

## Susceptibility of six chemical insecticides against brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae) in Kallar tract of Punjab, Pakistan; 2015-2019

### *Susceptibilidad de seis insecticidas químicos contra el saltamontes marrón, Nilaparvata lugens (Hemiptera: Delphacidae) en el tracto Kallar de Punjab, Pakistán; 2015-2019*

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#### Abstract

Brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) is an economically important insect pest of rice crop worldwide including Pakistan. The use of chemicals insecticides to control the BPH population has been considered an effective strategy. However, long-term and intensive use of insecticides has resulted in resistance against this pest. In this study, six recommended insecticides were tested against BPH over the period of 2015-2019 to determine the susceptibility level. From 2015-19, susceptibility of BPH was reduced to all tested insecticides. Increasing trend of LC<sub>50</sub> values was recorded in fipronil (18.23-35.11 mg/L), imidacloprid (64.22-128.8 mg/L), buprofezin (185.01-315.0 mg/L), chlorantranilipyrrole (199.2-263.3 mg/L), pymetrozine (248.2-315.5 mg/L), and nitenpyram (21.07-34.3 mg/L) respectively from 2015 to 2019. Correlation coefficient values indicated a significant relation ( $P < 0.05$ ) between fipronil, imidacloprid, and buprofezin. The study will be helpful for resistance management strategies to prevent the resistance development in BPH against insecticides.

#### Keywords

Insecticide resistance, Kallar tract, Chemical control, Basmati rice, Brown planthopper

#### Resumen

El saltamontes marrón (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), es una plaga de insectos económicamente importante del cultivo de arroz en todo el mundo, incluido Pakistán. El uso de insecticidas químicos para controlarla ha constituido una estrategia eficaz; sin embargo, utilizarlos de forma prolongada e intensiva ha ocasionado que la plaga se vuelva resistente a dichos pesticidas.

En este estudio se probaron seis insecticidas recomendados contra la BPH durante el periodo 2015-2019 para determinar el nivel de susceptibilidad. De 2015 a 2019, la susceptibilidad a la BPH se redujo a todos los insecticidas probados. Se registró una tendencia creciente de los valores de LC<sub>50</sub> en fipronil (18.23-35.11 mg/l), imidacloprid (64.22-128.8 mg/l), buprofezina (185.01-315.0 mg/l), clorantranilipiról (199.2-263.3 mg/l), pimetrozina (248.2-315.5 mg/l) y nitenpiram (21.07-34.3 mg/l) respectivamente de 2015 a 2019. Los valores del coeficiente de correlación indicaron una relación significativa ( $P < 0.05$ ) entre fipronil, imidacloprid y buprofezin.

El estudio será útil tanto para llevar a cabo estrategias de manejo de la resistencia como para prevenir el desarrollo dicha resistencia de la BPH hacia los insecticidas.

#### Palabras clave

Resistencia a insecticidas, tracto Kallar, control químico, arroz basmati, chicharrita parda

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## 1. Introduction

Rice, *Oryza sativa* L. (Graminales: Poaceae), is one of the most important cereal crop and a staple food for over half of the world's population (Heong et al., 2015). Asia is a major rice-producing continent including China, India, Bangladesh, and Pakistan. Pakistan is among the top ten rice-producing countries in the world (Khan et al., 2022). After cotton, rice is the 2nd most important cash crop of Pakistan which is grown on 11% of the total cropped area of the country. In Pakistan, Kallar tract (i.e., Gujranwala, Sialkot, Sheikhpura, Narowal, Lahore, Hafizabad, Nankana) is famous for the cultivation of aromatic rice (Khan et al., 2022). Pakistan is the 4th largest rice exporter, earning \$2.05 billion in foreign exchange annually (Pakistan Economic Survey, 2020-21). With a rapidly growing world population, it is imperative to increase the production of rice in the world including in Pakistan (Miao et al., 2011). Unfortunately, this crop is facing major threats such as climate change and the attack of various insect pests and diseases. The brown planthopper (BPH), *Nilaparvata lugens* (Stål), (Homoptera: Delphacidae) is one of the most serious insect pest of rice crop in all rice-growing countries including Pakistan (Rizwan et al., 2019; Sabir et al., 2019). Both nymphs and adults are damaging and cause severe economic losses to the rice crop. They suck the cell sap from the plant material and lead to the situation known as "hopper burn" (Liu et al., 2008, Normile, 2008, Horgan, 2009, Vanitha et al., 2011, Atta et al., 2019). Adults are Macroptereous due to which they migrate over a mile causing more damage to crops and also transmitting various kinds of diseases (Heong and Hardy, 2009).

It is estimated that 10-30% of yield loss in rice crop is attributable to the BPH attack (Harris and Trisyono, 2019). The control strategy of BPH has predominantly relied upon synthetic chemicals (Garrood et al., 2016). Chemical insecticides of major classes such as carbamate, organophosphorus, and neonicotinoids are widely used against this pest (Wang, Gao, Zhu, et al., 2008, Liu et al., 2015, Matsumura et al., 2017). BPH has been reported to develop resistance to many insecticides (Wang, Gao, Xu, et al., 2008, Wen et al., 2009, Punyawattoe et al., 2013). The development of resistance in BPH to insecticides has been attributed to the extensive use of chemicals including organophosphates, carbamates, pyrethroids, neonicotinoids, insect growth regulators (IGRs), and phenylpyrazoles (Punyawattoe et al., 2013, Zhang et al., 2016).

The use of synthetic chemical insecticides has been increased to suppress the population of rice insect pests in Pakistan. Monitoring the insecticide resistance in BPH is an essential part of the integrated pest management. It is necessary to evaluate the susceptibility of this pest regularly to estimate the effectiveness of recommended chemicals for its control. This insect has developed resistance to almost 33 different active ingredients of insecticides with 421 reported cases throughout the world (Mota-Sanchez and Wise, 2022). Thus, monitoring of resistance is a crucial part to comprehend the current status of susceptibility of the BPH population to various insecticides in the field. Little is known about the status of insecticide resistance in BPH in Pakistan. Thus, our main objective of the study was to determine the susceptibility of commonly used insecticides against BPH.

## 2. Methodology

### 2.1. Insect culture

The immature and adults of BPH were collected from the rice fields (31°43'25.5"N 74°16'17.4"E) of Kallar area using an aspirator each year from 2015 to 2019. The collected insects were reared

in a growth chamber. The collected insects were reared on Basmati 515 seedlings in rearing cages (40 × 50 × 80 cm) under controlled conditions of  $28 \pm 2^\circ\text{C}$ , 70-80% relative humidity, and a 16:8 (L: D) h photoperiod. The 3rd instars nymphs of F1 generations of the collected population were used for susceptibility assay.

## **2.2. Insecticides**

Following insecticides were purchased from the local market; imidacloprid 20%SC, nitenpyram 25%SP, pymetrozine 50%WG (neonicotinoids) fipronil 80%WG (phenylpyrazole), buprofezin 25%WP (IGR), and chlorantranilipyrrol 0.4%GR (anthranilic diamides) and tested against BPH. Five serial dilutions of each insecticide were prepared by following the methodology of Atta et al. (2015, 2020).

## **2.3. Bioassay**

The dose-response assay was carried out using rice-stem dipping methodology (Wang, Gao, Zhu, et al., 2008; Ali et al., 2017) under laboratory conditions. Rice plants (55-70 days old) were pulled out of the soil, washed carefully, cut into 12 cm pieces, and air-dried for half an hour. Five stems were congregated together and immersed in the solution for half minute, and air-dried for 30 minutes. The rice stems were wrapped from the top and bottom with moistened cotton and put into a plastic jar (3L). Thirty 3rd-instar nymphs were collected using aspirator and transferred into plastic jars containing treated rice stems. There were five replications for each dose of a single insecticide. In control treatment, water was used. Mortality data were recorded at 72 and 96 h of insecticide application. The insects were observed with camel hair brush, and assumed dead if they were unable to move.

## **2.4. Data analysis**

Probit analyses were carried out to calculate the  $LC_{50}$  values for each insecticide against BPH for the year 2015-19 using POLO-Plus 2.0 software (Liao et al., 2021). Pearson correlation coefficients between  $LC_{50}$  values were calculated for all pair-wise comparisons of six insecticides using Minitab 17.0 software (Arshad et al., 2019).

# **3. Results**

## **3.1. Variations in susceptibility ratios to six insecticides**

An increasing trend of  $LC_{50}$  values was recorded each year for each insecticide against BPH. There was a considerable increase (64.22 to 125.81 mg/L) in  $LC_{50}$  value for imidacloprid over five years. The susceptibility to imidacloprid decreased greatly from 2015 to 2019 ( $LC_{50}$  =64.2-125.8 mg/L). The susceptibility to buprofezin reduced drastically during the fourth and fifth years. The  $LC_{50}$  value increased from 185.0 to 315.0 mg/L showing a significant reduction in susceptibility to buprofezin. Susceptibility to fipronil decreased significantly from 2015 to 2019. The toxicity of fipronil to BPH reduced over five years as  $LC_{50}$  increased from 18.2 to 35.1 mg/L. After three years,  $LC_{50}$  value was dramatically increased ( $LC_{50}$  =38.58 mg/L) in the case of fipronil and then the value was reduced ( $LC_{50}$  =35.11 mg/L) for last year. The susceptibility to chlorantraniliprole

varied each year. The susceptibility decreased each year from 1<sup>st</sup> to 3<sup>rd</sup> year ( $LC_{50}$  =199.2-275.8 mg/L), then increased in 4<sup>th</sup> year ( $LC_{50}$ =225.8 mg/L), and again the value was decreased in last year ( $LC_{50}$  =263.3 mg/L). The susceptibility to pymetrozine was reduced each year. The susceptibility of the BPH field population to nitenpyram insecticide decreased during the 3<sup>rd</sup> year and then it remained almost constant for the next two years (Table 1).

**Table 1.** Trend of  $LC_{50}$  values of brown planthopper for six insecticides over the past five years 2015-19

Year	$LC_{50}$ mg/L (slope)					
	Imidacloprid	Buprofezin	Fipronil	Chlorantraniliprole	Pymetrozine	Nitenpyram
2015	64.2±8.22 (0.67)	185.0±31.1 (0.96)	18.2±3.18 (1.45)	199.2±16.2 (1.41)	248.2±56.2 (0.96)	21.0±0.78 (0.85)
2016	85.5±7.84 (1.12)	201.1±35.9 (1.12)	22.6±4.17 (1.53)	222.3±25.1 (1.28)	262.0±61.5 (1.10)	25.1±2.49 (0.83)
2017	83.4±18.2 (0.92)	197.5±46.2 (0.89)	23.6±3.24 (1.38)	275.8±51.4 (2.01)	286.1±50.9 (0.86)	36.2±5.52 (1.00)
2018	108.6±17.3 (1.89)	255.6±62.5 (1.12)	38.5±4.81 (2.53)	225.8±20.4 (1.98)	271.4±44.0 (2.56)	32.4±3.84 (1.10)
2019	125.8±19.6 (3.48)	315.0±49.7 (2.27)	35.1±6.30 (1.29)	263.3±24.3 (1.78)	315.5±48.3 (3.36)	34.3±4.32 (1.13)

$LC_{50}$  = Lethal concentrations

### 3.2. Cross-resistance development to insecticides in BPH

The cross-resistance pattern of six insecticides was investigated by correlation based on  $LC_{50}$  values. Significant ( $P < 0.05$ ) relationship was found between fipronil and imidacloprid ( $r = 0.911$ ), imidacloprid and buprofezin ( $r = 0.962$ ). Chlorantraniliprole, nitenpyram, and pymetrozine showed no significant relation ( $P > 0.05$ ) to other insecticides (Table2).

**Table 2.** Correlation matrix evaluating cross-resistance in brown planthopper population collected from Kallar tract, Pakistan during 2015-19

Insecticide	Fipronil	Imidacloprid	Buprofezin	Chlorantraniliprole	Nitenpyram
Imidacloprid	0.911 (0.031) <i>b</i>				
Buprofezin	0.850 (0.067)	0.962 (0.008)			
Chlorantraniliprole	0.315 (0.605)	0.501 (0.390)	0.411 (0.491)		
Nitenpyram	0.631 (0.253)	0.670 (0.215)	0.551 (0.334)	0.906 (0.337)	
Pymetrozine	0.595 (0.289)	0.822 (0.087)	0.816 (0.091)	0.843 (0.723)	0.812 (0.094)

*b*  $P < 0.05$  shows the significance

## 4. Discussion

The use of insecticides for the control of BPH has increased since 2013 in the Kallar tract of Pakistan with the increased outbreak frequency of this notorious pest (Sabir et al., 2019). The extensive and frequent use of insecticides against BPH has evolved resistance to major classes of insecticides throughout Asia (Hemingway et al., 1999, Nagata et al., 2002, Punyawattoe et

al., 2013, Yang et al., 2014) until the neonicotinoids provided the main means of control (Hemingway et al., 1999, Nagata, 2002). Imidacloprid has been widely used against rice planthoppers in Asia during the 1990s. However, up to 300-fold resistance in BPH to imidacloprid in China has already been reported previously (Wu et al., 2018). BPH resistance to imidacloprid and thiamethoxam has been reported in many Asian countries (Gorman et al., 2008, Matsumura et al., 2008, Wang, Gao, Xu, et al., 2008, Matsumura et al., 2013, Zhang et al., 2014, Garrood et al., 2016, Zhang et al., 2016) but no cross-resistance to fipronil and buprofezin was reported (Khoa et al., 2018). In our study, BPH has developed resistance to imidacloprid, as  $LC_{50}$  values were much higher in later years of study as compared to former years of study. Moreover, it has developed cross-resistance to fipronil and buprofezin. Hence, the development of resistance in the field population of BPH to imidacloprid is widely and faster.

In South Asia and South-East Asia, organophosphates and carbamates have been commonly used in the past. Further, IGRs such as buprofezin were considered useful insecticide for BPH management (Uchida et al., 1985, Kanno, 1987). Our findings showed that BPH has developed high resistance to buprofezin also. Hence, it may be no more useful for BPH management in the Kallar tract.

Our results showed that resistance in BPH to fipronil increased every year. A high level of resistance in the BPH population to fipronil has been reported in other countries such as Thailand, China, Philippines, and Vietnamese (Matsumura et al., 2008; Yang et al., 2014; Garrood et al., 2016; Punyawattoe et al., 2013). A high level of resistance against fipronil elucidates that efficacy would be lost in the coming years.

Chlorantraniliprole is also an effective synthetic chemical for BPH management. No cross-resistance against this insecticide was found with other chemicals. Pymetrozine had also been widely used for effective management of sucking insect pests (Nicholson et al., 1996; Polston, 2003; Chang and Synder, 2008). It is a new chemistry insecticide as compared to fipronil, imidacloprid, and buprofezin and is still effective for BPH management, as  $LC_{50}$  value did not increase too much over five years in our study. The  $LC_{50}$  values describe that the BPH population is moderately susceptible to nitenpyram. The  $LC_{50}$  value was not increased in the case of nitenpyram during the course of study. Hence, it is still effective when compared with other chemicals used in this study. Moreover, no cross-resistance of nitenpyram was observed with other chemicals.

The resistance to imidacloprid, nitenpyram, and buprofezin has dramatically increased in recent years (Wu et al., 2018) as compared to previous reports (Peng et al., 2013, Zhang et al., 2016). BPH has developed resistance against traditionally used insecticides such as fipronil, buprofezin, and imidacloprid. Sublethal concentrations may suppress the insect pest to some extent or it may enhance the fecundity of females depending upon the sublethal impact caused by the insecticide under consideration (Ali et al., 2017). So the use of fipronil, imidacloprid, and buprofezin may exacerbate the pest situation instead of its management. As per our knowledge, there is no study available on resistance of BPH to insecticides in the rice fields in Pakistan. Thus, the study is very helpful for farmers and extension workers of agriculture department in recommending synthetic insecticide for BPH management. Chemicals to which BPH has developed resistance will not only increase the cost of production but may increase its population tremendously resulting in greater damage to the rice crop.

The manipulation of resistant genetic sources is very important for BPH long-term management. Chemicals used in this study belong to different classes i.e., neonicotinoids (imidacloprid, pymetrozine, nitenpyram), phenylpyrazole (fipronil), IGRs (buprofezin), and anthranilic diamides (chlorantraniliprole). Thus, resistant development in BPH is not specific to any class of insecticides.

## 5. Conclusions and recommendations

Our findings showed a dramatic increase of resistance development in the BPH population to tested insecticides from 2015 to 2019. The cross-resistance development depicted that insecticides must be used carefully to prevent resistance development to other insecticides. There is a need to develop new chemistries and a strategy to minimize the resistance and cross-resistance development in BPH against insecticides. Moreover, there is a need to evaluate the field efficacy of all recommended/registered insecticides for BPH management.

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