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Assessing the Role of Conservation Strips in Enhancing Beneficial Fauna in the Wheat-Cotton Agricultural System in Punjab, Pakistan

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ABSTRACT

Agricultural landscapes with strict monocultures are causing a decline in on-farm biodiversity. Conservation areas, consisting of grasses, shrubs, especially flowering plants, could help conserve on-farm biodiversity. In the current study, a conservation strip was established in the middle of an agricultural farm, with wheat and cotton as the main crops. The conservation strip was set up using flowering shrub holy basil (*Ocimum tenuiflorum* L.) and bermudagrass (*Cynodon dactylon* (L.)). Arthropod abundance and biodiversity were assessed using pitfall traps deployed within the conservation strip and at different distances (1 m, 10 m, and 20 m away from the conservation strip) in the field. Fortnightly sampling was conducted (eight times from June to October in cotton and seven times from January to March in wheat) for collection of arthropods. The captured arthropods were preserved in a 70% ethyl alcohol solution for further taxonomic identification. The abundance of arthropod fauna in the conservation strip and at different distances in the agricultural field were compared. Diversity indices were used to estimate the arthropod biodiversity of the conservation strip and the cultivated areas. The results showed significantly higher arthropod abundance in the conservation strips and in the field 1 m away from the strip, compared to arthropod samples collected 10 and 20 m away from the conservation strip. The abundance of different arthropod taxa, including Araneae, Coleoptera, and Orthoptera, was significantly higher in the conservation strip during the cotton season. The current evidence highlights the role of the conservation strip in supporting the populations of beneficial arthropods, especially predators.

INTRODUCTION

The increased demand for agricultural produce amid the global population rise is causing intensified farming, which in turn is causing a reduction in landscape heterogeneity and threaten the on-farm biodiversity (Rischen *et al*., 2021; Stein-Bachinger *et al*., 2022).

The modern intensive agricultural practices are leading to the loss of biodiversity and reduction in associated ecosystem services (Renard and Tilman, 2021; Jaiswal and Joseph, 2024). Arthropods, a diverse group of species, play crucial roles in ecosystem services like pollination, pest control, nutrient cycling, and decomposition, and these arthropods live in various habitats (Elizalde *et al*., 2020). Many arthropod species have been reported to decline due to increased agricultural intensification (Mulinge, 2023). Multiple studies have shown that diversification of agricultural farms would lead to better natural control of insect pests and reduced use of biopesticides.

Increasing on-farm plant diversity, using flowering and non-flowering species, is one way to diversify the agricultural landscapes, with the goal of providing more refuge spaces for natural enemies and pollinators (MacLeod *et al*., 2004). For instance, the perennial

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flowering strips provide foraging resources to the solitary and oligolectic bees, which are important pollinators of multiple crops (von Königslöw *et al*. 2021). Further, these beetle banks or conservation areas are known to improve abundance of beneficial arthropods, particularly predators, playing a role to increase the natural control of arthropod pest species (MacLeod *et al*., 2004; Howlett *et al*., 2021). The development and maintenance of these conservation areas within the agricultural farm is important because the modern farms lack suitable habitat and food resources for beneficial arthropods (Iuliano and Gratton, 2020), which may ultimately lead to increased pest pressure (Cardoso *et al*., 2020). The undisturbed conservation areas protect and enhance the arthropod biodiversity and strength of natural enemies (Grass *et al*., 2019), and supports the ecological balance.

Studies have reported that conservation areas, such as beetle banks or conservation strips consisting of non-crop vegetation planted in fields, could support the population and efficacy of beneficial arthropods (de Pedro *et al*., 2020; Tiwari *et al*., 2020; Iuliano and Gratton, 2020). However, ensuring this transformation in the abundance and diversity of arthropods depends significantly on the diversity of plant species within the conservation areas (Snyder, 2019). Development of beetle bank in wheat has been reported to increase populations of generalist predators such as carabids, staphylinids, linyphiids, and lycosids, while decreasing aphid populations in neighboring fields; however, the abundance of predatory arthropods decreases with increasing distance from the beetle bank (Collins *et al*., 2002). Further, an increase in the diversity of plant species has been reported to support the predatory arthropods and results in a higher activity in diverse landscapes (Khan and Joseph, 2022). Moreover, on-field conservation areas also support native bird, mammals and bee species, whose populations have decreased on farmlands due to agricultural intensification (Clough *et al*., 2020; Joseph *et al*., 2020).

Cotton-wheat cropping system has been practiced over a large area in southern Punjab, Pakistan and hosts beneficial organisms (Hussain and Afzal, 2005). The cotton crop has a higher number of pests, which reduces crop yield. Cotton farmers use pesticides as the primary option to manage cotton pests (Mizik, 2023). In wheat, aphid species are most common pests, yet their population is naturally managed by several beneficial arthropods (Saleem *et al*., 2009), leading to minimal or no pesticide usage in wheat. Currently, the wheat-cotton system lacks an on farm conservation plan in southern Punjab, Pakistan. Therefore, a lack of evidence exists regarding the effects of the conservation areas on changes in the on-farm biodiversity. In response to this knowledge gap, the current study was conducted to assess the effects of the conservation strip on beneficial arthropod abundance in the wheat-cotton system.

MATERIALS AND METHODS

Study site

The experiment was conducted on the local farm near MNS University of Agriculture Multan, Pakistan. For the last five years, wheat and cotton have been consistently grown along with the seasonal vegetables. Multan is located in the subtropical zone, with hot summers (35 to 40ºC), cold winters (8 to 10ºC), and annual rainfall ranging from 127 to 254 mm, and severe fog during winters (Ali *et al*., 2020). At the experimental farm, the soil type was clay loam. For wheat, the irrigation was done after 25 days of sowing, followed by irrigation at flowering and grain filling stages. For cotton, irrigation interval applied after 7-10 days. For wheat and cotton, diammonium phosphate (DAP), potassium sulfate, and urea were applied as fertilizer treatments.

Plant selection and sowing

The conservation strip was developed in the middle of the agricultural field. Measurements were taken with measuring tape and flags were deployed to mark the given area. Two plant species were selected for the establishment of conservation strip. One was bermudagrass *Cynodon dactylon* (L.) Pers. (Poaceae) and other was holy basil *Ocimum tenuiflorum* L. (Lamiaceae) (Fig. 1). These plants were selected because farmers do not consider them as weeds. Moreover, these plants were also not reported to host the main pests of cotton and wheat crops. Bi-directional ploughing was used to form the sowing bed. The strip was 152.4 m long, and 1 m wide. Sowing of *C. dactylon* and *O. tenuiflorum* was done manually in April. After the germination, pitfall traps were deployed. The strip was divided into six sub-strips of equal length (i.e., ~25 m × 1 m). In each block one pitfall trap was deployed in the center of each sub-strip. The conservation strip was manually irrigated after 1-2 days to ensure seed germination. After the plants started growing, they were irrigated along with the adjacent crop.

Pitfall traps

Pitfall traps were deployed within the conservation strip and at specific distances in the agricultural field following the method devised by Gireesh and Joseph (2021). A total of 24 traps were deployed: six within the conservation strip, and eighteen in the cultivated area, with six at each distance of 1m, 10m, and 20m from the strip. Disposable cups and plates were utilized to create pitfall traps. Each pitfall trap consisted of a disposable cup of 7.62 cm wide opening and was 15 cm deep, positioned in the soil with its opening parallel to the soil surface, i.e., at ground level. One-third of each disposable cup was filled with ethylene glycol, water, and three drops of dishwash liquid. The disposable cup was covered by a disposable plate to protect the trap contents from rain. Collection of trapped arthropods was made after one week of deployment, with two samplings done every month. A total of eight samplings were conducted from June to October in cotton, and seven times from January to March in wheat.

Arthropod collection, preservation, and identification

The collected samples were placed in glass vials of variable volumes filled with 75% ethanol, each properly tagged with a specific collection number. All samples were transported to the lab, cleaned with water to remove ethylene glycol, dirt particles, and other detritus adhered to their bodies before being stored in 75% ethanol. Order-level identification was carried out using a hand lens and microscope (Swift SW380T, China). The taxonomic identification of arthropods up to the order level was performed using the identification keys (Triplehorn and Johnson, 2005).

Diversity indices

Diversity indices are measures of species diversity based on species richness (number of recorded species) and abundance (number of individuals of a species) in a given population. In this study, diversity indices were calculated for the arthropods recovered from the pitfall traps. Shannon diversity indices (H), Shannon evenness measures (EH), Simpson's diversity indices (D), and Simpson's evenness measures (ED) were calculated for the diversity in and around the conservation strips separately for wheat and cotton. The formulas for the diversity indices and evenness measures used in the experiments are as follows:

$$H = -\sum_{i=1}^{S} pi \ln pi$$

$$EH = H/lnS$$

$$D = 1/\sum_{i=1}^{s} pi\,2$$

$$ED = D \times 1/S$$

Where, H, Shannon diversity index; E_H, Shannon evenness; S, richness of arthropods; *pi*, proportion of individual arthropods; ln, natural logarithm; D, Simpson diversity index and E_D, Simpson evenness.

Statistical analyses

The data were analyzed using Statistix 8.1 statistical package. The data was first log transformed and then ANOVA was performed. The means were separated using Tukey's test at $\alpha = 0.05$.

RESULTS

Diversity indices (Cotton)

Shannon and Simpson diversity and equitability indices for the different treatments, i.e., conservation strip (C0), 1m (C1), 10m (C2) and 20 m (C3) away from the conservation strip in the cotton field (Table I), were not significantly different (Table I).

Table I. Shannon diversity index (H), Shannon's equitability (EH), Simpson's diversity index (D), and Simpson's equitability (ED) for arthropods diversity in conservation strip and at different distances from conservation strip during cotton and wheat crops. The values are Means±SEM.

	H	EH	D	ED
Arthropod diversity				
Conservation strip (C0)	1.40 ± 0.09	0.75 ± 0.05	2.95 ± 0.59	0.46 ± 0.11
1m away from strip (C1)	1.26 ± 0.14	0.68 ± 0.07	2.86 ± 0.31	0.41 ± 0.03
10m (C2)	1.22 ± 0.05	0.69 ± 0.05	2.96 ± 0.21	0.46 ± 0.05
20m (C3)	1.11 ± 0.10	0.65 ± 0.06	2.24 ± 0.48	0.38 ± 0.08
F, df	2.15,6	0.77,6	0.61,6	0.25,6
P	0.1296	0.5261	0.6193	0.8574
Wheat crop				
Conservation strip (C0)	0.70 ± 0.12	0.53 ± 0.07	2.39 ± 0.19 b	0.66 ± 0.08
1m away from strip (C1)	0.74 ± 0.18	0.61 ± 0.07	2.37 ± 0.60 a	0.64 ± 0.08
10m (C2)	0.41 ± 0.19	0.50 ± 0.26	0.89 ± 0.31 ab	0.39 ± 0.15
20m (C3)	0.75 ± 0.18	0.66 ± 0.14	0.49 ± 0.23 b	1.13 ± 0.30
F, df	2.00, 3	0.30, 3	7.79, 3	3.19, 3
P	0.1576	0.8224	0.0023	0.0544

Fig. 1. Comparison of the conservation strip (A) prior to plant growth and (B) following establishment with plant growth.

For different months, Shannon diversity index (H) (*F*=2.1, df=6, *P*=0.1296), Shannon equitability (EH) (*F*=0.7, df=6, *P*=0.5261), Simpson's diversity Index (D) (*F*=0.6, df=6, *P*=0.6193) and Simpson's Equitability (ED) (*F*=0.2, df=6, *P*=0.8574) showed no significant differences (Fig. 2A).

Arthropod abundance (Cotton)

Arthropod abundance as observed in the conservation strip (C0) and 1m away from conservation strip in the cotton field (C1) was significantly higher as compared to the arthropod abundance data recorded from 10m (C2) and 20 m (C3) away from the conservation strip in the cotton field ($F = 32.1$, df = 11, $P < 0.001$) (Fig. 3A).

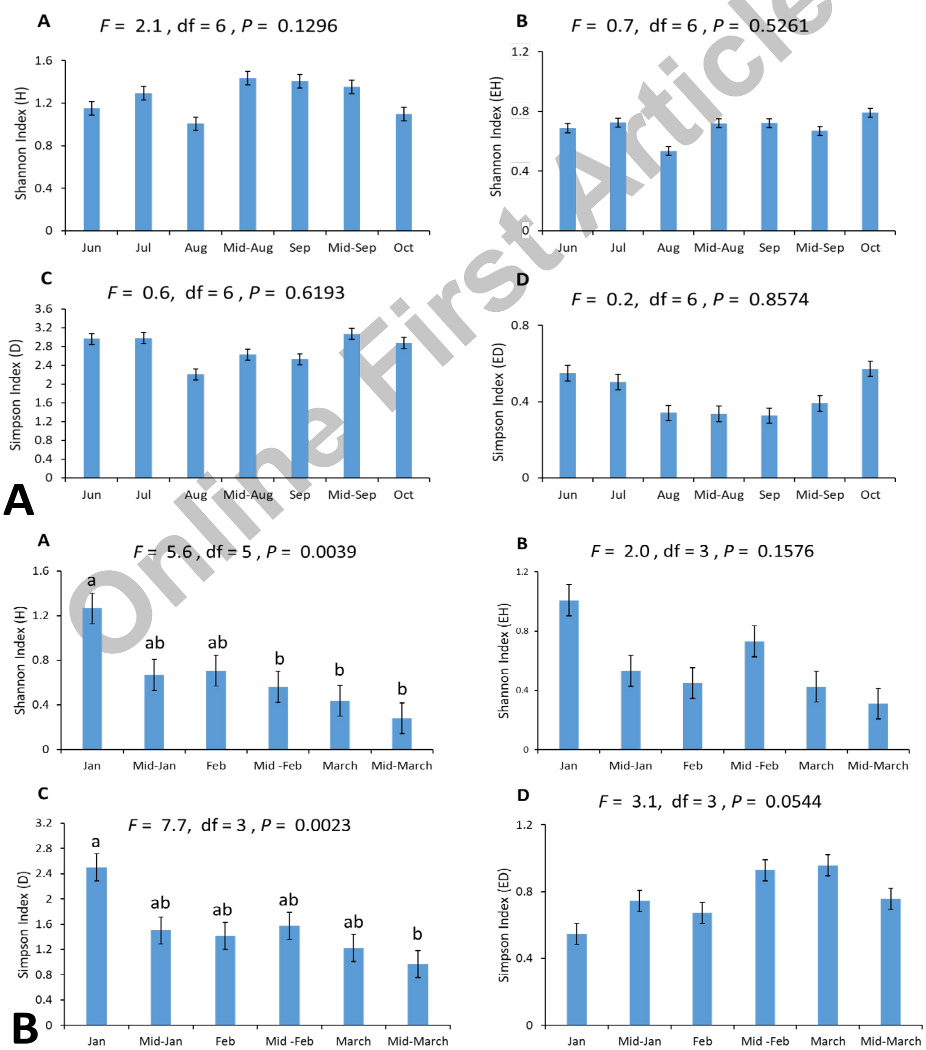

Fig. 2. Shannon diversity index "H" (A) Shannon equitability "EH" (B) Simpson's diversity Index "D" (C) and Simpson's equitability "ED" (D) for calculating arthropod diversity in conservation strip and cotton crop across different months from June to October 2022 (A), wheat crop across different months from January to mid-March 2023 (B).

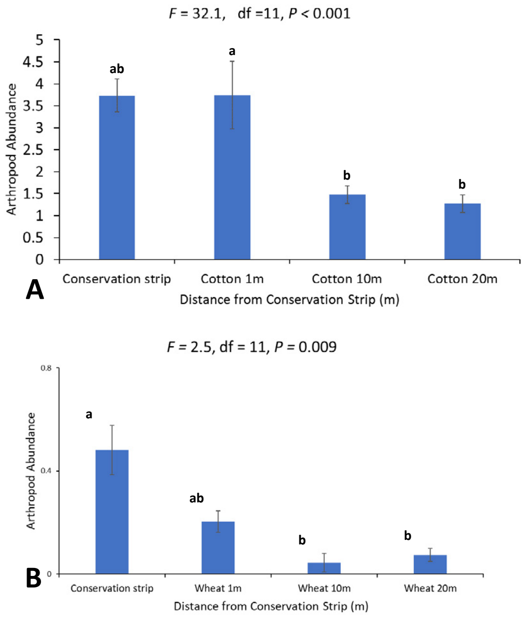

Fig. 3. Arthropod abundance recorded from conservation strip and different distances from the conservation strip, i.e., 1m, 10m and 20m in the cotton field, during June to October 2022 (A) and January to Mid-March 2023.

A significantly higher number of Araneae was observed in C0 and C1 as compared to C2 and C3 (F = 6.9, df = 3, P = 0.0002). Similarly, a higher number of hymenopterans were found in C0 and C1 as compared to C2 and C3 (F = 2.8, df = 3, P = 0.0397). Coleopterans were significantly higher in conservation strips, as compared to other treatments (F = 6.4, df = 3, P = 0.0004). Homopterans were significantly higher in C0 and C1 as compared to C2 and C3 (F = 3.1, df = 3, P = 0.0270). Similarly, significant differences were found for lepidopterans (F = 3.4, df = 3, P = 0.0174) and orthopterans (F = 7.7, df = 3, P = < 0.001). No significant differences were observed for dipterans (F = 0.42, df = 3, P = 0.7384), isopods (F = 1.0, df = 3, P = 0.395), and odonatans (F = 1.4, df = 3, P = 0.2424), hemipterans (F = 1.0, df = 3, P = 0.3949), Blattodea (F = 2.0, df = 3, P = 0.1055), and dermapterans (F = 2.4, df = 3, P = 0.0690) (Fig. 4).

Diversity indices (Wheat)

Arthropod diversity as estimated by the Simpson diversity index was significantly higher in wheat crop 1 m away from the conservation strip as compared to the other treatments. The other diversity and evenness measures showed no significant differences between the different treatments, i.e., conservation strip, 1m, 10m and 20m away from conservation strip in the wheat field (Table I).

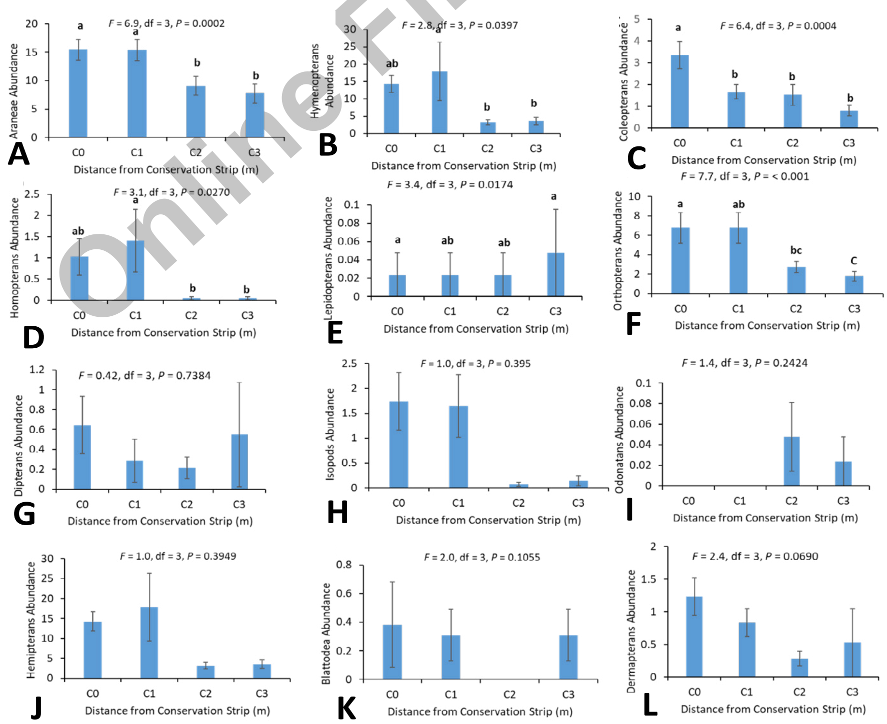

Fig. 4. Arthropod abundance recorded from conservation strip and cotton: (A) araneae, (B) hymenopterans, (C) coleopterans, (D) homopterans, (E) lepidopterans, (F) orthopterans, (G) dipterans, (H) isopods, (I) odonatans, (J) hemipterans, (K) blattodea, and (L) dermapterans.

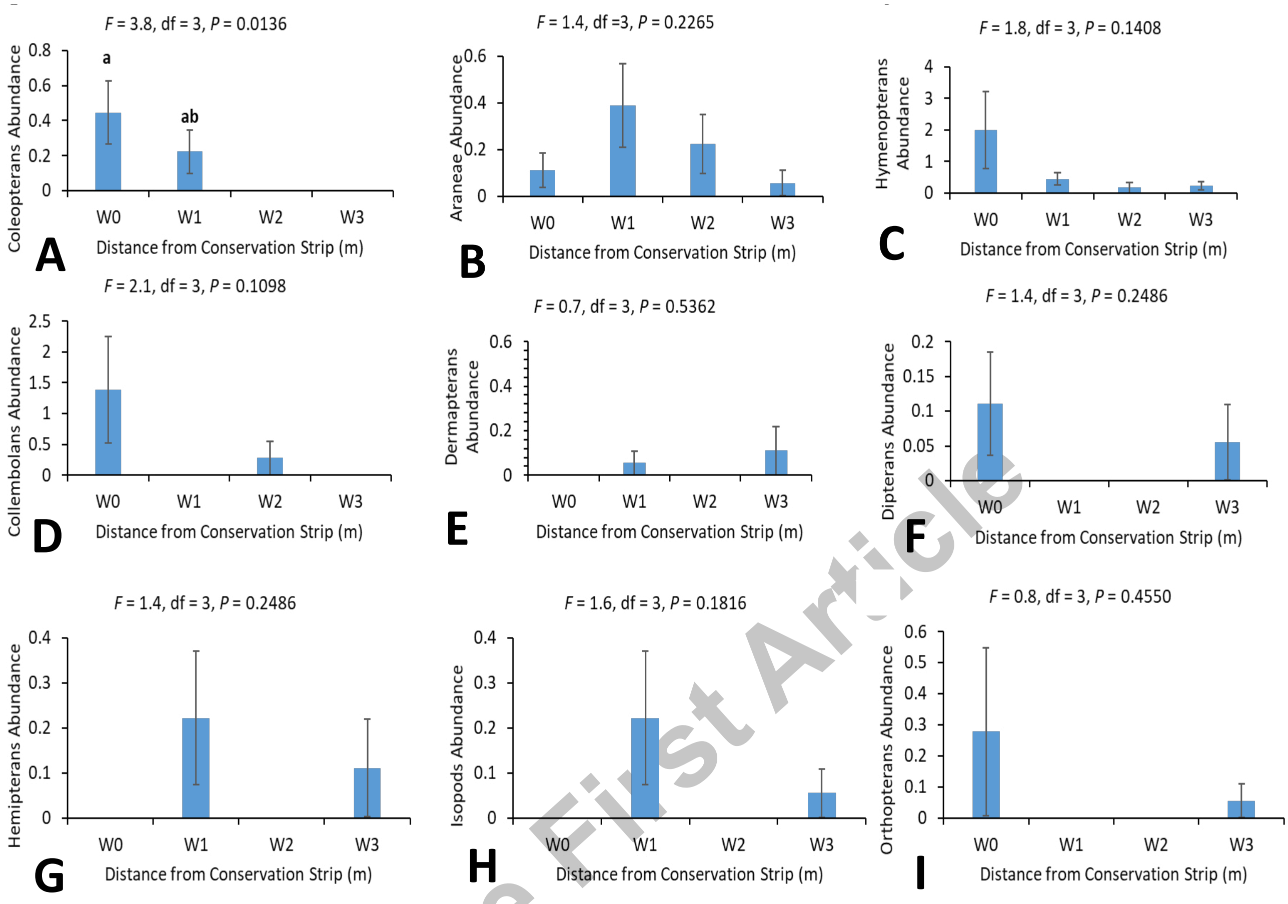

Fig. 5. Arthropod abundance recorded from conservation strip and wheat: (A) Coleopterans, (B) Araneae, (C) Hymenopterans, (D) Collembolans, (E) Dermapterans, (F) Dipterans, (G) Hemipterans, (H) Isopods, and (I) Orthopterans.

For different months, Shannon diversity index (H) was significantly higher for January as compared to Mid-February, March, and Mid-March (F = 5.6, df = 6, P = 0.0039). Shannon Equitability (EH), (F = 2.00, df = 6, P = 0.1576). However, Shannon Equitability (EH) (F = 2.00, df = 6, P = 0.1576) and Simpson's Equitability (ED) (F = 3.1, df = 6, P = 0.0544) were not significantly different for different months (Fig. 2B).

Arthropod abundance (Wheat)

Arthropod abundance, as captured by the pitfall traps deployed in the conservation strip (W0), was significantly higher compared to those deployed 1m (W1), 10m (W2), and 20m (W3) from the conservation strip in the wheat field (F = 2.5, df = 11, P = 0.009) (Fig. 3B). The number of coleopterans were significantly higher in and around the conservation strip as compared to the treatments located in the wheat field (F = 3.8, df = 3, P = 0.0136). However, no significant differences were found for Araneae (F = 1.4, df =3, P = 0.2265), Hymenoptera (F = 1.8, df = 3, P = 0.1408), Collembola (F = 2.1, df = 3, P = 0.1098), Dermaptera (F = 0.7, df = 3, P = 0.5362), Diptera (F = 1.4, df = 3, P = 0.2486), Hemiptera (F = 1.4, df = 3, P = 0.2486), Isopoda (F = 1.6, df = 3, P = 0.1816), and Orthoptera (F = 0.8, df = 3, P = 0.4550) (Fig. 5).

DISCUSSION

The current work shows the successful establishment of a conservation strip, or beetle bank, in the agro-ecosystem of Pakistan. During this study, a high number of arthropods belonging to eleven taxa were recorded from the cotton and wheat agricultural landscape in Multan, Pakistan. Araneae came out to be the most dominant taxa in conservation strip. An increased number of arthropods were captured in and around the conservation strips, which might be due to their role in providing food and refuge (Thomas *et al.*, 2000). An increased number of predatory taxa were recorded, which shows the potential of the conservation strip in providing the natural pest control in the field (Collins and Montgomery, 2002).

In the current study, conservation strip was established in May 2022. After a few months, it provided a refuge and food source for beneficial arthropods. Further, a higher number of spiders, beetles, and ants were captured within and around the conservation strips, consistent with previous studies (MacLeod *et al*., 2004; Killewald *et al*., 2024). Conservation strips, or beetle banks, have been reported to attract coleopterans in a short time (MacLeod *et al*., 2004), indicating higher population density of beneficial arthropods within a short period. The conservation strip captured a higher number of spiders, which have been reported to be under threat in agroecosystems (Samu *et al*., 2023). As spiders play a crucial role in predation and bioindication of human-based disturbances, conserving the spider community is beneficial for agricultural landscapes (Marc *et al*., 1999).

While the current study highlights several aspects of the conservation strip towards the conservation of beneficial fauna in agricultural landscapes, it also has limitations. Firstly, the beetle bank was newly established, and arthropod population assessment was conducted for only two seasons. Long-term studies are required for a clearer understanding of beetle banks in agricultural landscapes (MacLeod *et al*., 2004). Secondly, the study only utilized pitfall traps as the primary focus was on estimation of ground-dwelling arthropod populations, especially predators. However, employing multiple sampling methods may ensure a clear understanding of changes in the arthropod communities, particularly flying insects (McCravy, 2018). Future studies might incorporate various sampling techniques, including live observations (Bowers *et al*., 2021), video monitoring (Joseph, 2022), pan traps (Ulyshen *et al*., 2022), sweep netting (Roulston *et al*., 2007), and clay models (Khan and Joseph, 2021, 2022). Thirdly, we did not replicate the experiment across different agroecosystems, due to resource limitations. Future studies could deploy conservation strips in multiple agroecosystems to observe arthropod community changes with time. Finally, the study did not assess the population of pests in nearby crops. Evaluating pest populations could offer useful insights into the direct contribution of conservation strips toward natural control of crop pests.

Long term maintenance of these strips in agricultural landscapes is essential to protect the arthropod predators and pollinators. In the UK, damage to hedgerows over the past 50 years has led to a decline in on-farm diversity of beneficial fauna and decreased floral richness (Goulson, 2019; Tscharntke *et al*., 2021). This evidence shows that conservation regions are important in on-farm biodiversity management. The use of flowering and non-flowering plants for establishment of conservation strips has been reported to support beneficial fauna, including pollinators, predators, and parasitoids (Snyder, 2019). Long-term research is required to evaluate the practical applicability of conservation areas in different agroecosystems (Angelella *et al*., 2021). Maintaining these conservation belts over an extended period and in different agricultural landscapes is essential for protecting beneficial fauna. Moreover, the conservation strips should be incorporated in different integrated pest management programs as it provides promotes the population of predatory communities and hence leading to natural control of the field pests. This could be possible through informing the farmers about the natural control provided by beneficial arthropods (Khan *et al*., 2021). Thus, these conservation areas may be established in different cropping systems and must be maintained for a prolonged period.

DECLARATIONS

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IRB approval

The study was reviewed and approved by the Institutional Review Board (Board of Studies at Institute of Plant Protection, MNS University of Agriculture, Multan, Pakistan).

Ethical statement

All applicable ethical guidelines and regulations were followed during this research. No experiments involving human, or animal subjects were conducted.

Statement of conflict of interest

The authors have declared no conflict of interest.

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