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Baseline toxicity study of sulfoxaflor against rice brown planthopper, *Nilaparvata lugens* (Stål) populations of Tamil Nadu

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Abstract
Rice brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is a serious pest and inflicts a severe threat to rice cultivation throughout East and Southeast Asia. Susceptibility of BPH against sulfoxaflor 21.8 SC insecticide was investigated with four different populations of Tamil Nadu *viz.*, Coimbatore, Bhavani, Nagapattinam and TNAU susceptible strains. The bioassay method followed was the seedling dip method (IRAC method No. 5) under three replications. Results revealed that the Nagapattinam population registered the highest LC 50 value of 5.40 ppm followed by Bhavani (4.17 ppm), Coimbatore (3.06 ppm) and susceptible population (1.61 ppm) for the insecticide sulfoxaflor 21.8 SC. The resistance ratio values were 3.35 fold for the Nagapattinam population, 2.59 fold for the Bhavani population, and 1.90 fold resistance for the Coimbatore population. The order of toxicity of sulfoxaflor 21.8 SC to all four populations based on LC50 was Nagapattinam> Bhavani > Coimbatore > TNAU susceptible.

Keywords: Baseline susceptibility, insecticide resistance, resistance ratio, rice brown planthopper, sulfoxaflor 21.8 SC

Introduction
Rice is the world's most important staple food and estimates 20% of calorific intake worldwide [10]. Rice is a major grown crop in many Asian countries and certain abiotic and biotic stress have caused a massive reduction in productivity. The biotic stress includes insects, diseases, weeds which are major hindrances in rice growing regions. Among the insects, brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) is a serious pest and inflicts severe threat to rice cultivation throughout East and Southeast Asia. The planthopper is a typical phloem feeder and damage plants by sucking plant sap, ovipositing plant tissues, and causing hopper burn symptoms. BPH has also been reported to transmit various plant virus diseases like grassy stunt virus, ragged stunt virus, etc [16]. The estimation of single season losses by *Nilaparvata lugens* in Thailand and Vietnam was about $US 30million [10]. Due to the competence of being high adaptability, superior reproductive potential and capability of long distance migration make chemical control as precedence for the management of planthoppers [6]. In the early 1940s organochlorines were widely used insecticides to control rice planthoppers which then replaced by organophosphorus (OPs) and carbamate insecticides in the 1960s [18]. The foremost concern of environmental hazards, resistance developed to older compounds have suggested neonicotinoids and phenylpyrazoles as alternatives in the early 1990s to successfully reduce the resistant population densities of *N. lugens* with long-lasting effects [8]. However, the selection pressure imposed due to unremitting, excess and indiscriminate use of insecticides caused the outbreak of insects, development of insecticide resistance in pest populations resulting in pest resurgence [14]. The imidacloprid, a major use insecticide in the neonicotinoid group, becomes resistance in populations of *N. lugens* collected from across Asia with resistance factors of 600–800-fold recently after a decade of use [5]. Thus nitenpyram and thiamethoxam were used to control *N. lugens* throughout Asia since then BPH developed resistance to imidacloprid. The BPH exhibits moderate levels of resistance for the china population to thiamethoxam with a resistant ratio of 13.9 - 36.7 fold in 2011 and there was a further increase in the next year [24].
It is one of the greatest challenges for the scientific community to manage the resistant population outbreaks.

BPH has a high capability of migration which leads to resistant genotypes spread across Asia and also several biotypes developed due to various selection pressure of insecticides. In order to combat them, early detection and measurement of insecticide resistance helps to avoid ineffective molecule and assists newer molecules in resistant management strategies. Thus, the newer molecule like sulfoxaflor which is less exposed to neonicotinoid insecticide and its base-line data for insecticide susceptibility is necessary for resistance monitoring and further applications. Sulfoxaflor is a new novel molecule, systemic insecticide, exhibits low mammalian toxicity and the only member of the sulfoximine group [13]. It acts as an agonist at nicotine acetylcholine receptors (nAChRs) and it serves as the best alternative for the insects developed resistance to other neonicotinoid class of insecticides [9].

The present study was carried out during 2019-2020 to establish baseline toxicity data for sulfoxaflor 21.8 SC against four BPH populations of various regions of Tamil Nadu which are essential for valuable location specific resistant management strategies.

Materials and Methods

Collection of BPH from various localities of Tamil Nadu

The test insect *N. lugens* was collected from three different rice growing areas of Tamil Nadu *viz.*, Coimbatore, Bhavani and Nagapattinam. More than 50 healthy female adults and 500 nymphs were collected at each site. The collected insects were reared on TN-1 rice-seedlings under standard conditions of 27 + 2 °C and 12 hours of light. The one to two days old female adults of all populations were used for bioassay. A susceptible strain of *N. lugens* was collected from a Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore and reared on rice seedlings in the glasshouse without exposure to any of the insecticides.

Mass culturing of brown planthopper

The test insects required for bioassay were mass cultured on 35-45 day old TN-1 seedlings in wooden cages covered with wire mesh [11]. The wooden cages were placed in glasshouse and cage stands were placed in trays with water to prevent ants from entering cages. TN-1 seeds were sown at weekly intervals in plastic pots and on 10 DAS, the six to ten seedlings were transplanted to each pot, which then placed in a larger crate filled with puddled soil and water. Cages for the presence of natural enemies or other insects were examined periodically and removed using an aspirator. The potted rice plants of about 35–45 days old were inoculated with the field collected planthoppers (initial culture) and reared for further generations. The new plants replaced the old dried plants to sustain the BPH population. The insecticide solutions required for bioassay were prepared from the formulated products using distilled water. Preliminary range-finding tests were done to fix the test dose causing 20-80% mortality approximately for constructing log-concentration-probit mortality (LCPM) lines.

Bioassay

The bioassay method followed for BPH was the seedling dip method developed and recommended by the Insecticide Resistance Action Committee - IRAC Method 5 [12]. More than six concentrations were used for each insecticide under three replications with water treated control. The observations on mortality were assessed 24 hr after treatment and the results were expressed as percentage mortality. Female adults considered dead if they were unable to move after gentle prodding with a fine brush. The TN-1 seeds were sown in disposable plastic cups. As the germination progressed, 10-12 days old seedlings in pots were used for bioassay. Agar of 15g was boiled in one lit of water for about 15 mins and allowed to cool down to 40-45^{0}C. Then the agar was poured into the plastic container with 10 days old seedlings and allowed to solidify for about 15-20 mins which prevented the falling of soil while inverting the cup. The seedlings were dipped into chemical solutions of different concentrations for about 10-30 secs and dried for 15 min. After the treatment, the seedling was covered with mylar film cages. Adult female hoppers were collected from holding cages and transferred into treated cages using an aspirator. The LC_{50} values were calculated by subjecting the data to Finney's probit analysis [7] after test mortalities were corrected against untreated mortality using Abbott's formula [1]. If the mortality in control exceeded 20 percent, the experiment was repeated and conducted once again.

Assessing the level of resistance

The susceptible population was considered as a baseline population and cultured continuously without exposure to any insecticides. The collected field population was cultured and further generations were used for the study following the above-mentioned bioassay method. The resistance ratio (RR) was calculated by dividing the LC50 value of field population by that of LC50 of BPH susceptibility baseline population collected from our laboratory where the insecticide was not used for generations.

$$\text{Resistance Ratio (RR)} = \frac{LC_{50} \text{ of the resistant population}}{LC_{50} \text{ of the susceptible population}}$$

Resistance ratio was classified on the basis of the standard [24, 3, 16] as: susceptible (RR < 3), decreased susceptibility or minor resistance (RR = 3-5), low resistance (RR = 5-10), moderately resistance (RR = 10-40), highly resistance (RR = 40-160), and very highly resistance (RR > 160).

Results and Discussion

The results for the brown planthopper, *Nilaparvata lugens* populations collected from the various sites of Tamil Nadu *viz.*, Coimbatore, Bhavani, Nagapattinam and susceptible strain are presented in Table 1. The order of toxicity of the sulfoxaflor chemical to all four populations based on LC50 was Nagapattinam> Bhavani > Coimbatore > TNAU susceptible population.

The median lethal concentration values for sulfoxaflor 21.8 SC were 1.61, 3.06, 4.17, 5.40 ppm and LC95 values were 5.11, 6.90, 9.17, 11.29 ppm for susceptible(figure 1), Coimbatore(figure 2), Bhavani(figure 3), and Nagapattinam(figure 4) respectively. The values disagreed with the findings of [13] who reported susceptibility of sulfoxaflor against six populations of Karnataka with LC50 values of range 22.39 – 31.83. The variation in the susceptibility might be due to the earlier exposure of similar mode of action chemicals to the location studied, the insects generation studied, geographical factors and even genetic variation in populations as they are widely separated might be the cause of variation and thus insect exhibits changes in resistance.

Table 1: Baseline toxicity of sulfoxaflor to planthopper,*Nilaparvata lugens* populations.

Location	X^2	LC_{50}	Fiducial limits		LC_{95}	Fiducial limits		Resistance Ratio(RR)
			LL	UL		LL	UL	
Susceptible	0.14	1.61	1.45	1.79	5.11	3.65	7.16	-
Coimbatore	0.17	3.06	2.84	3.29	6.90	5.43	8.77	1.90
Bhavani	0.18	4.17	3.89	4.48	9.17	7.20	11.68	2.59
Nagapattinam	0.09	5.40	5.06	5.76	11.29	8.98	14.19	3.35

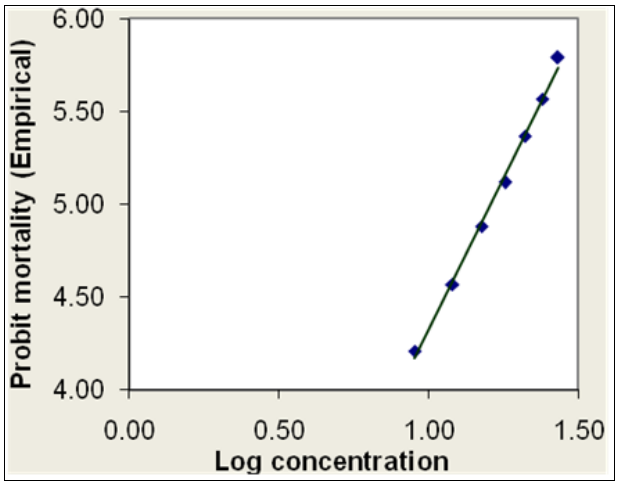

Fig 1: LC – Probit Mortality response for susceptible population of *N.lugens* to sulfoxaflor

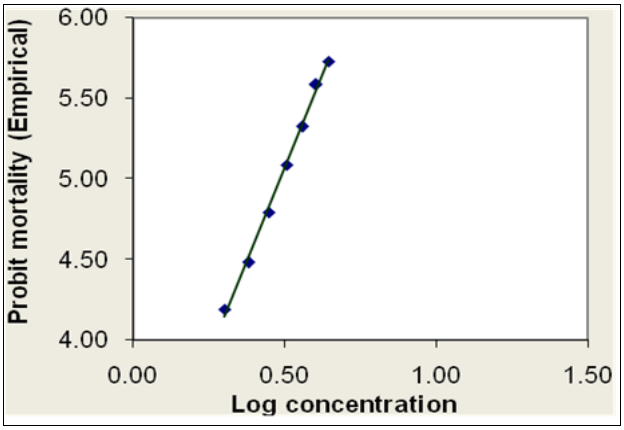

Fig 2: LC – Probit Mortality response for Coimbatore population of *N.lugens* to sulfoxaflor

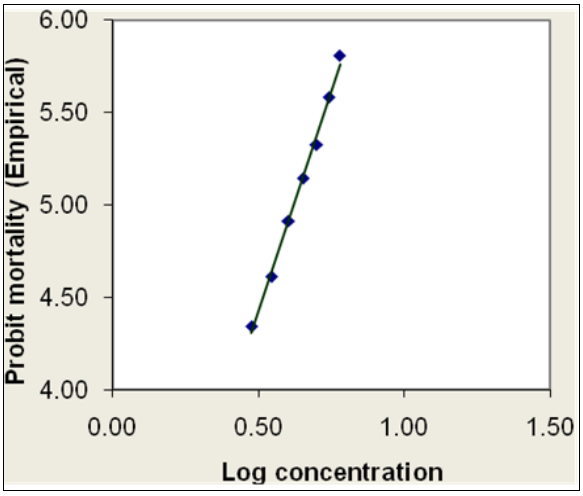

Fig 3: LC – Probit Mortality response for Bhavani population of *N.lugens* to sulfoxaflor

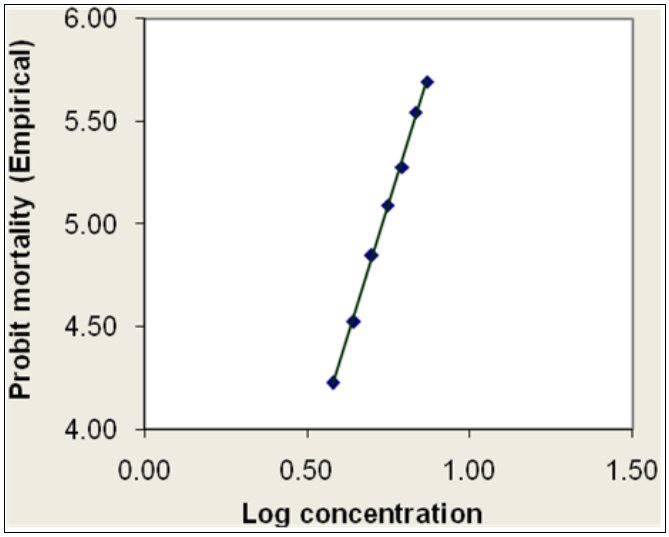

Fig 4: LC – Probit Mortality response for Nagapattinam population of *N.lugens* to sulfoxaflor

The bioassay study of all four populations (susceptible, Coimbatore, Bhavani and Nagapattinam) of BPH against sulfoxaflor 21.8 SC revealed that there has been a marked difference in their susceptibility compared with susceptible laboratory strain (figure 5). The Nagapattinam population observed a high value of toxicity to insecticide compared with other populations. The resistance developed might be due to excess and indiscriminate use of insecticides throughout the crop season and also due to exposure of insecticides with a similar mode of action. The Bhavani population exhibited a moderate value of toxicity to sulfoxaflor 21.8 SC and a low value of toxicity was observed in the Coimbatore population. The variation in BPH susceptibility of insecticides was studied in Vietnam and observed 2.4 fold for buprofezin, 3.3 fold for etofenprox, 1.5 fold for fenobucarb, 2.3 fold for fipronil and 2.1 fold for pymetrozine [15]. Basanth *et al* [4] studied the susceptibility in different populations of *N. lugens* and reported the resistant population show high resistance to older molecules and low resistance to new molecules.

The resistance ratio values observed were 3.35 fold for the Nagapattinam population, 2.59 fold for the Bhavani population. Similarly, the Coimbatore population exhibited 1.90 fold to sulfoxaflor insecticide. The resistance ratio values of all populations indicate their level of resistance based on the standard classification. The minor level of resistance observed in the Nagapattinam population and all other populations exhibit still lower values of resistance which indicates increased susceptibility to sulfoxaflor insecticide.

The predominant reason for the rapid resistance development in *N. lugens* against neonicotinoids is not only its extensive and intensive application of insecticides but also due to the existence of cross-resistance between neonicotinoid insecticides [22]. The lack of cross-resistance to sulfoxaflor was observed in multiresistant strains of many insects which further support sulfoxaflor as a novel molecule against a broad range of insecticide resistant strains [25]. Cytochrome P450 monooxygenases play important role in imidacloprid resistance in *N. lugens* whereas sulfoxaflor with its unique characteristic it not susceptible to monooxygenase degradation [23]. Thus, baseline data of sulfoxaflor helps in early detection of resistance and appropriate measures can be taken accordingly to delay the resistance development.

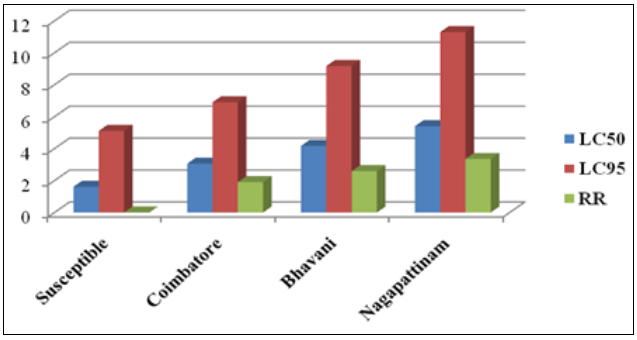

Fig 5: Insecticide Susceptibility of BPH populations to sulfoxaflor 21.8 SC insecticide

Conclusion

The current investigation was undertaken to study the baseline toxicity study and assess levels of insecticide resistance in different BPH populations collected from different localities of Tamil Nadu. Each population has differed in their susceptibility values to insecticide sulfoxaflor. The TNAU susceptible strains exhibited much lower toxicity value and the Nagapattinam population records the highest toxicity value compared to all other populations. The Bhavani population records moderate toxicity value and the Coimbatore population exhibits low toxicity value. The sulfoxaflor the only member of sulfoximine group has very distinct features and thus its susceptibility data provides better knowledge in resistant management programs.

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