discussions

3.1 Bioassay of insecticides to WBPH susceptibility

Bioassay of insecticides to WBPH susceptibility was carried out to WBPH from West Java (Karawang, Subang, and Indramayu) and to the standard WBPH of laboratory. The WBPH populations susceptibility to insecticide was monitored by a standard topical application method. The LD_{50} value, 95% confidence interval and slope of regression line were calculated by Polo PC program. Control mortality was corrected by using Abbott's formula for each probit analysis. The result WBPH susceptibility from West Java as follows:

3.2 Susceptibility WBPH population from Laboratory to some insecticides

The values for the regression line of triflumezopyrim to WBPH - laboratory was 0.1036 (intercept), 0.136 (natural response), and 0.961 (slope) with their standard error (Table 1). In case the t-ratio of slope was 0.961/0.136 = 7.0661 > 1.96, that show regression was significant, so this indicate the treatment has effect to WBPH-laboratory. In the other hand heterogeneity was 0.47 <1, this indicate that data bioassay was fit for model probit regression to WBPH-laboratory.

Topical LD_{50} of triflumezopyrim to WBPH-laboratory was 0.786 ng/g (ng insecticide/g body weight) with 95% lower and upper fiducial limit were 0.082 and 2.058 ng/g. The value LD_{50} and confidence interval limit of 95% was acceptable as effective dose because g=0.247 less than 0.4.

The values for the regression line of imidacloprid to WBPH-laboratory was -1.2878 (intercept), 0.137 (natural response), and 0.645 (slope) with their standard error (Table 1). In case the t-ratio of slope was 0.645/0.137= 4.7080 > 1.96, that show regression was significant, so this indicate the treatment has effect to WBPH-laboratory. In the other hand heterogeneity was 0.78 <1, this indicate that data bioassay was fit for model probit regression to WBPH-laboratory.

Topical LD_{50} of imidacloprid to WBPH-laboratory was 102.442 ng/g with 95% lower and upper fiducial limit were 8.724 and 330.008 ng/g respectively. The value LD_{50} and confidence interval limit of 95% was acceptable as effective dose because g=0.221 less than 0.4.

Table 1: The LD_{50} values (ng/g) of WBPH-laboratory in Rice Research Institute, Sukamandi.

Insecticide	LD_{50} (ng/g)	Fiducial Limit 95% (ng/g)		Intercept	Slope	Natural response
		Lower	Upper			
Triflumezopyrim	0.786	0.082	2.058	0.1036	0.961±0.244	0.136±.063
Imidacloprid	102.442	8.724	330.008	-1.2878	0.645±0.155	0.137±.064
Cyazypyr	80.459	5.774	280.337	-1.0695	0.566±0.143	0.137±.064

The values for the regression line of cyazypyr to WBPH-laboratory, was -1.0695 (intercept), 0.137 (natural response), and 0.566 (slope) with their standard error (Table 1). In case the t-ratio of slope was 0.566/0.137 = 4.1313 > 1.96, that show regression was significant, so this indicate the treatment has effect to WBPH-laboratory. In the other hand heterogeneity was 0.76 <1, this indicate that data bioassay was fit for model probit regression to WBPH-laboratory.

Topical LD_{50} of cyazypyr to WBPH-laboratory was 80.459 ng/g with 95% lower and upper fiducial limit were 5.774 and 280.337 ng/g. The value LD_{50} and confidence interval limit of 95% was acceptable as effective dose because g=0.245 less than 0.4.

In the vital aspect of quantal data analysis there were tested the hypothesis of WBPH population from laboratory to triflumezopyrim, imidacloprid, and cyazypyr in the sameness of the slopes and intercepts of the each regression lines were rejected. This indicate the lines were significantly different (Fig 1).

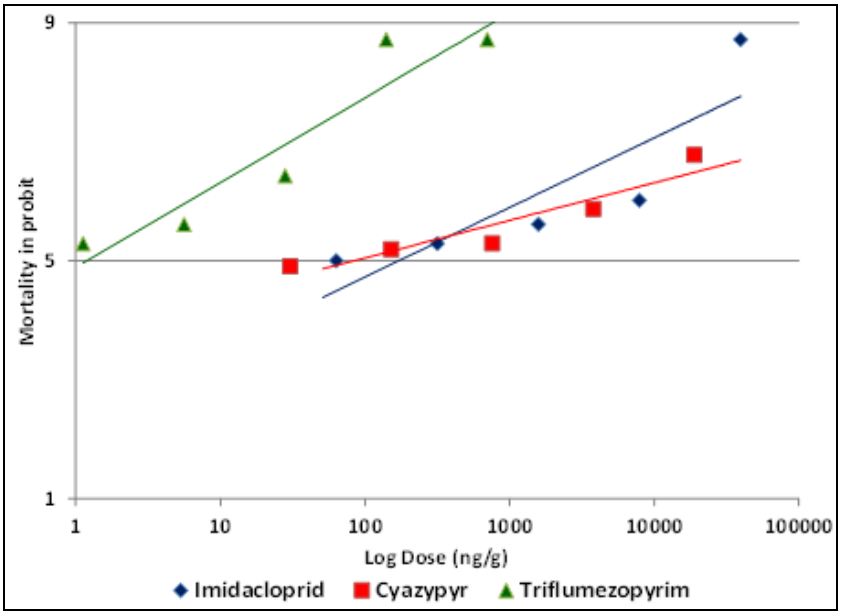

Fig 1: Relationship between log dose (ng/g) and mortality probit of WBPH of laboratory

3.3 Susceptibility WBPH population from Karawang-West Java to some insecticides

WBPH adult was collected from Ciherang variety in September 2014 from Tempuran-Purnajaya - Karawang-West Java (village-sub District-District-Province) with 06.20976° S Latitude, 107.47210° E Longitude, and Altitude was 16 m from sea level.

The values for the regression line of triflumezopyrim, was -0.2626 (intercept), 0.138 (natural response), and 0.743 (slope) with their standard error (Table 2). The value t-ratio of slope was 0.743/0.168 = 4.423 > 1.96, show that the regression was significant, so this indicate the treatment has effect to WBPH from Karawang. In the other hand heterogeneity was 0.72 <1, this indicate that data bioassay was fit for model probit regression to WBPH from Karawang.

Topical LD_{50} of triflumezopyrim to WBPH from Karawang was 2.311 ng/g with 95% lower and upper fiducial limit were 0.297 to 6.554 ng/g. The value LD_{50} and confidence interval limit of 95% is acceptable as effective dose because g=0.197 less than 0.4.

The values for the regression line of imidacloprid, was -1.4247 (intercept), 0.136 (natural response), and 0.680 (slope) with their standard error (Table 2). The value t-ratio of slope was 0.680/0.157 = 4.331 >1.96, show that the regression was significant, so this indicate the treatment has effect to WBPH from Karawang. In the other hand Heterogeneity was 0.71<1, this indicate that data bioassay was fit for model probit regression to WBPH from Karawang.

Topical LD_{50} of imidacloprid to WBPH from Karawang was 128.158 ng/g with 95% lower and upper fiducial limit were 14.576 to 384.387 ng/g. The value LD_{50} and confidence interval limit of 95% were acceptable as effective dose because g=0.204 less than 0.4.

The values for the regression line of cyazypyr, was -1.4550 (intercept), 0.140 (natural response), and 0.623 (slope) with their standard error (Table 2). The value t-ratio of slope was 0.623/0.149 = 4.181 >1.96, show that the regression was significant, so this indicate the treatment has effect to WBPH from Karawang. In the other hand heterogeneity was 0.94<1, this indicate that data bioassay was fit for model probit regression to WBPH from Karawang.

Topical LD_{50} of cyazypyr to WBPH from Karawang was 236.149 ng/g with 95% lower and upper fiducial limit were 14.576 to 732.148 ng/g. The value LD_{50} and confidence interval limit of 95% is acceptable as effective dose because g=0.220 less than 0.4.

Table 2: The LD_{50} values (ng/g) of WBPH population collected from Karawang - West Java

Insecticide	LD_{50} (ng/g)	Fiducial Limit 95% (ng/g)		Intercept	Slope	Natural response	Hetero-geneity	g-value	RR $_{cal.}$
		Lower	Upper						
Triflumezopyrim	2.311	0.297	6.554	-0.2626	0.743±0.168	0.138±0.064	0.72	0.197	2.94
Imidacloprid	128.158	14.576	384.387	-1.4247	0.680±0.157	0.136±0.064	0.71	0.204	1.25
Cyazypyr	236.149	30.827	732.148	-1.4550	0.623±0.149	0.140±0.066	0.94	0.220	2.94

*Remarks: RR = Resistance ratio., RR $_{cal.}$=RR $_{calculate}$, LD_{50} of WBPH laboratory population to triflumezopyrim = 0.786 ng/g, imidacloprid = 102.442 ng/g, and cyazypyr = 80.459 ng/g.

Another vital aspect of quantal data analysis there were tested the hypothesis of WBPH from Karawang to triflumezopyrim, imidacloprid, and cyazypyr in the sameness of the slopes and intercepts of the each regression lines were rejected. This indicate the lines were significantly different (Fig 2).

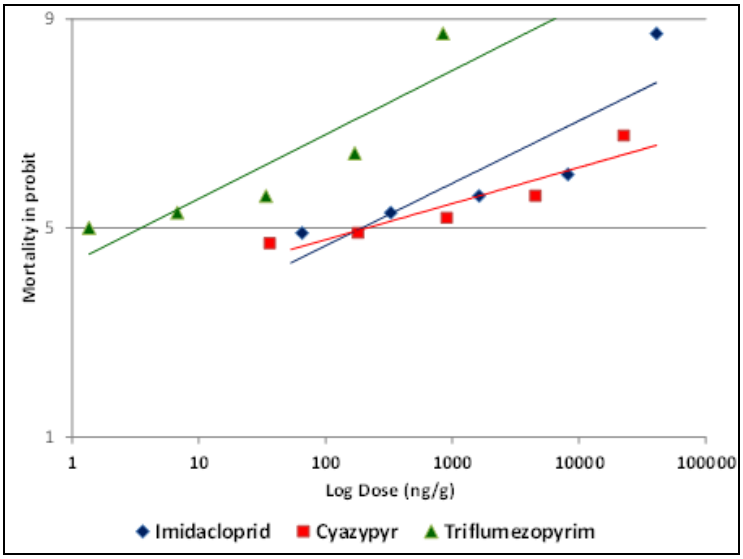

Fig 2: Relationship between log dose (ng/g) and mortality probit of WBPH from Karawang-West Java

Response WBPH from Karawang to imidacloprid with $RR_{calculate}$ = 1.25, near to $RR_{standar}$ = 1, it mean the WBPH from Karawang tend to susceptible category to imidacloprid. Response WBPH from Karawang to cyazypyr and triflumezopyrim with $RR_{calculate}$ = 2.94 and 2.94 respectively, The $RR_{calculate}$ near to $RR_{standar}$ =3-5, it mean the BPH from Karawang tend to decreased susceptibility category to cyazypyr and triflumezopyrim (Table 2).

3.4 Susceptibility WBPH population from Subang-West Java to some insecticides

In Sukamandi Jaya-Ciasem-Subang-West Java (village-sub District-District-Province) with 06.35238º S Latitude, 107.65063º E Longitude, and Altitude was 44 m from sea level, the WBPH adult was collected from Inpari 16 variety in August 2014. The values for the regression line of triflumezopyrim, was -0.2565 (intercept), 0.138 (natural response), and 0.793 (slope) with their standard error (Table 3). In case the t-ratio of slope was 0.793/0.178 = 4.657 > 1.96, show that the regression was significant, so this indicate the treatment has effect to WBPH from Subang. In the other hand heterogeneity was 0.98 < 1, this indicate that data bioassay was fit for model probit regression to WBPH from Subang.

LD_{50} of triflumezopyrim to WBPH from Subang was 2.152 ng/g with 95% lower and upper fiducial limit were 0.305 and 5.838 ng/g. The value LD_{50} and confidence interval limit of 95% were acceptable as effective dose because g=10.194 more than 0.4.

The values for the regression line of imidacloprid, was -1.1186 (intercept), 0.101 (natural response), and 0.592 (slope) with their standard error (Table 3). In case the t-ratio of slope was 0.592/0.144 = 4.111 > 1.96, show that the regression was significant, so this indicate the treatment has effect to WBPH from Subang. In the other hand heterogeneity was 0.19 <1, this indicate that data bioassay was fit for model probit regression to WBPH from Subang.

LD_{50} of imidacloprid to WBPH from Subang was 78.446 ng/g with 95% lower and upper fiducial limit were 5.174 and 268.741 ng/g. The value LD_{50} and confidence interval limit of 95% is acceptable as effective dose because g=0.228 less than 0.4.

Table 3: The LD_{50} values (ng/g) of WBPH population collected from Subang - West Java

Insecticide	LD_{50} (ng/g)	Fiducial Limit 95% (ng/g)		Intercept	Slope	Natural response	Hetero-geneity	g-value	$RR_{cal.}$
		Lower	Upper						
Triflumezopyrim	2.152	0.305	5.838	-0.2565	0.793± 0.178	0.138± 0.064	0.98	10.194	2.74
Imidacloprid	78.446	5.174	268.741	-1.1186	0.592± 0.144	0.101± 0.055	0.19	0.228	0.77
Cyazypyr	194.808	2.700	887.827	-1.7399	0.773± 0.166	0.142± 0.066	1.01	0.471	2.42

*Remarks: RR = Resistance ratio, $RR_{cal.}$=$RR_{calculate}$, LD_{50} of WBPH laboratory population to triflumezopyrim = 0.786 ng/g, imidacloprid = 102.442 ng/g, and cyazypyr = 80.459 ng/g.

The values for the regression line of cyazypyr, was -1.7399 (intercept), 0.142 (natural response), and 0.773 (slope) with their standard error (Table 3). In case the t-ratio of slope was 0.773/0.166 = 4.657 > 1.96, show that the regression was significant, so this indicate the treatment has effect to WBPH from Subang. In the other hand heterogeneity was 1.01 ≤ 1, this indicate that data bioassay is fit for model probit regression to WBPH from Subang.

LD_{50} of cyazypyr to WBPH from Subang was 194.808 ng/g with 95% lower and upper fiducial limit were 2.700 and 887.827 ng/g. The value LD_{50} and confidence interval limit of 95% were unacceptable as effective dose because g=0.471 more than 0.4.

Another vital aspect of quantal data analysis there were tested the hypothesis of WBPH from Subang to triflumezopyrim, imidacloprid, and cyazypyr in the sameness of the slopes and intercepts of the each regression lines were rejected. This indicate the lines were significantly different (Fig 3).

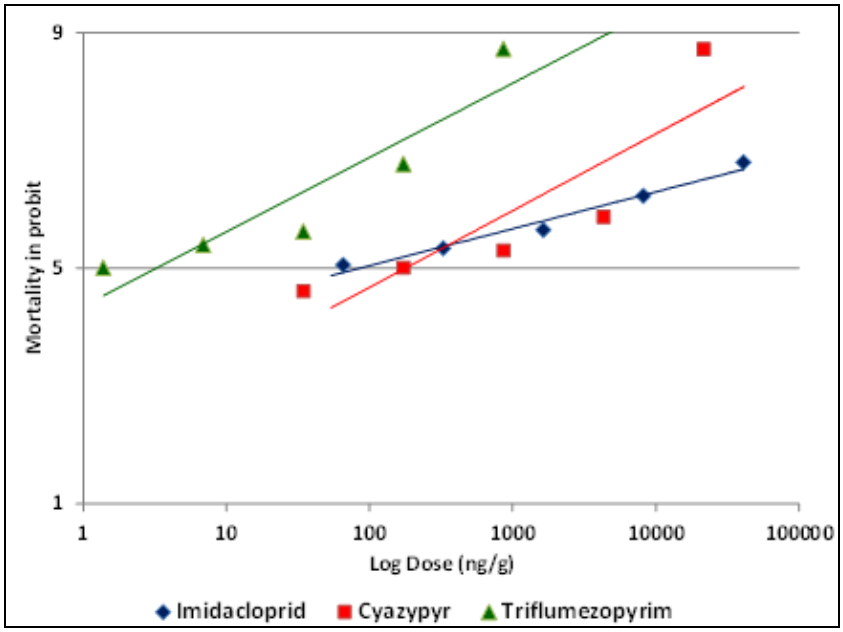

Fig 3: Relationship between log dose (ng/g) and mortality probit of WBPH from Subang-West Java

Response WBPH from Subang to imidacloprid was highly susceptible with $RR_{calculate}$ = 0.77, response WBPH from Subang to triflumezopyrim and cyazypyr with RR= 2.74 and $RR_{calculate}$ =2.42 respectively. The RR $_{calculate}$ near to $RR_{standar}$ =3-5, it mean the BPH from Subang tend to decreased susceptibility category to cyazypyr and triflumezopyrim (Table 3).

3.5 Susceptibility WBPH population from Indramayu-West Java to some insecticides

WBPH adult was collected from IR42 variety in September 2014 from Kandang Haur-Karang Sinom - Indramayu -West Java (village-sub District-District-Province) with 06.39048º S Latitude, 108.09794º E Longitude, and Altitude was 16 m from sea level.

The values for the regression line of triflumezopyrim, was -0.1594 (intercept), 0.102 (natural response), and 0.641 (slope) with their standard error (Table 4). In case the t-ratio of slope was 0.641/0.151= 4.245 >1.96, show that the regression was significant, so this indicate the treatment has effect to WBPH from Indramayu. In the other hand heterogeneity was 0.72<1, this indicate that data bioassay was fit for model probit regression to WBPH from Indramayu.

Topical LD_{50} of triflumezopyrim to WBPH from Indramayu was 1.791 ng/g with 95% lower and upper fiducial limit were 0.157 to 5.654 ng/g. The value LD_{50} and confidence interval limit of 95% were acceptable as effective dose because g=0.213 less than 0.4.

The values for the regression line of imidacloprid, was -1.1544 (intercept), 0.135 (natural response), and 0.616 (slope) with their standard error (Table 4). In case the t-ratio of slope was 0.616/0.153= 4.026 > 1.96, show that the regression was significant, so this indicate the treatment has effect to WBPH from Indramayu. In the other hand heterogeneity was 0.89 <1, this indicate that data bioassay is fit for model probit regression to WBPH from Indramayu.

Topical LD_{50} of imidacloprid to WBPH from Indramayu was 76.193 ng/g with 95% lower and upper fiducial limit were 4.567 to 265.743 ng/g. The value LD_{50} and confidence interval limit of 95% is acceptable as effective dose because g=0. 237 less than 0.4.

Table 4: The LD_{50} values (ng/g) of WBPH population collected from Indramayu - West Java

Insecticide	LD_{50} (ng/g)	Fiducial Limit 95% (ng/g)		Intercept	Slope	Natural response	Hetero-geneity	g-value	RR $_{cal.}$
		Lower	Upper						
Triflumezopyrim	1.791	.157	5.654	-0.1594	0.641±0.151	0.102±0.056	0.72	0.213	2.28
Imidacloprid	76.193	4.567	265.743	-1.1544	0.616±0.153	0.135±0.063	0.89	0. 237	0.74
Cyazypyr	207.606	43.703	522.142	-1.8875	0.835±0.177	0.145±0.068	0.95	0.173	2.58

*Remarks: RR = Resistance ratio, RR $_{cal.}$=RR $_{calculate}$, LD_{50} of WBPH laboratory population to triflumezopyrim = 0.786 ng/g, imidacloprid = 102.442 ng/g, and Cyazypyr = 80.459 ng/g,

The values for the regression line of cyazypyr, was -1.8875 (intercept), 0.145 (natural response), and 0.835 (slope) with their standard error (Table 4). In case the t-ratio of slope was 0.835/ 0.177= 4.718 >1.96, show that the regression is significant, so this indicate the treatment has effect to WBPH from Indramayu. In the other hand heterogeneity was 0.95 <1, this indicate that data bioassay was fit for model probit regression to WBPH from Indramayu.

Topical LD_{50} of cyazypyr to WBPH from Indramayu was 207.606 ng/g with 95% lower and upper fiducial limit were 43.703 to 522.142 ng/g. The value LD_{50} and confidence interval limit of 95% was acceptable as effective dose because

g=0.173 less than 0.4.

Another vital aspect of quantal data analysis there were tested the hypothesis of WBPH from Indramayu to triflumezopyrim, imidacloprid, and cyazypyr in the sameness of the slopes and intercepts of the each regression lines were rejected. This indicate the lines were significantly different (Fig 4).

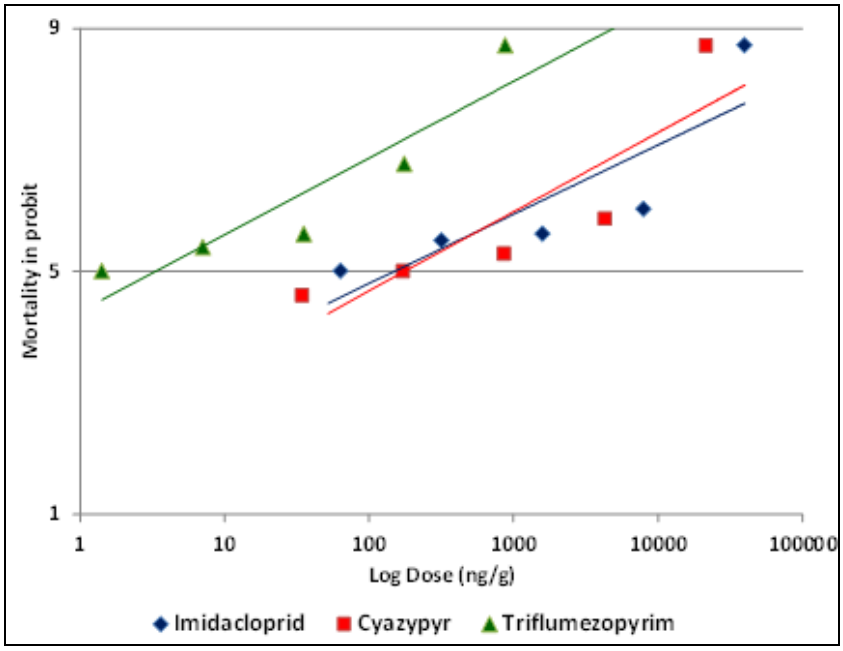

Fig 4: Relationship between log dose (ng/g) and mortality probit of WBPH from Indramayu-West Java

Response WBPH from Indramayu to imidacloprid was highly susceptible with $RR_{calculate}$ = 0.74. Response WBPH from Indramayu to triflumezopyrim and cyazypyr with RR= 2.28 and $RR_{calculate}$ = 2.58 respectively. The $RR_{calculate}$ near to $RR_{standar}$ =3-5, it mean the BPH from Indramayu tend to decreased susceptibility category to cyazypyr and triflumezopyrim (Table 4).

WBPH has a natural defense to insecticides that have been marketable or newly released or still in code form. Resistance level is possible depends on several factors, including how quickly the insects reproduce, the migration and host range of the pest, the crop protection product's persistence and specificity, and the rate, the timing and number of applications made (www.irac-online.org /content/uploads/resistance-the-facts.pdf). The rather r-strategic WBPH insects, especially the r-strategic BPH are typically faster resistant to insecticides than the k-strategic insects, due to rapid build-up in numbers of resistant mutants from abundance offspring. The crop protection product's persistence and specificity as natural phytotoxins have been exposed to pests for a long time, resulted in the development of the physiological capability to detoxify or tolerant to insecticides. In the other hand the rate, timing and number of applications insecticides of humans culture for pests control increases artificial selection against pests resistance.

LD_{50}-values for the WBPH populations collected from West Java (Karawang, Subang, and Indramayu) to new triflumezopyrim were 1.791-2.311 ng/g, with the RR were 2.28-2.94 fold. This indicate that the BPH of West Java is still susceptible to triflumezopyrim. LD_{50}-values for the WBPH populations collected from West Java (Karawang, Subang, and Indramayu) to imidacloprid were 76.193-128.158 ng/g, with the RR were 0.74-0.77 fold. This indicate that the WBPH of West Java is still susceptible to imidacloprid. LD_{50}-values for the WBPH populations collected from West Java (Karawang, Subang, and Indramayu) to cyazypyr were 194.808-236.149 ng/g, with the RR were 2.42-2.94 fold. This indicate that the WBPH of West Java is still susceptible to cyazypyr.

In the case of imidaclopirid to all the WBPH populations from Japan, China, Taiwan, Vietnam and Philippines had small LD_{50} values (0.11–0.34 µg/g) and large slopes of regression lines (2.7–4.6), except for one population in Japan (Japan-KM-A) (1.06 µg/g). Especially for WBPH from Phillippines had LD_{50} values 0.6-0.27 µg/g [13]. Most WBPH populations in Eastern of China have developed moderate resistance to buprofezin up to 25 fold. Approximately 32% of WBPH field populations exhibited moderate resistance to imidacloprid, while in other field populations showed the minor changes was 7.6 fold in their susceptibility to this insecticide [4]. It shows that resistance of WBPH to insecticides through a long period. The development of resistance to certain insecticides in response to their long application history may also be seen in other insects [20, 21].

All the WBPH populations from Japan, Taiwan, Vietnam, Philippines, and approximately 68 % of WBPH field populations from China exhibited had small LD_{50} values, especially for WBPH from Philippines had smaller LD_{50}, but LD_{50} of WBPH population from West Java was the smallest compare to others country. During 2006, WBPH from Godavari delta exhibited RR of 2.7 fold for clothianidin but remained susceptible to thiamethoxam, acephate, monocrotophos and fipronil recording RR values of around 1.0 or less than1.0 fold. Low to moderate level of resistance

was recorded in Godavari WBPH to carbamate insecticide BPMC wit RR was 2.7 fold and insect growth regulator buprofezin with RR was 2.9 fold [22].

WBPH control with chemicals must be avoided to use the insecticides that have been identified resurgence and resistance. Therefore necessary to regulate the use of triflumezopyrim in the insecticides resistance management (IRM) to control WBPH, as a complement and rotation of the others insecticide in the field. Response WBPH from Karawang to imidacloprid tend to susceptible category ($RR_{calculate}$ = 1.25), response WBPH from Subang and Indramayu to imidacloprid was highly susceptible with $RR_{calculate}$ = 0.77 and 0.74-fold respectively. Response WBPH from Karawang, Subang and Indramayu to triflumezopyrim tend to decreased susceptibility category with $RR_{calculate}$ were 2.94, 2.74, 2.28-fold respectively. Response WBPH from Karawang, Subang and Indramayu to cyazypyr tend to decreased susceptibility category with $RR_{calculate}$ were 2.94, 2.42, 2.58-fold respectively.

The imidacloprid and triflumezopyrim not only good for suppress WBPH but also good for handle BPH. The LD_{50} for BPH of East Java (Ngawi, Lamongan, and Banyuwangi districts) to imidacloprid were 96.608-216.000 ng/g body weight, and to triflumezopyrim were 1.227-1.703 ng/g body weight. Response BPH from all sites to triflumezopyrim and imidacloprid were susceptible with Resistance Ratio (RR) <3 [14].

Responce BPH from Subang was highly susceptible category to triflumezopyrim a 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 triflumezopyrim 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 triflumezopyrim 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 triflumezopyrim with LD_{50} was 3.730 ng/g and $RR_{calculate}$ was 5.48 fold. BPH from Indramayu and Pemalang were highly susceptible to imidacloprid 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 imidacloprid 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 imidacloprid with LD_{50} was 313.243 ng/g body weight and $RR_{calculate}$ was 2.84-fold [19]. Triflumezopyrim was fantastic insecticide to provide a low LD_{50} and didn't induce resistance to WBPH almost of all sites. This insecticides can be used to solve the problem resistance of WBPH against to the other insecticides.

4. Conclusions

The regression line of topical LD_{50} of imidacloprid, cyazypyr, and triflumezopyrim were significant, to indicate the insecticides treatment have effect to WBPH from Laboratory, and WBPH from the field of West Java. In the other hand Heterogeneities were less than of 1, this indicate that the data bioassay were fit for model probit regression to WBPH from all sites. Topical LD_{50} for WBPH from all sites with 95% lower and upper fiducial limit to imidacloprid, cyazypyr, and triflumezopyrim were acceptable as effective dose because g less than of 0.4. The null hypothesis of equality tests the sameness of the slopes and intercepts of the regression lines were rejected for WBPH from all sites. This indicate the regression lines were significantly.

Response WBPH from Karawang to imidacloprid tend to susceptible category with LD_{50} was 128.158 ng/g and $RR_{calculate}$ = 1.25. Response WBPH from Subang and Indramayu to imidacloprid was highly susceptible with LD_{50} were 78.446 and 76.193 ng/g and $RR_{calculate}$ = 0.77 and 0.74-fold respectively. Response WBPH from Karawang, Subang and Indramayu to triflumezopyrim tend to decreased susceptibility category with LD_{50} were 2.311, 2.152, 1.791 ng/g and $RR_{calculate}$ were 2.94, 2.74, 2.28-fold respectively. Response WBPH from Karawang, Subang and Indramayu to cyazypyr tend to decreased susceptibility category with LD_{50} were 236.149, 194.808, 207.606 ng/g and $RR_{calculate}$ were 2.94, 2.42, 2.58-fold respectively. Triflumezopyrim was fantastic insecticide to provide a low LD_{50} and didn't induce resistance to WBPH almost of all sites. This insecticides can be used to solve the problem resistance of WBPH against to the other insecticides.

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