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# Olfactory response of insect predators of rice brown plant hopper (*Nilaparvata lugens* (Stal.)) to flower volatiles

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## Abstract
Flowers of five different plant species *viz.*, green gram (*Vigna radiata*), marigold (*Tagetes erecta*), sunhemp (*Crotalaria juncea*), cowpea (*Vigna unguiculata*), okra (*Abelmoschus esculentus*) along with control (only air without flowers) were tested for their effectiveness in attracting predatory mirid bug *Cyrtorhinus lividipennis* (Reuter) and coccinellid predators - *Micraspis discolor*, *Harmonia octomaculata* and *Coccinella transversalis* in a six arm olfactometer at Indian Institute of Rice Research, Hyderabad, Telangana, India. Flowers of different plant species acted as an odour source to attract different predators. *C. lividipennis* attraction was high in sunhemp followed by cowpea, marigold and okra and the lowest population was recorded in green gram. All three predatory coccinellids were attracted more to cowpea followed by okra and green gram and the lowest population was recorded in sunhemp followed by marigold flowers. These flowering plant species could be used in rice systems as bund /border crop to attract brown planthopper predatory mirid bug and coccinellids and suppress plant hopper infestation in rice main crop.

**Keywords:** Brown plant hopper, coccinellids, *Cyrtorhinus lividipennis*, Flower volatiles, olfactory response

## Introduction
Rice planthoppers (Hemiptera: Delphacidae) including the brown planthopper (*Nilaparvata lugens* (Staal)), white-backed planthopper (*Sogatella furcifera* (Horvath)), and small brown planthopper (*Laodelphax striatellus* (Fallen)), are the most destructive insect pests of rice in Asia and outbreaks have occurred frequently in the last decade as a result of insecticide resistance and a break down in resistance genes in rice cultivars [1]. Planthopper impact is so severe that it is now considered to be a substantial threat to world food security [2]. In Asian rice systems, the predatory mirid bug, *Cyrtorhinus lividipennis* (Reuter) (Heteroptera: Miridae) is an important natural enemy of eggs and young nymphs of rice planthoppers [3]. Natural enemy performance can be increased by growing potential nectar plants which can act as carbohydrate sources to parasitoids in rice crops.

Conservation biological control of plant hoppers by way of attracting parasitoids and predators through nectar plants is a novel ecofriendly approach. However, three key issues influence the use of food plants in agricultural systems as a means to boost biological control of pests. First, not all plants provide equivalent benefit to natural enemies. Nectar, for example, must have an appropriate profile of sugars and be produced by flowers that are attractive to the predator or parasitoid and that allow physical access to the nectaries [4]. Second, the plant food must not benefit pest species such as moths. This necessitates the identification of ‘selective’ food plants that support feeding only by key natural enemy species [5, 6, 2]. Third, the plants selected should ideally have benefits beyond pest management such as constituting secondary crops or providing complementary ecosystem services [7].

Sesame (*Sesamum indicum*) nectar greatly improved longevity, fecundity and handling time for *A. nilaparvatae* and *A. optabilis* [8] and longevity, predation and handling time in predatory mirid bug (*Cyrtorhinus lividipennis*). Also reported that sesame has potential to enhance biological control of rice planthoppers [9, 10].

Coccinellidae respond to various cues when they seek food, shelter or oviposition sites [8]. It has been proposed that lady beetles find their prey using visual stimuli [11, 6, 2]

and that adults and larvae of lady beetles can perceive color contrasts [3, 2]. Proposed that coccinellids can visually identify their prey by their size and shape. Other studies suggest that lady beetles use olfactory stimuli to find their prey [13, 14, 10]. Showed that *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) and *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) can detect prey by odor [13, 15].
Accordingly, the present study was conducted to study the effectiveness of certain flowering/nectar plant species in attracting *C. Lividipennis*, coccinellids through their odour, so that suitable plants could be grown on the bunds surrounding rice fields to promote biological control of planthopper pests.

## Materials and methods

Laboratory studies were conducted to study the response of plant volatiles of five flowering plants *viz*., green gram (*Vigna radiata*), marigold (*Tagetes erecta*), sun hemp (*Crotalaria juncea*), cowpea (*Vigna unguiculata*), okra (*Abelmoschus esculentus*) on natural enemies by using olfactometer at Indian Institute of Rice Research (IIRR) Rajendranagar, Hyderabad. The attractiveness of different flowers to the natural enemies was assessed with the help of six arm olfactometer by connecting each arm to a particular odour source.

### Design of olfactometer

The six arm olfactometer consisted of a release chamber at the centre and was connected by a pure air inlet tube for creating pure air; the inlet tube was connected to a blower through an inlet chamber fitted with a filter and an air flow meter. The blower unit consisted of a battery operated mini fan fitted with a glass tube to generate pure air.

### Natural enemies used in the olfactometer studies

The most common and abundantly found natural enemies of rice BPH *viz.*, predatory bug, *Cyrtorhinus lividipennis* (Reuter) (Heteroptera: Miridae) and predatory coccinellids, like *Coccinella transversalis*, *Micraspis discolor* and *Harmonia octomaculata* were tested for their preference to the volatiles of flowering plants.

### Response of natural enemies to flower volatiles in the olfactometer

The flowers of each treatment were picked before commencing assays each day and the cut end of each flower was embedded into a cotton wool swab soaked in distilled water to keep the freshness of the cut flower. The studies were done between 9:00 and 15:00 in the climate room (26 ± 1 $^0$C). About 10 flowers each of green gram, cowpea, sunhemp and okra and one flower of marigold were inserted separately into each arm and the sixth arm was maintained as a control (air without any flowers). After inserting the flowers in to the arms of the olfactometer, an airstream was generated and led through a flow meter with activated charcoal. The predators used in the study were allowed to acclimatize to the laboratory for 30 minutes before using them in assays. Twenty predators of each species were released into the central chamber of the olfactometer through the hole. The observations were recorded on number of predator/ parasitoids settled on each arm at 5, 10, 15 and 20 minutes after release (MAR). The experiment was replicated three times. After conducting the experiment with each predator, the glassware was cleaned and rinsed with double distilled water, before reusing it.

### Statistical analysis

The data obtained from the flower volatile studies were pooled and subjected to CRD analysis of variance (ANOVA) after subjecting the values to square root transformations.

## Results and discussion

### Response of mirid bug, *Cyrtorhinus lividipennis* to flower odours

The olfactory experiments of mirid bug showed that the flowers of all the five plants *viz.*, green gram, cowpea, okra, marigold and sunhemp were highly attractive to the mirid bug when compared to air (control) showing the innate preference of the mirid bug to the flowering plants (Table 1). Among the different flowers, the mirid bug showed significantly more preference towards four flowering plants *viz.*, sunhemp, cowpea, marigold and okra when compared to control at 5 minutes after release (MAR) of the predator into the olfactometer where the number of mirid bugs attracted to these four treatments varied from 2.33 to 3.33 when compared to 1.67 adults attracted to green gram. The same trend continued at 10, 15 and 20 MAR of the predator. The mean values also showed the similar preference to sunhemp flowers (4.00) and the number of mirid bugs attracted to various other flower odours varied from 1.67 to 3.17 while significantly lowest numbers of adults (0.58) were present in the arm containing air without flowers (Fig 1). The order of preference of flowers to the mirid bug in descending order was sunhemp > cowpea > marigold > okra > green gram.
Thus, the results clearly demonstrated that all flower species were attracted to the predator, *C. lividipennis* of rice BPH when compared to control. The results also indicated that although all the flowers were more attractive when compared to control, some of them were more attracted than others. Among the various treatments, the specific predator of BPH *C. lividipennis* was more attracted to the flowers of sunhemp, cowpea and marigold [16]. Reported that the predation performance of *C. lividipennis* on BPH eggs was greatly enhanced after the parental adult was allowed to feed on flowers of four plants *viz.*, *Sesamum indicum*, *Tagetes erecta*, *Trida procumbens* and *Emilia sonchifolia*. They stated that *S. indicum*is was well suited for use as an ecological engineering plant in the margins of rice crops [13]. These results were supported by who reported that, *Sesamum indicum*, *Emilia sonchifolia*, and *Impatiens balsamena* appeared potentially suitable for supporting *Anagrus optabilis* and *Anagrus nilaparvatae* to the extent that adults were attracted to the odours of these flowers. The olfactometer studies conducted by [5] revealed that rice BPH predatory mirid bug, *C. lividipennis* attraction was higher towards the leaves and flowers of sunflower followed by gingelly and cowpea.

### Response of predatory coccinellids to flower odours

**Response of *Coccinella transversalis* to flower odours:** The olfactory studies indicated that out of 5 flowering plants tested, the flowers of 3 plants *viz.*, cowpea, okra and marigold attracted *C.transversalis* starting from few minutes after release and similar trend continued even after 20 MAR. The sunhemp flowers had not attracted the beetles at 5 minutes and 10 minutes and no colonization was found but at 20 MAR, 2.33 adults were attracted to sunhemp when compared 4.67 adults attracted to cowpea (Table 2, Fig. 2). The odour preference of *C.transversalis* to flowers of different plants in descending order was cowpea > okra > green gram > marigold > sunhemp.

**Response of *Micraspis discolor* to flower odours:** The results on olfactory response of *M. discolor* adults to flower odours (Table 3) indicated more colonization of beetles on cowpea, okra and green gram in which the number of adults attracted to flower odours varied from 1.33 to 2.00 compared to few adults attracted to sunhemp (0.33) and marigold (0.67) at 5 MAR. Similar response was recorded at 10 MAR wherein cowpea (2.67), okra (2.33) and green gram (1.67) attracted significantly more number of adults when compared to sunhemp (1.00) and marigold (1.33). The number of adult beetles attracted to okra, cowpea and green gram flowers at 15 MAR varied from 2.67 to 3.33 while the sunhemp (1.67) and marigold (2.00) attracted less number of beetles. Similar trend was observed even after 20 MAR, where in sunhemp attracted a significantly less number of beetles (2.33) when compared to other treatments (Fig 2). The order of preference of flower odour to *M. discolor* was in descending order was copea > okra > green gram > marigold > sunhemp.

**Response of *Harmonia octomaculata* to flower odours:** The predatory beetle *H. octomaculata* was attracted to flower odours of all the flowering plants except sunhemp and the number of adults attracted to these treatments at 5 MAR varied from 1.00 to 2.67 beetles and significantly less number of adults were attracted to sunhemp (0.67) and control (0.33) (Table 4). Similar trend was noticed even after 10 MAR where the number of adults attracted to these treatments *viz.*, marigold, okra, cowpea and green gram varied from 1.67 to 3.33 beetles and only 1.33 adults were colonized in the arm containing sunhemp flowers. Even at 15 MAR also, sunhemp attracted a significantly less number of adults (2.33) when compared to other treatments. The number of adults observed in various treatments except in sunhemp varied from 2.67 to 4.67 at 20 MAR and in sunhemp only 2.00 adults were attracted (Fig 2.). The mean values also showed the similar trend and the order of preference of flower odours to *H. octomaculata* in descending order was cowpea > okra > green gram > marigold > sunhemp.

The present finding is in accordance with [5] who studied the olfactory response of predators like miridbug, rove beetle and cocoinellids towards leaf and flower samples of cowpea, sunflower, gingelly, tomato, brinjal and okra (non-rice crops) by using six arm olfactometer. He reported that there was more number of predators attracted towards the leaves and flowers of sunflower followed by gingelly and cowpea. This may be due to the presence of 2- phenyl ethanol in these plants. Also reported that numbers of predators were attracted towards the leaves and flowers of cowpea. This result was further supported by [17] who revealed that the order of preference of flowers for the coccinellids was maximum in cowpea followed by French bean, lab lab, cluster bean and green gram [7].

## Conclusion

From the present olfactometer studies, it can be concluded that, mirid bug attraction was higher towards sunhemp, cowpea, and marigold flowers and coccinellids like *M. discolor*, *H. octomaculata* and *C. transversalis* were attracted more to cowpea followed by okra and green gram. And hence these flowering plant species could be used in rice systems as bund /border crop, which could attract brown planthopper predatory mirid bug, *C. lividipennis* and coccinellids which leads to the suppression of rice plant hopper infestation in rice main crop. Alternate food sources to natural enemies throughout the crop season and biological control can be achieved by such relatively simple cultural practices.

**Table 1:** Olfactory response of Mirid bug, *Cyrtorhinus lividipennis* to flowers of different plants

| Treatments | Mean number of *C. lividipennis* attracted per arm | | | | |
|---|---|---|---|---|---|
| | 5 MAR* | 10 MAR | 15 MAR | 20 MAR | MEAN |
| Green gram | 1.67 (1.44) | 2.33 (1.66) | 1.33 (1.34) | 1.33 (1.34) | 1.67 |
| Cowpea | 3.00 (1.86) | 2.67 (1.74) | 3.67 (2.02) | 3.33 (1.95) | 3.17 |
| Okra | 2.33 (1.68) | 2.00 (1.56) | 2.67 (1.74) | 2.67 (1.77) | 2.42 |
| Marigold | 2.67 (1.77) | 2.67 (1.94) | 3.00 (1.84) | 3.00 (1.87) | 2.84 |
| Sunhemp | 3.33 (1.95) | 3.67 (1.76) | 4.00 (2.09) | 5.00 (2.30) | 4.00 |
| Control | 1.00 (1.22) | 0.33 (0.88) | 0.33 (1.25) | 0.67 (1.05) | 0.58 |
| C.D. (p=0.01) | (0.35) | (0.69) | (0.98) | (0.51) | |
| SE(m) ± | (0.11) | (0.22) | (0.31) | (0.16) | |

Figures in parentheses were square root transformed values
*MAR=Minutes After Release

**Table 2:** Olfactory response of *Coccinella transversalis* to flowers of different plants

| Treatments (Flowers) | Mean number of *C. transversalis* attracted per arm | | | | |
|---|---|---|---|---|---|
| | 5 MAR | 10 MAR | 15 MAR | 20 MAR | MEAN |
| Green gram | 1.00 (1.17) | 0.67 (1.05) | 1.67 (1.44) | 3.00 (1.77) | 1.59 |
| Cowpea | 1.67 (1.46) | 2.67 (1.77) | 3.33 (1.93) | 4.67 (2.27) | 3.09 |
| Okra | 1.00 (1.17) | 1.67 (1.44) | 2.00 (1.56) | 3.33 (1.86) | 2.00 |
| Marigold | 0.33 (0.88) | 1.33 (1.34) | 2.00 (1.56) | 2.67 (1.95) | 1.58 |
| Sunhemp | 0.00 (0.71) | 1.00 (1.22) | 1.67 (1.46) | 2.33 (1.68) | 1.25 |
| Control | 0.00 (0.71) | 0.00 (0.71) | 0.33 (0.88) | 1.00 (1.22) | 0.33 |
| C.D. (p=0.01) | (0.51) | (0.43) | (0.59) | (0.32) | |
| SE(m) ± | (0.16) | (0.14) | (0.19) | (0.10) | |

Figures in parentheses were square root transformed values
*MAR=Minutes After Release

**Table 3:** Olfactory responses of *Micraspis discolor* to flowers of different plants

| Treatments (Flowers) | Mean number of *M. discolor* attracted per arm | | | | |
|---|---|---|---|---|---|
| | 5 MAR | 10 MAR | 15 MAR | 20 MAR | MEAN |
| Green gram | 1.33 (1.34) | 1.67 (1.44) | 2.67 (1.77) | 3.00 (1.87) | 1.33 |
| Cowpea | 2.00 (1.58) | 2.67 (1.77) | 3.33 (1.93) | 4.00 (2.11) | 2.00 |
| Okra | 1.67 (1.46) | 2.33 (1.64) | 2.67 (1.76) | 3.67 (2.02) | 1.67 |
| Marigold | 0.67 (1.05) | 1.33 (1.34) | 2.00 (1.58) | 3.33 (1.95) | 0.67 |
| Sunhemp | 0.33 (0.88) | 1.00 (1.17) | 1.67 (1.46) | 2.33 (1.68) | 0.33 |
| Control | 0.00 (0.71) | 0.33 (0.88) | 0.33 (0.88) | 0.67 (1.05) | 0.33 |
| C.D. (p=0.01) | (0.39) | (0.61) | (0.50) | (0.46) | |
| SE(m) ± | (0.12) | (0.19) | (0.16) | (0.15) | |

Figures in parentheses were square root transformed values
*MAR=Minutes After Release

**Table 4:** Olfactory response of *Harmonia octomaculata* to flowers of different plants

| Treatments (Flowers) | Mean number of No. of *H. octomaculata* attracted per arm | | | | |
|---|---|---|---|---|---|
| | 5 MAR | 10 MAR | 15 MAR | 20 MAR | MEAN |
| Green gram | 2.00 (1.56) | 2.33 (1.66) | 3.33 (1.95) | 3.67 (2.03) | 2.83 |
| Cowpea | 2.67 (1.76) | 3.33 (1.93) | 4.33 (2.19) | 4.67 (2.27) | 3.75 |
| Okra | 2.33 (1.64) | 2.67 (1.74) | 3.67 (2.02) | 4.00 (2.11) | 3.17 |
| Marigold | 1.00 (1.17) | 1.67 (1.46) | 3.00 (1.84) | 2.67 (1.77) | 2.08 |
| Sunhemp | 0.67 (1.05) | 1.33 (1.34) | 2.33 (1.68) | 2.00 (1.56) | 1.58 |
| Control | 0.33 (0.88) | 0.67 (1.05) | 0.33 (0.88) | 0.67 (1.05) | 0.50 |
| C.D. (p=0.01) | (0.63) | (0.65) | (0.45) | (0.48) | |
| SE(m) ± | (0.20) | (0.22) | (0.15) | (0.13) | |

Figures in parentheses were square root transformed values
*MAR=Minutes After Release

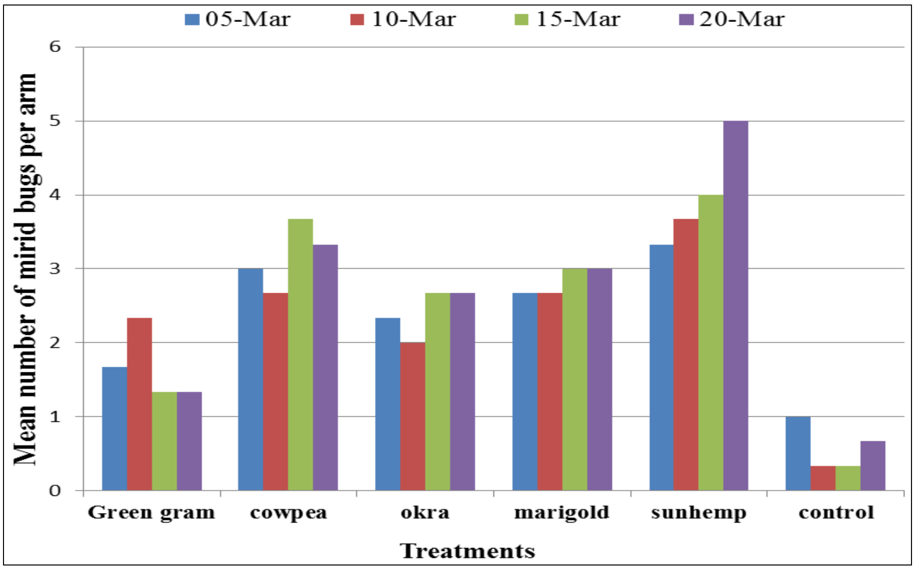


**Fig 1:** Olfactory response of mirid bug, *Cyrtorhinus lividipennis* to flowers of different plants

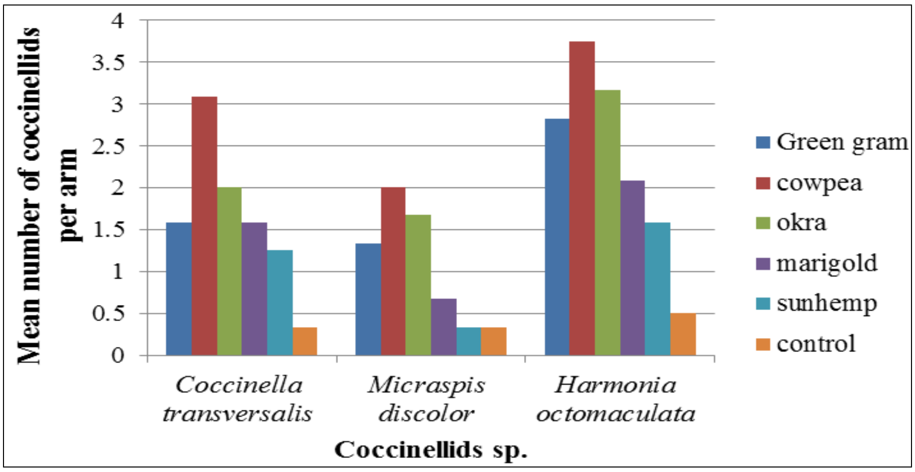


**Fig 2:** Olfactory response of predatory coccinellids to flowers of different plants

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