RESEARCH ARTICLE

# ASSESSMENT OF HONEY DEW EXCRETION BY NON -TARGET BPH, *NILAPARVATA LUGENS* STAL.ONDIFFERENT IR-64 *BT* RICE EVENTS

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**Abstract:** The experiment was undertaken at greenhouse of Entomology and Department of Plant molecular biology &biotechnology, CoA, Raipur during 2014 and 2015.Area marked due to honey dew excretion by BPH under different IR64 Bt rice events ranged from 15.52 to 24.85 $mm^2$. The maximum marked area (24.85 $mm^2$) was observed in IR-64-C followed by TN-1-C (23.58 $mm^2$) with minimum in Ptb-33-C (15.52 $mm^2$) during 2014. Whereas during 2015, new starved female was released and new filter paper was kept inside the funnel to receive the honey dew in all the rice events were ranged from 11.72 to 20.43 $mm^2$. The maximum marked area (20.43 $mm^2$) was observed in IR-64-4 followed by IR-64-1 and TN-1-C (23.58 $mm^2$), respectively and minimum in Ptb-33-C (11.72 $mm^2$). On the basis of two years, pooled mean of honey dew area marked under different rice events was ranged 13.62 to 21.43 $mm^2$. The highest honey dew excreted on IR64 Bt events was noticed (21.43 $mm^2$) in IR-64-4 followed by TN-1-C (20.84 $mm^2$) and minimum in Ptb-33-C (13.62 $mm^2$) within 24hrs. releasing of BPH. The descending order of honey dew excretion by starved female on Bt events was as IR-64-4> TN-1-C > IR-64-C> IR-64-1> IR-64-2> Ptb-33-C. The area of honey dew excretion by female on *Bt* rice and on non-transgenic control rice plants did not differ significantly.

**Keywords***:* Bt protein, Non-target insect BPH, Honey dew excretion

## INTRODUCTION

Rice is the most remunerative crop stands first among all food grain and is staple food for more than half of world's population. Insect pest are one of the major constraints of high tech agriculture and pesticides use is necessary .The transgenic plants expressing insecticidal properties are becoming environmentally safe alternatives to chemical pesticides. Genetically modified crop containing crystal protein from the bacterium *Bacillus thuringiensis* (Bt) was grown on 26.3 million ha worldwide in the year 2005 (James, 2005). Bt rice has the potential to eliminate yield losses caused by lepidopteron pests up to 2%-10% of Asia's annual rice yield of 523 million tons (High *et al.,* 2004). Genetically modified crops had provided economic benefits to growers and also offer a promising alternative to chemical insecticides for control of lepidopteran pests in rice (Zhu, 2001; High *et al.,* 2004). Zhou *et al*., (2004) had detected the impact of Bt rice on non -target *Nilaparvata lugens* and he did not find any difference in feeding and oviposition behavior. In recent years, rice stem borers had developed resistance to some most commonly used insecticides in China and other rice growing countries. The transgenic rice on target lepidopteran pests is a important tool both for pest management and insecticide resistance management. The Bt rice has effectively controlled the three species of stem borers (*C. suppressalis, S. incertulas, S. inferens*) and leaf folder (*C. medinalis*) as reported by Tu*et al.* (2000).The rice field has highly diverse and interlinked insect pest species of herbivores, predators, and parasitoids. These are an essential component of biological control and are one of the fundamentals of insect management strategies in rice (Schoenly *et al* 1998). This importance was previously exemplified by the outbreaks of brown plant hopper, *Nilaparvata lugens* (Homoptera:Delphacidae) that resulted from the excessive use of insecticides in early 1997 (Gallagher *et al*1994). For biological control in managing the balance of insect pest population in rice fields, it is essential to assess the effect of novel insecticides such as *Bt* on non-target insects and their predators/parsitoids. Mirid bug, *Cyrtorrhinus lividipennis* (Hemiptera: Miridae) survives predaciously by feeding on *N. lugens* larvae and nymphs in the rice ecosystem. Insight into the potential effect of the deployment of transgenic *Bt*rice on the population dynamics of other non-target insects and their predatory organisms can be gained by evaluating the effects of Bt toxins on life-history parameters of brown planthopper and mirid bug feeding on *Bt* rice and control non-*Bt* rice.

This study was undertaken to assess the effect of Bt toxins on life-history parameters of brown plant hopper, a non-target insect of rice to understand the secondary exposure of Bt toxins. *N. lugens* is a major pest of rice and an important constituent of the

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population structure of the rice growing area of Asian countries.

## MATERIAL AND METHOD

### Experimental details

The experimentalwas undertaken at glasshouse of Entomologyand Department of Plant molecular biology & biotechnology, College of Agriculture, Raipur during 2014 and 2015.

### Mass culture of BPH

The macropterous females of *N. lugens*50 to 100 per field were collected from Entomological rice fields of IGKV,Raipur. These females were pooled and allowed to oviposit on caged rice plants of Taichung Native-1 (TN-1), a rice genotype that does not contain any genes with resistance against these pest species. From the resulting progenies,colonies weremaintained on susceptible rice variety TN-1.The culture of BPH is being maintained throughout the experimental period in the air cooled glass house in the Department of Entomology at 30°C ± 5°C on potted TN1 variety of rice. BPH were reared on 40 to 45 days old potted TN1 plants inside a rearing cage of 75 x 75 x 75 cm size, consisting of wooden frame with small window on front side and fine wire mesh on top and other sides. Cages were mounted on cemented platform having water level of 7.5 cm. Potted TN1 plants were placed inside the rearing cages for egg laying along with at least 60 pairs of BPH per pot. After 2-3 days the females starts egg laying inside the leaf sheath at the basal portion of paddy plants. After the emergence of nymphs from plants BPH pairs were transferred to another TN1 pots with the help of aspirator for egg laying.The colonies were grown on an artificial diet before releasing them onto transgenic *Bt* rice plants.The BPH population was taken from mass culture maintained in the glasshouse. Standard evaluation technique developed by IRRI was adopted to evaluate different Bt/non Bt lines. Observations were recorded on the honey dew excretion for all the rice events.

### Collection and quantification of honeydew of *N. lugens*

The parafilm sachet method as described by Pathak *et al.* (1982) was used to collect honeydew from the female adults of *N. lugens* fed on transgenic *Bt*rice and their counterpart control rice lines. Three sachets were attached to the stems of each plant. Plants were grown in the transgenic greenhouse arranged in a complete block design (CBD) at 25-30°C under a natural photoperiod of approximately L12:D12. Three fifth instar nymphs were randomly selected from *N. lugens* colonies maintained on caged TN-1 rice plants and starved for 2 hrs were then placed singly into inverted plastic cups enclosing a plant stem and allowed to feed for 24 hrs. After 24hrs, the filter papers were collected and the area of honeydew spots on the filter paper was determined by placing the transparent sheets with 1 mm grids on top of the filter paper. Blue and white spots, produced by alkaline and acidic honeydew deposition, respectively, were determined separately. The honeydew excreted was collected with the help of a micropipette and placed into 1.5-ml micro centrifuge tubes. The honey from each plant was pooled and stored at -20°C/-80°C. There were three replicates for each transgenic line and three for their control plants.The presence of Cry protein in the honeydew secretions was analyzed using the CryIAb/CryIAc ELISA kit (Envirologix, USA) at 450 nm as per the manufacturer's instructions Reading for each replication was recorded separately. Honeydew production on rice plants at the maximum tillering stage was quantified using the bromocresol green technique (Pathak and Heinrich 1982).

**Table 1.** Honeydew excretion by BPH on differenttransgenic rice (including control) lines during 2014 and 2015

| Transgenic rice | Honeydew excretion by BPH within 24h ($mm^2$) | | |
|---|---|---|---|
| | 2014 | 2015 | Pooled mean |
| IR-64-1 | 18.98 (4.41) | 18.10 (4.31) | 18.54 |
| IR-64 -2 | 19.10 (4.43) | 17.89 (4.29) | 18.49 |
| IR-64-3 | 20.18 (4.55) | 14.93 (4.93) | 17.56 |
| IR-64-4 | 22.42 (4.79) | 20.43 (4.58) | 21.43 |

| | | | |
|---|---|---|---|
| IR-64-C | 24.85 (5.03) | 14.74 (4.90) | 19.80 |
| TN1-C | 23.58 (4.91) | 18.10 (4.31) | 20.84 |
| Ptb33-C | 15.52 (4.00) | 11.72 (3.50) | 13.62 |
| **SEM** | **4.32** | **2.90** | |
| **CD at 5%** | **7.57** | **5.09** | |
| **CV (%)** | **36.23** | **30.37** | |

*The values in parenthesis are square root transformed values

* Three replications for each treatment

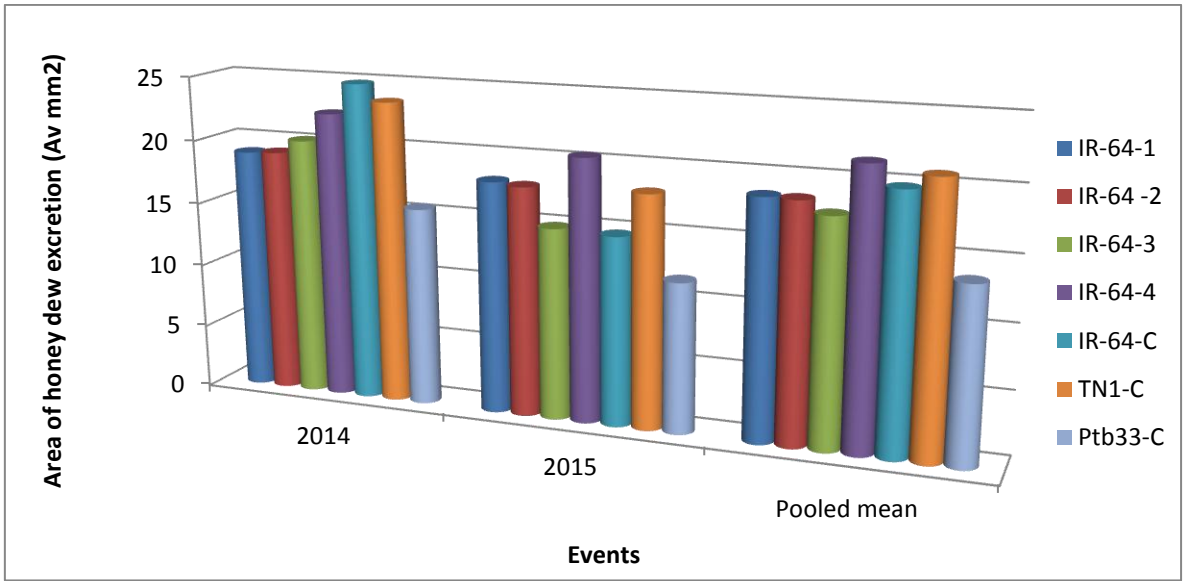


**Fig.1.** Honeydew excretion by BPH on differenttransgenic rice (including control) lines during 2014 and 2015

## RESULT AND DISCUSSION

Area marked due to honey dew excretionby BPH under different IR64 Bt and non-Bt events ranged from 15.52 to 24.85 $mm^2$. The maximum marked area (24.85 $mm^2$) was observed in IR-64-C followed by TN-1-C (23.58 $mm^2$) with minimum in Ptb-33-C (15.52 $mm^2$) during 2014. Whereas during 2015, new starved female was released and new filter paper was kept inside the funnel to receive the honey dew in all the treatment and replications were ranged from 11.72 to 20.43 $mm^2$. The maximum marked area (20.43 $mm^2$) was observed in IR-64-4 followed by IR-64-1 and TN-1-C (23.58 $mm^2$), respectively and IR-64-4 (22.42 $mm^2$)and minimum in Ptb-33-C (11.72$mm^2$). On the basis of two years, pooled mean of honey dew area marked under different treatments was ranged 13.62 to 21.43 $mm^2$. Maximum honey dew excreted onIR64 Bt and non-Bt events was noticed (21.43 $mm^2$) in IR-64-4 followed by TN-1-C (20.84 $mm^2$) and minimum in Ptb-33-C (13.62 $mm^2$) within 24hrs releasing of BPH (table-1 & Fig.-1).

The honeydew excreted by females of *N. lugens* was quantified by measuring the area of white and blue spots on bromocresol green-treated filter paper. White spots, indicating the deposition of acidic honeydew from xylem feeding, were significantly fewer in the transgenic *Bt* rice lines than in the corresponding control plants. whereas, the area of blue spots indicating the feeding from the phloem region was significantly higher in transgenics than in the control rice plants. Our results clearly indicate that BPH feeding on *Bt* rice has shown a preferred feeding behavior from phloem tissues. The results observed with the honeydew excreted by *N. lugens* females feeding on *Bt* rice observed are similar to those in an earlier study by Bernal *et al* (2002) where toxic protein was detected in honeydew and phloem tissues of *Bt* rice plants.

On the basis of honey dew area marked under different treatments at 48hrsreleasing of BPH, it may be stated that starved female feeding on IR-64-4Bt plant, honey dew excretion increased gradually and it was higher to other Bt and non- Bt control whereas, in case ofPtb-33-C treated plant, the insect start

feeding subsequent but thequantum of feeding was low as compared to other Bt events within 24hrs.The descending order of honey dew excretion bystarved female on Bt events wasas IR-64-4> TN-1-C> IR-64-C> IR-64-1> IR-64-2>Ptb-33-C. Are of honey dew excretion by female on *Bt* rice and on non-transgenic control rice plants did not differ significantly.

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