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Feeding Behavior of Imidacloprid Resistant Brown Planthopper, *Nilaparvata lugens* (Stål)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A study was conducted to investigate the feeding behavior of imidacloprid resistant (RS) and laboratory (LS) strains of brown planthopper, *Nilaparvata lugens,* by using standard honeydew and probing tests. The honeydew production was higher in RS strain (7.12 and 14.63 cm^2) as compared to LS strain (5.44 and 1.86 cm^2) in both the imidacloprid treated and untreated conditions, respectively. The probing test revealed an increased probing activity by both the LS and RS strains on imidacloprid treated seedlings as compared to the untreated. The number of probing marks by LS and RS strains on the treated seedlings were 16 and 31, whereas in untreated seedlings 7 and 11 respectively. These results established the higher feeding capacity of imidacloprid resistant *N. lugens* strain as compared to the laboratory strain, measured in terms of probing behaviour and honeydew production.

Keywords: Honeydew; probing; insecticide; imidacloprid; resistance; rice; brown planthopper.

1. INTRODUCTION

The brown planthopper, *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae), is one of the most notorious pests of rice. Due to the short life cycle and high fecundity, the population of *N. lugens* can increase rapidly under favourable conditions inflicting heavy crop losses. Besides, *N. lugens* also acts as a vector of grassy stunt, ragged stunt and wilted stunt viruses [1,2,3]. Application of chemical insecticides has been a primary tool for managing this pest. However, due to extensive use, *N. lugens* has developed resistance to almost all the major classes of insecticides. Currently, *N. lugens* has developed resistance to 36 active ingredients of insecticides, with 432 reported cases across the globe, 11 posing serious challenges for the development of new insecticides and pest resistance management [4]. Increased insecticide resistance has limited the efficacy of insecticides leading to a significant increase in production costs and control failure. Imidacloprid, the first commercialised neonicotinoid insecticide, was introduced for planthopper control in the early 1990s. It is a systemic insecticide that translocate rapidly through the plant tissue and proved extremely effective against sucking pests. It disrupts the insect nervous system by competitive modulation of nicotinic acetylcholine receptors (nAChR) [5]. Insecticide resistant insects often exhibit increased energy consumption or disturbances in their metabolic balance, resulting in certain fitness cost and change of feeding behaviour [6]. Resistant insect populations show slower developmental rates, reduced survival rates and fecundity [7,8,9]. Since, the feeding behaviour and dietary habits of an insect directly influence the fitness parameters such as developmental characteristics and fecundity [10], it is worthwhile to study the feeding behaviour of insecticide resistant populations of *N. lugens*. Insecticides also impact pest dispersal, locomotion, reproduction, feeding and host-finding behavior [11]. Exposure of the potato psyllid, *Bactericera cockerelli* to imidacloprid resulted in reduced probing time, increased periods of rest and the ultimate abandonment of leaflets [12]. The probing behavior of *Frankliniella fusca* was altered on imidacloprid and cyantraniliprole treated peppers [13]. In this context the present study was conducted to understand the feeding behaviour of imidacloprid resistant *N. lugens* population.

2. MATERIALS AND METHODS

2.1 Insects

The laboratory strain (LS) of *N. lugens* was obtained from Bayer Biosciences, Hyderabad which was being maintained in glasshouse for about five years without exposure to any insecticides. Further, LS was maintained in the glass house at ICAR-Indian Institute of Rice Research, Hyderabad for 10 generations without exposure to any insecticides. The resistant strain (RS) was continuously selected from the LS for 10 generations by exposing to sub-lethal doses of imidacloprid at each generation. The third instar nymphs were sprayed with sub-lethal doses of imidacloprid using a pneumatic hand sprayer and the surviving nymphs were advanced to the next generation. These strains were maintained in insect proof cages on 45-day old potted rice plants (TN-1) at 27 ±1 °C temperature, 70-80 per cent relative humidity and 16:8 h light:dark photoperiod.

2.2 Insecticide

Commercial imidacloprid 17.8SL formulation (Confidor, Bayer Crop Sciences) was used for the experiments.

2.3 Feeding test

The feeding behaviour of LS and RS strains of *N. lugens* was assessed by measuring honeydew excretion, which serves as an indicator of their feeding preferences and efficiency. Feeding capacity of *N. lugens* was determined by ninhydrin method [14] with suitable modifications. The lower 10 cm stems from 50-days old rice plant were cut, shade-dried and dipped in the imidacloprid solution (300 ppm) for 30s. Distilled water without any insecticide served as a control. A treated stem was inserted through the centre of a 15 cm diameter Whatman No. 1 filter paper, which was placed on a plastic card on top of a cup. A layer of water was maintained in the cup to touch the plant roots. Finally, each stem was enclosed within an inverted cup. Three newly formed brachypterous females were released per treatment. There were five replications per treatment. The insects were allowed to feed for 24 h. The filter papers with honeydew deposition were collected and treated with 0.001% ninhydrin in acetone solution followed by oven drying at 100°C for 5 minutes. Due to their amino acid content honeydew stains appeared violet or purple and these coloured areas were copied on a tracing paper and measured using Image J software.

2.4 Probing test

One seven-day-old rice seedling (variety TN-1) was placed in a test tube (25 mm X 150 mm) containing a 5 mm layer of water at the bottom. One newly formed brachypterous female was introduced into the tube and the tube opening was covered with a muslin cloth. After 24 hours, the insects were removed and the seedlings were stained with 1% aqueous solution of Erythrosine-B dye for an hour. Each treatment was replicated five times. Subsequently, the feeding punctures, also known as ‘probing marks’ were examined and counted under a stereo zoom microscope (Olympus, SZX 10).

2.5 Statistical Analysis

Data were subjected to one-way ANOVA and treatment means were separated by LSD (P=0.05) (SAS Institute, 2008).

3. RESULTS AND DISCUSSION

The area of honeydew production in LS strain was 5.44 and 1.86 cm^2 in imidacloprid treated and untreated conditions, respectively. Whereas, in RS strain it was 7.12 and 14.63 cm^2 respectively. Thus, the honeydew production was significantly higher in RS strain compared to LS strain in both the treated and untreated conditions. Homopteran insects feed on phloem sap that is rich in water and excrete excess water through the ‘filtration chamber’ mechanism in the form of honeydew containing sugars, amino acids, lipids, and waxes. Quantification of honeydew excretion serves as an indirect but precise measure of *N. lugens* feeding activity, offering a straightforward bioassay for *N. lugens* feeding behaviour. The energy requirements of RS insects may be higher due to the diversion of energy to meet the demands of detoxification pathways [6]. A similar impact on the feeding behavior was reported in other sap-feeding insects. In *Myzus persicae* at low concentrations of imidacloprid, a reduction in honeydew excretion, loss of weight, restless behaviour, and movement from treated to untreated leaves was observed indicating antifeeding properties of this compound [15]. *Bemesia tabaci* feeding on imidacloprid treated cotton leaf discs showed significantly low honeydew excretion and fecundity compared to the untreated control [16]. In contrast, Chen et al. [17] observed that imidacloprid-susceptible *Diaphorina citri* feeding on citrus exhibited significantly more bouts associated with intercellular pathway, phloem penetration, phloem salivation, and non-probing activities than imidacloprid-resistant counterparts. However, there were no differences observed in the frequency or duration of phloem ingestion or xylem feeding between susceptible and resistant *D. citri*.

Table 1. Feeding behaviour of Laboratory (LS) and Resistant (RS) strains of *N. lugens*

Treatment	Area of Honeydew (cm^2)	No. of probes
LS-Untreated	5.44^{ab}	7^{a}
LS-Treated	1.86^{a}	16^{a}
RS-Untreated	14.63^{c}	11^{a}
RS-Treated	7.12^{b}	31^{b}
LSD (P=0.05)	**4.94**	**9.68**

In a column means with same letters do not differ significantly, LSD (P=0.05)

The probing test revealed an increased probing activity in both the LS and RS strains in imidacloprid treated seedlings compared to the untreated. The number of probing marks by LS and RS strains in treated seedlings were 16 and 31, whereas in untreated seedlings were 7 and 11, respectively. In addition, the number of probes by RS under treated conditions was significantly higher as compared to the rest of the treatments. In homopterans, the piercing organ is the stylet that secretes a sheath after penetration and it remains within the plant tissues even after withdrawal of the stylets and could be easily visualized by histological staining. Before the insect stylet reaches the phloem, multiple attempts are made to find a suitable site for feeding. Insect feeding is modulated by the complex mechanisms that respond to internal and external signals [18]. Wang et al. [19] reported that imidacloprid resistant aphids showed increased activity in searching for a suitable feeding site. Imidacloprid resistant aphids showed a higher frequency of apoplastic stylet probing compared to the susceptible aphids. The duration of phloem ingestion was notably increased in resistant aphids on imidacloprid treated plants in contrast to control plants. Whereas, imidacloprid significantly reduced the capacity of susceptible aphids to locate and feed from the phloem. Zhu et al. [20] revealed that *Sogatella furcifera* when exposed to plants treated with triflumezopyrim concentrations of LC_{10}, LC_{50} and LC_{90} through direct contact method revealed reduction of 27.5, 33.5 and 34.3 per cent probing frequencies, respectively compared to the untreated control.

4. CONCLUSION

Our results revealed that RS strain of *N. lugens* has higher feeding capacity in terms of probing behaviour and honeydew production as compared to the LS strain. The higher feeding capacity might be due to higher energy requirements in resistant strains to meet the demands of detoxification pathways. Higher metabolic energy demand in resistant populations influence the developmental parameters negatively, imposing fitness cost. Thus provide an opportunity for the reversal of the insecticide resistance in the crop ecosystem on withdrawal of the selection pressure.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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