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RESEARCH ARTICLE

Floral resources as ecological tools to enhance natural enemies for pest management in rice ecosystem

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Abstract

Conservation biological control in rice ecosystems can be promoted through habitat management by providing natural enemies with various resources, such as nectar, pollen, physical refugia, alternative prey and hosts. Laboratory and field studies were conducted to identify the most attractive flowering plants to natural enemies in paddy fields and to evaluate their efficacy in pest management. Olfactometric studies revealed that flowers of marigold, cowpea and sesame attracted major predators in paddy fields, such as *Micraspis discolor*, *Ophionea nigrofasciata* and *Cyrtorhinus lividipennis* and parasitoids *Trichogramma chilonis* and *T. japonicum*. Habitat management with flowering plants, around the paddy bunds, significantly increased the abundance and diversity of key natural enemies, followed by a reduction in pest population. A higher population of natural enemies *viz*., coccinellids, ground beetles, rove beetles, mirid bugs, damselflies and spiders were recorded in plot with flowering plants. This plot recorded the highest yield of 529.73 g m^{-2}, while the lowest was in the control plot, with a yield of 263.46 g m^{-2}. The cost-benefit ratio of paddy cultivation showed the highest benefit-cost ratio (BCR) in the plot with flowering plants (2.16), followed by conventional (1.96) and farmers' practice (1.83). The lowest BC ratio of 0.95 was recorded in the control. The study highlighted the role of flowering plants in enhancing the natural enemy population in paddy fields and the augmentation of biocontrol services.

Keywords: ecological engineering; habitat manipulation; natural enemies; olfactometer; rice ecosystem

Introduction

Rice, *Oryza sativa* L., is a vital source of sustenance for over 65 % of the global population. India ranks second in position and next to China in the cultivation and consumption of rice (1). Many biotic and abiotic factors affect rice production and huge economic losses are faced by farmers (2). Insect pests are the second most important biotic stress limiting crop production after weeds, with twenty species recognized as major pests (3). The average reduction in yield due to insect pests can go up to 25 to 30 %. The reliance on broad spectrum insecticides in rice accounts to nearly 15 % of the total pesticides used in crop production (4). With increased consciousness about the hazards of pesticides in agriculture, there is a remarkable shift towards integrated pest management with emphasis on biological control (5).

The major natural enemies found in the rice ecosystem include predators under four orders *viz*., Coleoptera, Hemiptera, Odonata and Araneae and parasitoids under Hymenoptera and Diptera. The indigenous natural enemies have a strong impact on pest populations and hence their conservation is an essential part of sustainable pest management (6).

Ecological Engineering (EE) is an emerging approach to pest management that focuses on designing and managing agricultural systems through cultural practices aimed at promoting the activity of natural enemies within the agroecosystem (7). It is based on ecological principles that enhance biological control services and reduce external inputs, such as insecticides, to protect biodiversity. It includes methods such as habitat management practices for the conservation and augmentation of beneficial organisms in pest control (8). This can be achieved through the modification of vegetation to enhance biological control, regulate insect herbivores and reduce the use of plant protection chemicals (9). Habitat management methods focus on enhancing the natural enemies by providing supplementary resources in the form of food such as pollen, nectar, extrafloral nectar, alternate pests, host and physical refugia. Several studies on successful pest management employing ecological engineering practices have been conducted in different crops worldwide (10-14).

Identifying the best floral resources for population build-up and enhancing the biocontrol potential of natural enemies is a crucial step in developing habitat management tactics. Hence, the present study was carried out to identify the best floral resources for ecological engineering and to assess the efficacy of selected flowering plants in field conditions for a sustainable pest management in rice.

Materials and Methods

Olfactometer for behavioural studies

The olfactometer consisted of an acrylic chamber of 18 cm diameter with six arms, each with a length of 9 cm. It was closed from the top with a lid and had a release chamber of 5 cm diameter at the center, through which the insects were released. This was attached to an air inlet tube of diameter 6 mm, which was further connected to an air pump through which air was uniformly passed to each arm of olfactometer at 220-240 V. The air flow was 2×3 L min^{-1} at a pressure of 0.012 MPa. The studies were conducted at a temperature of 28±1 °C.

Predators

Three major predators in the rice ecosystem, *viz*., lady bird beetle *Micraspis discolor*, ground beetle *Ophionea nigrofasciata* and green mirid bug *Cyrtorhinus lividipennis* were used for the behavioural studies. *Micraspis discolor* and *O. nigrofasciata* were collected from the rice fields of Regional Agricultural Research Station (RARS), Pattambi, Kerala, India and *C. lividipennis* were reared inside the net house on Brown Plant Hopper (BPH) using potted seedlings. Ten adults of each predator were used for the study.

Parasitoids

The parasitoids used were two species of *Trichogramma*, *viz*., *T. japonicum* and *T. chilonis*. Tricho cards were collected from All India Coordinated Research Project (AICRP) on Biological Control of Crop Pests (BCCP), Kerala Agricultural University (KAU), Thrissur, Kerala, India and State Biocontrol Laboratory, Thrissur, Kerala, India. The tricho cards were cut into 2×2 cm size and were kept in plastic vials till adult emergence. The olfactometric studies with each parasitoid were performed on the day of their emergence into adults.

Flowers as a source of odour

Flowers of *Tagetus erecta* (marigold), *Vigna unguiculata* (cowpea), *Sesamum indicum* (sesame), *Abelmoschus esculentus* (okra) and *Cosmos sulphureus* (cosmos) were used as the odour source for the behavioural studies.

Behavioural studies with predators and parasitoids

The olfactometer studies were carried out by inserting ten flowers each of cowpea, sesame and cosmos and one flower each of marigold and okra, inside the five arms. The sixth arm without flowers served as control. The lid of the olfactometer was closed tightly after the insertion of flowers and air was passed inside the acrylic chamber for five min. before the release of insects to saturate it with flower odour. Ten predators were released at the center of the chamber, through the release portal. The observations were recorded on the number of insects settled in each arm at 5, 10, 15 and 20 min. after release (MAR). Similarly, 20 numbers of parasitoids were separately released into the olfactometer and observations were recorded. The experiment was conducted separately for each predator and parasitoid and replicated three times with fresh flowers. Each time, the arms were cleaned with cotton impregnated with hexane before and after the assay.

Planting flowering plants on paddy bunds

Flowers to which the highest number of predators and parasitoids were attracted, in olfactometer experiments, were selected to evaluate their efficacy under field conditions. The experiment was carried out at RARS, Pattambi, Kerala, India, using the variety Jyothi (PTB 39), during the Rabi season 2022-23. The experiment was conducted in an exploded block design comprising four treatments T_1- Package of Practices (PoP) of Kerala Agricultural University, Kerala, India (15), T_2- PoP + flowering plants, T_3- farmers' practice and T_4- untreated control. Each of the treatment plot was laid out in an area of 500 m^2. The flowering plants were raised at the polyhouse of KAU and transported to the field at the flowering stage. The paddy bunds under the treatment T_2 were planted with flowering plants of marigold, sesame and cowpea at a spacing of 45 cm after transplantation of paddy seedlings. The crop was raised as per the PoP of KAU in T_1 and T_2. However, pesticide application was withdrawn in plot with flowering plants due to the increased activity of natural enemies. *Trichogramma* egg cards against rice leaf folder and rice stem borer were kept in T_1 and T_2 at 0.25 cc per plot. Plant protection measures were adopted against stem borer during the early tillering and panicle initiation stages in plots with farmers' practice (T_3). No plant protection measures were taken in the untreated control.

Field evaluation of flowering plants for habitat management

The observations were taken on the incidence of stem borer and leaf folder and the number of leafhoppers from 15 quadrants in all the treatments. The observations on major predators such as coccinellids, ground beetles, rove beetles, mirid bugs, spiders and damselflies were recorded and expressed as number per m^2 and parasitoids were expressed as number per 5 sweeps. Egg masses of the yellow stem borer were collected from the field and the number of parasitized and non-parasitized egg masses was counted. The observation of pests and their natural enemies was recorded at 15, 30, 45, 60, 75 and 90 days after transplanting (DAT), at an interval of 15 days. The yield per plot was recorded at the time of harvesting and the benefit-cost ratio was calculated.

Statistical analysis

The data obtained from the olfactometer studies were subjected to square root transformation and CRD Analysis of Variance (ANOVA). The treatment means were compared using Least Significant Difference (LSD). Statistical analysis on the per cent incidence of stem borers and leaf folders was done in RBD analysis of variance after transforming to arcsin percentage values. Similarly, the data on the number of pests and natural enemies were subjected to square root transformation and RBD Analysis of Variance (ANOVA). Critical difference values were calculated at 5 % probability level and the treatment values were separated using Least Significant Difference (LSD).

Results

Behavioural response of predators

Significant attraction of predators towards flowers was observed when compared to the control. The mean number of *M. discolor* attracted to different flowers revealed a significantly higher attraction towards cowpea (2.08±0.14 per arm) and okra (2.00±0.20 per arm), which were statistically on par with each other ($p < 0.05$). For *O. nigrofasciata*, the highest preference was towards cowpea with 1.83±0.14 per arm, followed by marigold (1.17±0.14) and sesame with 0.92±0.07 per arm. The response of

C. lividipennis towards flowers revealed that significantly highest attraction was found towards sesame flowers with 2.16±0.24 adults per arm (Table 1).

Behavioural response of parasitoids

Similar to predators, parasitoids also showed a significant attraction towards flowers. Mean data on response of *T. chilonis* to flowers revealed that significantly highest attraction was found towards cowpea with 2.66±0.18 per arm followed by sesame (1.25±0.23 per arm), marigold (1.00±0.12 per arm), okra (0.75±0.18 per arm) and cosmos (0.42±0.18 per arm) ($p < 0.05$). In the case of *T. japonicum*, the highest response was observed towards cowpea (2.50±0.20) and sesame (2.16±0.24), which were comparable to each other. Significantly lower response towards control was recorded for all the natural enemies assessed (Table 1).

Effect of flowering plants on pest population

A significantly lower incidence of pests was recorded from the plot with flowering plants when compared to PoP, farmers practice and control. The mean incidence of stem borer *Scirpophaga incertulas* (Walker), was highest in the control (8.59±0.29 %) at 60 DAT, while PoP + flowering plants (T_2) recorded a significantly lower damage of 3.09±0.21 %, followed by PoP with 4.05±0.36 % damage (Fig. 1). Similarly, leaf folder *Cnaphalocrocis medinalis* Guenee recorded the highest mean per cent incidence in control (T_4) (2.07 %), followed by farmers practice (T_3) (1.50 %) (Fig. 2). Higher population of leafhoppers *Nephotettix virescens* (Distant) were recorded during 45 and 60 DAT only. The highest population of leafhoppers recorded was in control plot with 1.27±0.14 m^{-2}, followed by farmers practice with 0.93±0.22 m^{-2} and PoP (0.73±0.17 m^{-2}). Significantly lowest population of leafhoppers was recorded from PoP + flowering plants (0.47±0.16 m^{-2}).

Effect of flowering plants on population of predators

The study recorded predators under four orders, *viz.,* Odonata, Hemiptera, Coleoptera and Araneae. The coccinellids recorded from the field where *M. discolor*, *Coccinella transversalis*,

Table 1. Number of predators and parasitoids settled per arm in olfactometer

Treatments	*Micraspis discolor*	*Ophionea nigrofasciata*	*Cyrtorhinus lividipennis*	*Trichogramma chilonis*	*Trichogramma japonicum*
Marigold	0.67±0.14^{b}	1.17±0.14^{a}	0.75±0.12^{b}	1.00±0.12^{b}	1.08±0.24^{ab}
Cowpea	2.08±0.14^{a}	1.83±0.14^{a}	0.92±0.07^{b}	2.66±0.18^{a}	2.50±0.20^{a}
Sesame	0.33±0.14^{bc}	0.92±0.07^{ab}	2.16±0.24^{a}	1.25±0.23^{b}	2.16±0.24^{ab}
Okra	2.00±0.20^{a}	0.58±0.07^{bc}	0.58±0.17^{bc}	0.75±0.18^{b}	0.92±0.07^{ab}
Cosmos	0.25±0.11^{bc}	0.25±0.00^{bc}	0.50±0.12^{bc}	0.42±0.18^{bc}	0.83±0.14^{bc}
Control	0.08±0.07^{c}	0.08±0.07^{c}	0.00±0.00^{c}	0.16±0.14^{c}	0.08±0.07^{c}
CD	0.232	0.142	0.203	0.276	0.258
SEM	0.075	0.046	0.066	0.090	0.084

CD- Critical Difference, SEM- Standard Error Mean

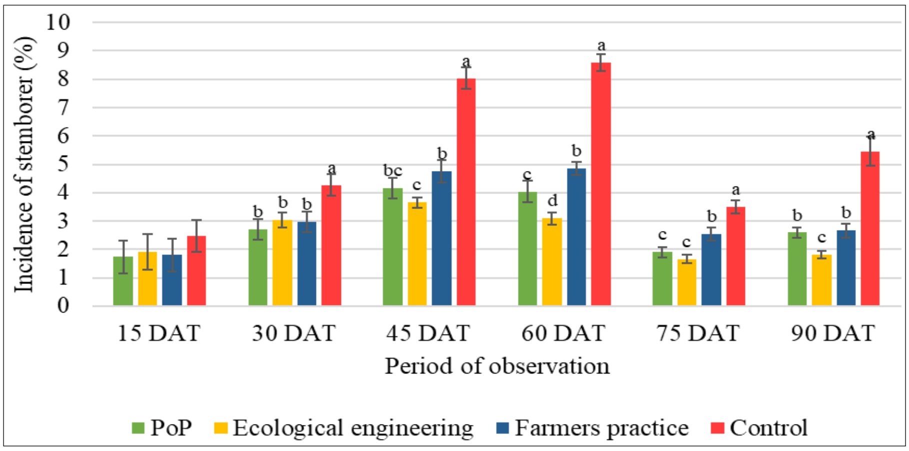

Fig. 1. Effect of treatments on the incidence of stem borer *Scirpophaga incertulas*. Letters indicate differences according to analysis of variance (p=0.05); ns, not significant.

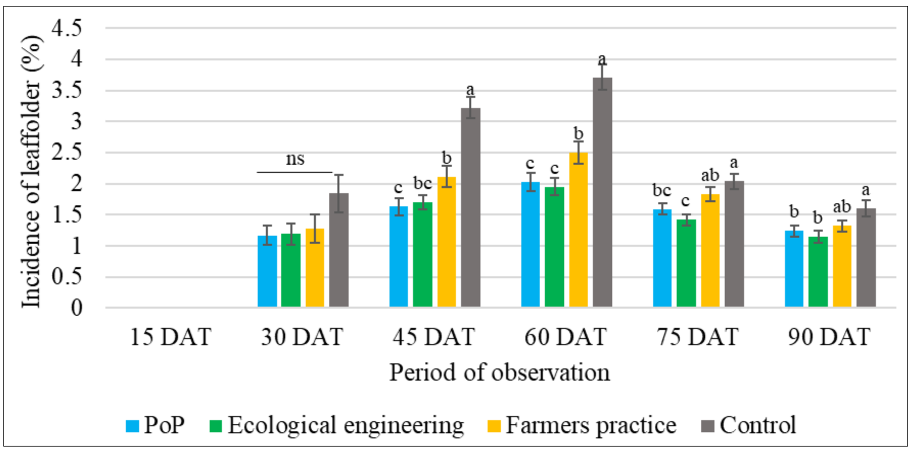

Fig. 2. Effect of treatments on the incidence of leaf folder *Cnaphalocrocis medinalis*. Letters indicate differences according to analysis of variance (p=0.05); ns, not significant.

Harmonia octomaculata, *C. sexmaculata* and *Brumoides suturalis*. All five species were recorded in PoP + flowering plants, while PoP, farmers practice and control, recorded *M. discolor*, *C. transversalis*, *H. octomaculata* and *B. suturalis* only. The mean population of coccinellids was highest in PoP + flowering plants (T_2) (1.73 m^{-2}), followed by control (T_4) (1.03 m^{-2}). Only a few populations of mirid bugs were recorded during the study; however, significantly higher value was obtained from PoP + flowering plants (T_2) (0.85 m^{-2}), followed by the control (T_4) (0.40 m^{-2}). The mean population of ground beetles ($F = 60.995$, $Df = 3$, $P < 0.05$) and rove beetles ($F = 38.618$, $Df = 3$, $P < 0.05$) were highest in PoP + flowering plants (1.60 and 2.94 m^{-2}), followed by farmers practice (0.45 and 1.26 m^{-2}) and PoP (0.57 and 1.32 m^{-2}). Damselflies were highest in PoP + flowering plants (T_2) (1.41 sq. m^{-2}), followed by the control (T_4) (0.70 m^{-2}) ($F = 19.132$, $Df = 3$, $P < 0.05$) (Fig. 3). In this study, seven species of spiders under four families were recorded in the PoP + flowering plants ($F = 32.361$, $Df = 3$, $P < 0.05$). *Tetragnatha javana*, *T. mandibulata*, *Pardosa pseudoannulata, Neoscona theisi*, *N. elliptica*, *Argiope catenulate* and *Oxyopes javanus* were recorded from the plot where ecological engineering was adopted. However, only four species were recorded from PoP, farmers practice and control *viz.*, *T. mandibulata*, *P. pseudoannulata*, *N. theisi and O. javanus*. The mean number of spiders in different treatments is depicted in Fig. 3.

Effect of flowering plants on the population of parasitoids

The study recorded major parasitoids in Hymenoptera under four families, Ichneumonidae, Braconidae, Scelionidae and Eulopidae with fourteen species (Fig. 4). The highest mean population was recorded in PoP + flowering plants (T_2) with 2.20 per 5 sweeps, followed by control (T_4) (1.06 per 5 sweeps) (Fig. 5). The highest per cent parasitism on the eggs masses of yellow stem borer collected from the field was recorded in PoP with flowering plants (T_2) (92.50 %), which was significantly higher compared to the other plots. Control (T_4) recorded a parasitism of 72.50 per cent, followed by PoP (65 %) and farmers practice (57.50 %). The parasitism recorded from PoP (T_1), farmers practice (T_3) and control (T_4) were on par with each other. The parasitoids that emerged from the egg masses of rice yellow stem borer were identified as *Tetrastichus* sp. and *Telenomus* sp.

Yield and benefit cost ratio

The mean yield of paddy obtained from PoP + flowering plants (T_2) and PoP (T_1) was found to be significantly higher, with 529.73 and 513.80 g m^{-2}, respectively, compared to farmers' practice (T_3) (471.07 g m^{-2}) and control (T_4) (263.46 g m^{-2}) ($F = 54.409$, $Df = 3$, $P < 0.05$). The cost-benefit ratio of paddy cultivation showed the highest benefit-cost ratio (BCR) in PoP + flowering plants (2.16), followed by PoP (1.96) and farmers' practice (1.83), as against 0.95 in control.

Discussion

The flowering plants had a significant effect in attracting the beneficial insects towards them. The presence of volatile compounds released from different flowers could be responsible for their specific attraction. Several studies have recorded the olfactory response of predators and parasitoids to flowers and their volatiles. The present study indicated a higher attraction of predators *viz.,* coccinellids, towards cowpea and okra, which could be due to the volatile compounds released from them. This finding is supported by earlier studies (16), which reported the attraction of *Coccinella septumpunctata* to the leaves of *Berberis vulgaris* and flower heads of *Tripleurospermum inodoratum* in a Y-tube olfactometer, suggesting the preference of coccinellids to plant odours. Additionally, compounds like beta caryophyllene and limonene attracted multicolored lady beetle in a four-arm olfactometer (17). The attraction of mirid bugs to sesame can be attributed to an array of volatiles. An earlier study reported the attraction of *C. lividipennis* to volatiles from rice (18). Similar results have shown that the compounds isocaryophyllene and trans-2-dodecenol from the aerial parts of *Coriandrum sativum* and *Nerium indicum* were attractive to *C. lividipennis* (19). The attraction of insects to flowers may also be influenced by the presence of floral rewards such as pollen and nectar, which play a role in nutritional requirements of predators (20). The plant volatile compounds can also act as the signals to locate pollen, nectar and other food resources (21). This supports the attraction of ground beetles towards flowers as carnivorous natural enemies also rely on plant-derived food sources during certain stages of their life cycle (22).

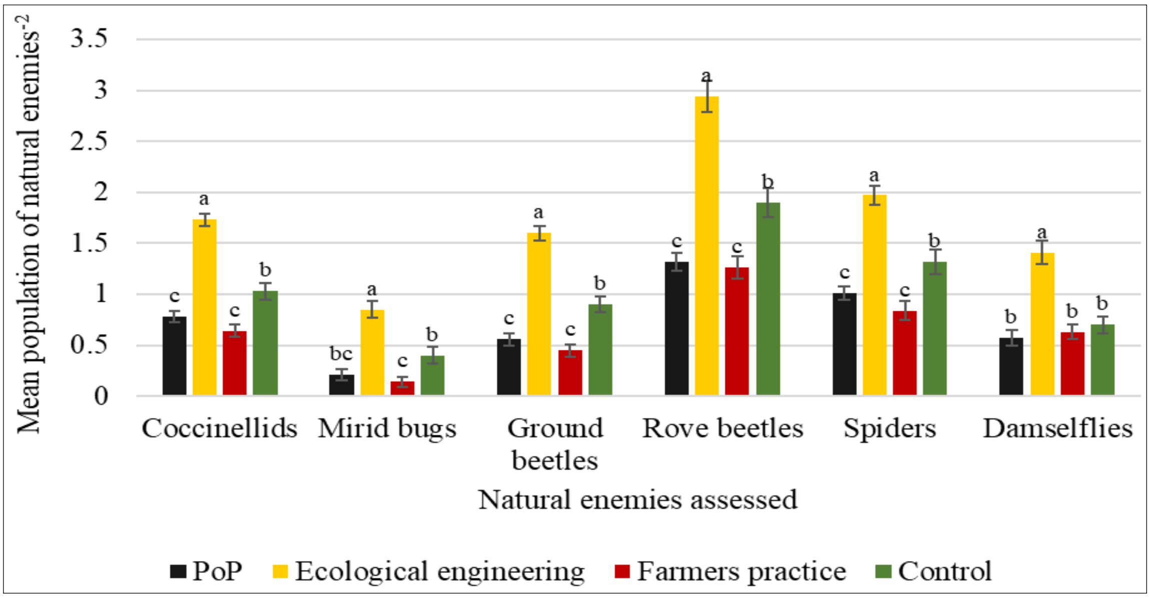

Fig. 3. Effect of treatments on population of predators. Letters indicate differences according to analysis of variance (p=0.05).

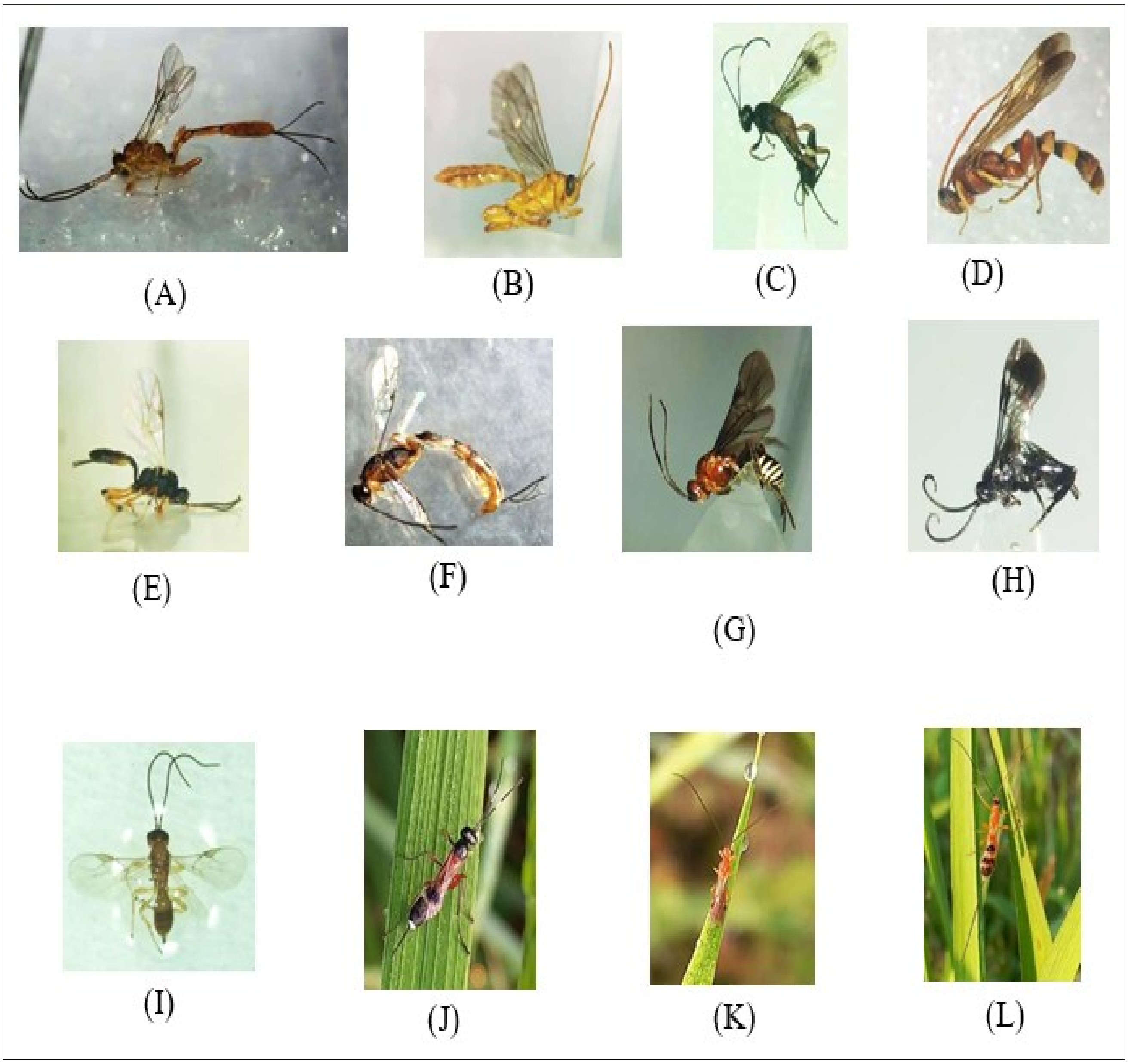

Fig. 4. Hymenopteran parasitoids recorded from ecological engineering plot; (A) *Trathala flavoorbitalis*, (B) *Xanthopimpla* sp., (C) *Isotima* sp., (D) *Metopius rufus*, (E) Phygadeuontinae subfamily, (F) Cremastinae subfamily, (G) *Amyosoma* sp., (H) *Cardiochiles* sp., (I) Opiinae subfamily, (J) *Goryphus* sp., (K) *Tropobracon* sp. and (L) *Stenobracon nicevelli.*

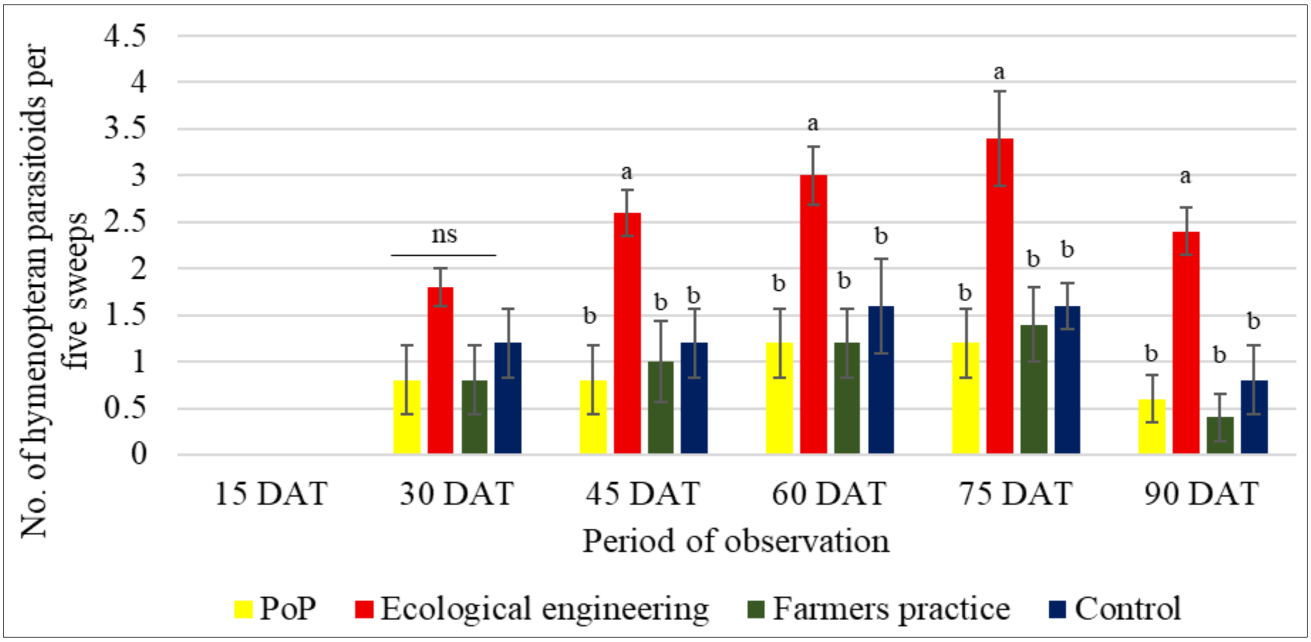

Fig. 5. Effect of treatments on population of hymenopteran parasitoids. Letters indicate differences according to analysis of variance (p=0.05).

Higher attraction of *Trichogramma* was recorded towards cowpea flowers. Similar attraction of *T. chilonis* towards weeds was reported in an olfactometer study. A higher response of *T. chilonis* was recorded towards *Echinochloa crusgalli* followed by *Sacciolepis interrupta* when compared to leaf folder damaged leaves (23).

The higher natural enemy population observed in the plot with flowering plants had a key role in curtailing the pest incidence. Previous studies reported reduction in stem borer incidence, white ears and lowest incidence of *N. lugens* and white backed planthoppers when flowering plants were planted around the field (24-26). The subsequent reduction of pests in plot surrounded by flowering plants can be attributed to the higher number of predators such as ground beetles, rove beetles, coccinellids, damselflies and spiders. The presence of stem borer eggs, as well as larvae, was lower in plot with flowering plants than without them.

The plants used in the present study as floral resources *viz*., cowpea, marigold and sesame were found to support natural enemies. Ladybird and rove beetles were seen on open flowers as well as flower buds, feeding on nectar produced by the flowers (Fig. 6, 7). *Harmonia axyridis* ingests nectar and pollen from flowering plants and the floral resources are used by coccinellids to increase survival, as an energy source and to enhance reproductive capacity (27). A higher relative abundance of *C. transversalis* (Fab.), *Menochilus sexmaculatus* (Fab.) and *Hippodamia variegata* were recorded in the ecologically engineered field (28). The increased population of spiders in PoP + flowering plants is supported by a previous study, which reported the attractiveness of spiders towards the flowers of *Salvia farinacea*, *Foeniculum vulgare*, *Fagopyrum esculentum*, *Mentha spicata* and *Anethum graveolens* (29).

A previous study on the parasitism of insect herbivores in rice fields diversified with patches of flowering plants showed that a higher proportion of stemborer eggs were parasitized by *Tetrastichus* spp. and *T. japonicum* in the diversified plot as against the control plot (30). Higher population of parasitoids can be attributed to the availability of carbohydrate-rich diets in plots with flowering plants, which optimize their longevity and reproductive capacity (31). These dietary sources are present in floral nectar, extra-floral nectar and honeydew, all of which encompass monosaccharides, disaccharides and oligosaccharides (32). This improved effectiveness in the parasitoid host behaviour is associated with the enhanced energy levels of the parasitoid, from the consumption of pollen and nectar (Fig. 8). A similar effect of honey, sucrose, glucose and fructose was observed on *Telenomus remus* and recorded an increased longevity (5 days) in females fed with carbohydrate, in contrast to one day in those fed with water (33). These findings support the results obtained in this study. The rate of parasitism of *Aphidius platensis* was found to increase after their exposure to marigold flowers, probably due to the feeding of pollen and nectar from flowers (34).

The higher yield (529.73 g m^{-2}) and BC ratio (2.16) obtained in PoP + flowering plants in our study conform with the findings of studies conducted for pest management elsewhere in India and abroad. A higher yield of rice was recorded when border cropped

Fig. 6. Coccinellids beetle on cowpea flower bud.

Fig. 7. Rove beetle feeding on pollen from marigold flower.

Fig. 8. *Stenobracon nicevelli* on cowpea flower bud.

with sunflower, followed by sesame and cowpea (35). Also, another study recorded a higher yield of rice from diversified habitat (4.99 t/ha) than with rice alone (4.36 t/ha) (36). Ecological engineering in Cambodian rice fields with mung bean, sesame and sponge gourd recorded a higher BC ratio of 2.02 against the plot with pesticide treatment (1.84) (37).

The plant protection measures undertaken during the initial stages of paddy, along with a higher population of natural enemies in PoP + flowering plants plot led to reduced pest incidence and higher yield. The availability of floral resources such as food, nectar and alternate prey eventually led to a higher number of predators and parasitoids in the field. The increased yield and reduced pesticide use contributed to a higher BC ratio in PoP + flowering plants plot than PoP alone, despite the additional expenditure incurred for raising the flowering plants. Growing cowpea and sesame in paddy bunds can further add to the income of the farmers.

Conclusion

The present study assessed the olfactory responses of major predators in the rice ecosystem *viz*., *M. discolor*, *O. nigrofasciata* and *C. lividipennis* and parasitoids *T. chilonis* and *T. japonicum* to flowers. The study revealed that marigold, cowpea and sesame flowers significantly attracted the natural enemies towards them when compared to the control. The validation of the lab experiment under field conditions was promising in reducing pest population and increasing the yield. This offers a promising strategy in natural pest suppression by incorporating flowering plants into the cropping system through habitat management. The flowering plants can attract natural enemies to the cropping area by providing floral resources and thereby paving the way for enhanced natural enemy population. Further research is needed to identify additional flowering plants that can attract natural enemies, as well as to examine the effects of floral resources on their growth and development.

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Authors' contributions

MK contributed to methodology, investigation, formal analysis and writing of the original draft. PS and KK contributed to conceptualization, resources, supervision, data curation and writing review and editing. SR contributed to writing review and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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