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Host selection behavior of leaf folder, *Cnaphalocrocis medinalis* in relation to volatiles of three rice varieties

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Abstract
In this study, we investigated the preference of adult rice leaf folder moths and third instar larvae towards three rice varieties: TN 1, W1263, and TKM 6. Ten adult moths were released into a cage containing rice plants of the three varieties. The moths showed a clear preference for TN 1, with a mean number of 1.67 moths choosing it after 15 minutes, and the preference increased over time, reaching 5.60 moths after 24 hours. W1263 was the second most preferred variety, with 4.40 moths choosing it after 24 hours. In contrast, TKM 6 was not preferred by any of the moths throughout the study period. Similarly, third instar leaf folder larvae were placed equidistant from the fresh leaves of the three rice varieties in a separate cage. The larvae also displayed a preference for TN 1, with 2.20 larvae choosing it within 30 minutes and 6.90 larvae preferring it after 24 hours. W1263 was preferred from one hour after release, with 3.10 larvae attracted to it after 24 hours. In contrast, TKM 6 was least preferred by the larvae, with only 0.10 larvae being attracted after 24 hours. Therefore the preference of *C. medinalis* to different rice varieties was in the order TN 1 > W1263 >TKM 6.

Keywords: Rice varieties, leaf folder, host plant, volatiles

Introduction
Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world, providing food for over half of the global population. However, rice production is often limited by various biotic stresses, including insect pests such as the rice leaf folder (*Cnaphalocrocis medinalis* Guenee) (Cheng *et al*., 2013) [4]. The rice leaf folder is a major pest of rice in many Asian countries, including India, China, and Japan, and can cause significant yield losses if not controlled. The larvae of the rice leaf folder feed on the leaves of the rice plant, causing characteristic "window-pane" damage that can reduce photosynthesis and ultimately lead to reduced yield (Jena and Kim 2010) [11].
Host selection behavior, a critical aspect of the insect-plant relationship, involves a complex interplay of ecological factors. Among these factors, plant volatiles have emerged as key mediators influencing the foraging and host-plant finding process of herbivorous insects (Bruce *et al*., 2005) [2]. These volatile organic compounds, released by plants into the atmosphere, play essential roles in interplant communication and defense mechanisms. They have been recognized as potent olfactory signals, allowing herbivores to perceive and process crucial information about host plant suitability (Hansson, 1995; Hildebrand and Shepherd, 1997) [8, 9].
The role of plant volatiles in herbivorous insect host selection has been extensively studied in various systems. Researchers have found that the perception of plant volatiles provides fundamental cues for herbivorous insects to distinguish between suitable host plants and non-host plants (Pickett *et al*., 1993; Hansson, 1995; Hildebrand and Shepherd, 1997; Dicke and Loon, 2000; Bruce *et al*., 2005; Binyameen *et al*., 2012) [2, 8, 9, 14, 5, 1]. Additionally, herbivorous insects have evolved a sensitive olfactory system to detect these chemical cues, enabling them to locate and assess potential hosts efficiently (Hildebrand and Shepherd, 1997) [9].
However, understanding the intricacies of the rice leaf folder's host selection behavior is paramount in developing effective and sustainable pest management strategies. Therefore, this research aims to investigate the host selection behavior of the rice leaf folder in relation to plant volatiles of different rice genotypes.

Materials and Methods
The study was conducted at the ICAR- Indian Institute of Rice Research during the years 2021- 2022.

The rice varieties used in the study were TKM 6, TN 1, and W1263. Plants of each variety that were used in the experiment were thirty days old. The experiment had three treatments and ten replications. For each replication ten insects were used.

Plant culture

The Rice cultivars used in this study were TN 1, PB 1, W 1263, TKM 6, BPT 5204 and DRRH3. These were cultured in trays for 12 days and were then transplanted into pots with single plant in each pot. Plants at thirty days after transplanting were used for the experiments. Plantings were continued at regular intervals so that enough plants of uniform age were available for experiments.

Table 1: Genetic background of rice varieties

SI. No.	Variety	Parentage	Pest response	Pest/disease	Reference
1	TKM 6	GEB 24 X Co 18.	Multiple Resistance	Stem borer, Green Leaf Hopper, Brown Plant Hopper, Leaf folder, Thrips, Bacterial Leaf Blight, Rice Tungro Virus and Grassy Stunt	Jayaraj and Uthamasamy, 1990 [10].
2	Taichung Native 1 (TN1)	Dwarf Chow-wu-gen x Tsai-Yuan-Chunj	Susceptible	Green leafhopper,leaf folder,White backed plant hopper, brown plant hopper, gall midge, rice stem borer, Bacterial Blight	Li *et al*., 2019
3	W1263	Donor accession	Resistant	Stem borer, Leaffolder	Padmavathi *et al*., 2019 [13]

Insect Culture

To establish the *C. medinalis* colony, naturally occurring adults were collected from rice fields and placed in cages on rice plants of the variety TN1 to mate and lay eggs. A cotton swab soaked in sucrose solution was provided as a food source. The eggs were left on the same plant to hatch, and once the larvae began feeding on the plants, they were moved to new potted plants for further development. Experiments were conducted using late third instar larvae.

Host selection behavior of leaf folder, *C. medinalis*

To study the effect of volatiles of different rice genotypes on the host selection behavior of leaf folder *C. medinalis*, the attraction of adult females and third instar larvae to different rice genotypes was tested.

The attraction of *C. medinalis* to the above three rice genotypes, namely TKM 6, TN 1, and W1263, was tested. Potted plants of each variety were placed in a cage (100 x 100 x 120 cm) at equidistance to each other, and an open jar containing ten leaf folder female moths was placed in the middle of the cage. The number of leaf folder moths landing on each plant of the respective variety was recorded at different time intervals of five minutes, 15 minutes, 30 minutes, one hour, three hours, five hours and 24 hours after the release of the insects.

To determine the attraction of larvae to the three rice varieties, a tray (45 x 30 cm) lined with moistened filter paper was taken, and leaves of each rice variety cut into pieces were placed on the edges of the tray. A petri plate containing twenty third instar larvae of leaf folder was placed in the middle of the tray without a lid. The number of larvae that moved on to each leaf of the respective variety was recorded at different time intervals of five, 15 and 30 minutes, one, three, five and 24 hours after the release of the larvae.

Results

The attraction of adult moths and third instar larvae of *C. medinalis* to different rice genotypes (TN 1, W1263, and TKM 6) was tested in a cage. The response of the insects was observed at various time intervals after their release.

Regarding the attraction of leaf folder moths, there was no significant preference observed in the first 15 minutes after their release (Figure 1). However, at 30 minutes, a mean number of 3.22 moths preferred TN 1 followed by W1263 (2.11 moths).

Table 2: Number of leaf folder moths attracted to three different rice varieties

SI. No.	Varieties	5 mins	15 mins	30 mins	1 hr	3 hrs	5 hrs	24 hrs
1	TN 1	0.00 (0.70)	1.11^a (1.55)	3.22^a (2.29)	3.60^a (2.39)	5.10^a (2.36)	6.50^a (2.64)	5.60^a (2.46)
2	W 1263	0.00 (0.70)	1.67^b (1.79)	2.11^b (1.95)	3.10^b (1.89)	3.40^b (1.97)	4.20^b (2.16)	4.40^b (2.21)
3	TKM 6	0.00 (0.70)	0.00^c (0.70)	0.00^c (0.70)	0.50^c (1.00)	0.60^c (1.04)	0.00^c (0.70)	0.00^c (0.70)
	SD		0.85	1.64	1.66	2.27	3.07	3.17
	CV		1.00	0.31	0.23	0.25	0.27	1.00
	CD		0.08	0.13	0.12	0.10	0.11	0.11

Figures in parentheses are $\sqrt{x} + 0.5$ transformed values for mean number of eggs parasitized

At one hour, TN 1 was preferred by 3.60 individuals followed by W1263 (3.10 moths) and TKM 6 (0.50 moths) (Table 2). At three hours after release, significantly more moths (5.10 individuals) made a choice towards TN 1 followed by W1263. The variety TKM 6 was not preferred by any of the moths at any of the time intervals. After five hours of release, the mean number of moths choosing TN 1 increased to 6.50 while W1263 attracted 4.20 moths. After 24 hours of initial release, the highest number of moths (5.60) preferred TN 1 followed by W1263 with 4.40 individuals. Therefore, the preference of the leaf folder moths towards different rice genotypes was in the order TN 1 > W1263 >TKM 6.

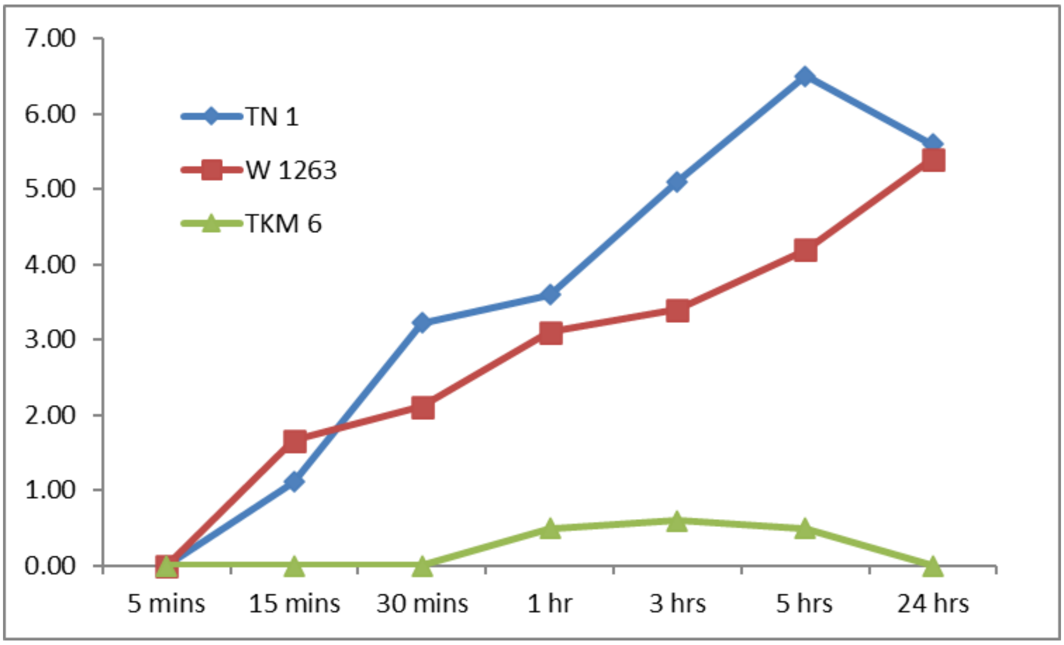

Fig 1: Attraction of *C. medinalis*moths to three different rice varieties

In the case of leaf folder third instar larvae, they showed a significant preference for TN 1 and TKM 6 within 30 minutes of their release (Figure 2). However, W1263 was preferred only from one hour after their release.

Table 3: Number of leaf folder moths attracted to three different rice varieties

SI. No.	Rice varieties	5 mins	15 mins	30 mins	1 hr	3 hrs	5 hrs	24 hrs
1	TN 1	0.00 (0.70)	0.00 (0.70)	2.20^a (1.64)	2.30^b (1.67)	5.30^a (2.41)	4.70^b (2.28)	6.90^a (2.72)
2	W 1263	0.00 (0.70)	0.00 (0.70)	0.00^c (0.70)	3.10^a (1.89)	3.30^b (1.95)	5.20^a (2.28)	3.00^b (1.87)
3	TKM 6	0.00 (0.70)	0.00 (0.70)	1.10^b (1.26)	0.00^c (0.70)	0.00^c (0.70)	0.90^c (1.18)	0.10^c (0.77)
	SD			1.10	1.61	2.68	2.35	3.18
	CV			0.33	0.30	0.31	0.22	0.32
	CD			0.06	0.06	0.08	0.23	0.12

Figures in parentheses are $\sqrt{x + 0.5}$ transformed values for mean number of eggs parasitized

After one hour, there was a significant difference in preference with 3.10 larvae, 2.30 larvae, and 0.00 larvae observed on varieties W1263, TN 1, and TKM 6, respectively (Table 3). At five minutes of release, the preference of the larvae was significant with 5.20, 4.70, and 0.90 larvae on the varieties W1263, TN 1, and TKM 6, respectively. Finally, at 24 hours after release, 6.90 larvae were attracted to TN 1, 3.00 larvae to W1263, and only 0.10 larvae to TKM 6. Therefore, the preference of the third instar larvae of the leaf folder was in the order TN 1 > W1263 >TKM 6 after 24 hours of their release.

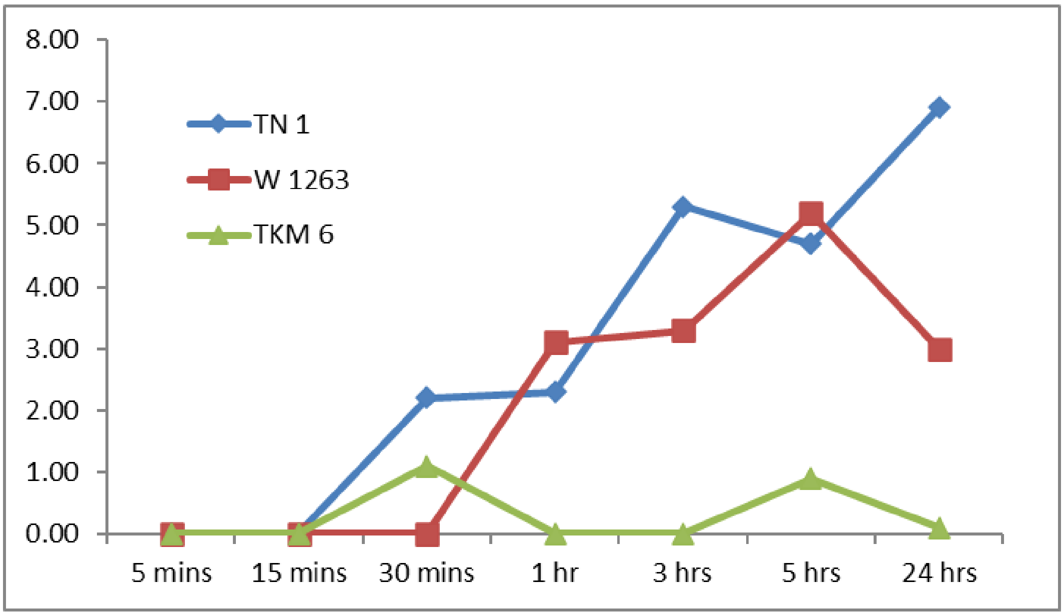

Fig 2: Attraction of *C. medinalis*third instar larvae to three different rice varieties

These results suggest that the rice genotype TN 1 is more attractive to both adult moths and third instar larvae of the leaf folder compared to W1263 and TKM 6. The preference of *C. medinalis* to different rice varieties was in the order TN 1 > W1263 >TKM 6.

Discussion

Olfaction is an important sense that herbivores use to locate their host plants for feeding and oviposition. Studies have reported that herbivores recognize their host plants based on the blends of volatiles emitted by the plant (Bruce and Pickett, 2011) [3]. Herbivores utilize their sensitive olfactory system to perceive plant volatiles as olfactory signals (Hansson, 1995; Hildebrand and Shepherd, 1997) [8, 9]. Plant volatiles consist of hundreds of compounds, including green leaf volatiles, terpenoids, acids, aliphatics and aromatics (Fraser *et al*., 2003) [6]. Detection of a unique compound is sufficient to elicit a response in a number of insect species (Fraser *et al*., 2003; Binyameen *et al*., 2012; Ghaniniaet *al*., 2014) [6, 1]. Saxena and Pathak (1979) [15] reported that both resistant and susceptible plants elicit behavioral responses in herbivores. The allelochemicals in different rice varieties were responsible for eliciting behavior responses of brown planthopper (Saxena and Pathak, 1979; Saxena and Puma, 1979) [15, 16]. Genomic and phenotypic evaluation of susceptible rice variety TN1 implies that the plant's response against insects and insect performance on the plant were the two indicators for determining the susceptibility of TN1 to *C. medinalis* and *N. lugens* (Li *et al*., 2019).

Our study suggests that *C. medinalis* larvae and moths exhibited a greater preference for settling on susceptible TN 1 rice plants compared to the resistant W1263 and TKM 6. Similarly, the number of eggs laid by *C. medinalis* adults was also lower on resistant plants compared to susceptible ones. The volatile profile of the plant cultivar has a role in host selection behavior of the herbivore. Medina and Heinrichs (1986) reported that the volatile allelochemicals have a role in imparting resistance to *C. medinalis*. The odors of resistant rice plants repelled *C. medinalis* larvae. Larvae feeding on leaf cuts treated with extracts of odors from resistant rice plants reported higher mortality of the herbivore. The variation in attraction of herbivores to different rice genotypes suggests that the difference in volatiles produced by plants resulted in behavioral responses in the insects.

Declaration of interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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