



# Spider Diversity and Abundance in Bt and Non-Bt Cotton Crops in Punjab, India

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**Abstract:** Bt cotton, expressing a gene for Bt-toxin specifically affects lepidopteran larvae, but little is known about its impact on spiders. This study recorded the diversity and abundance of spiders in both Bt (transgenic cotton PAU Bt 1) and non-Bt cotton (F 2228) crops along with the effect of other factors like crop stages and climate for two seasons. Spiders of nine genera and 11 species representing six families: Araneidae (37.68%), Oxyopidae (28.85%), Lycosidae (18.68%), Thomisidae (10.57%), Salticidae (3.80%), and Pisauridae (0.41%) were recorded with maximum species richness of family Araneidae followed by family Lycosidae, and families Oxyopidae, Thomisidae, Salticidae and Pisauridae. The Shannon-Weiner index and species evenness index were 1.42 and 0.59, respectively. Spider abundance was significantly higher in Bt cotton compared to non-Bt cotton indicating no adverse effect of Bt toxin on spiders. In both crops, the spider abundance was higher at the boll-bearing and boll-opening stages. Additionally, there was a significant effect on spiders of mean temperature. The study suggests that rich spider fauna of cotton crops be protected to utilize their full potential in the Integrated Insect Pest Management Program.

**Keywords:** Bt-toxin, Climatic factors, Cotton, *Gossypium hirsutum*, PAU Bt 1

Spiders belong to one of the most important arthropod predator groups in the world, ranking seventh in the world after six major insect orders (Mahalakshmi and Jeyaparvathi 2014). They help maintain ecosystem balance by preying on a wide variety of insects, many of which are agricultural pests, thus contributing to crop protection. The taxonomic study of spiders is advancing rapidly, with many new species discovered each year, but the world's fauna is still poorly understood. There are a total of 52,536 known spider species belonging to 134 families and 4,341 genera in the world (World Spider Catalogue 2024). Indian arachnid fauna accounts for 3.72% of the total world diversity (61 families, 493 genera, and 1947 species) (Caleb and Sankaran 2023).

Cotton is one of the most important commercial crops cultivated in India, accounting for around 23% of the total cotton production in the world. India has got first place in the world with a cotton growing area of around 40% to that of world's area of 32.42 million hectares in 2022-23. (Cotton Advisory Board 2024). Cotton crops harbour a wide variety of natural enemies, but their potential as biological control agents for insect pests has been largely overlooked in scientific research. Biological control has long been recognized as a safe, environment-friendly, and long-lasting solution for managing insect pests, but intensive agriculture and climate affect population dynamics and activities of natural enemies (Van den Berg et al., 1990).

Spiders play an important role in pest control by eating large numbers of prey animals in agricultural fields, reducing the need for chemical pesticides (Rajeswaran et al., 2005).

Most spider species are omnivorous and feed on multiple life stages of prey. The abundance of spiders in crops increases during the growing stage of the plant (Mallesh and Sravanthy 2021). Despite the large number of studies on the ecology of spiders in different ecosystems worldwide, the significance of spiders as natural control agents is still largely unknown. Little attention has been paid to their insect control benefits in India because little is known about their species diversity, population abundance, and ecology.

In 2002, the Indian government decided to launch genetically modified cotton (Bollgard I cotton) called Bt cotton by inserting the gene (from soil bacterium *Bacillus thuringiensis* var. *kurstaki*) coding for Bt-toxin into cotton as a transgene, causing it to produce this natural insecticide in its tissues (Choudhary and Gaur 2010). This protects cotton crop lepidopteran pests, particularly bollworm complex (American bollworm (*Helicoverpa armigera* Hub.), spotted bollworm (*Earias vitella* Fab.), and pink bollworm (*Pectinophora gossypiella* Saunders), increasing cotton yield both quantitatively and qualitatively. Despite their relative safety in comparison to conventional insecticides and economic benefits to growers, there is still a concern that Bt crops may affect the population and diversity of upper trophic level non-target organisms, such as spiders (Head et al., 2001). The consumption of prey that survive the ingestion of Bt-toxin and thus contain traces of the toxin could indirectly affect natural enemies occurring in cotton fields (Meissle and Lang 2005). The present study aimed to record the diversity and abundance of spiders in Bt

(transgenic cotton PAU Bt 1) and non-Bt cotton crops, as well as the influence of crop stages and climate on the spider community in Punjab, India.

### MATERIAL AND METHODS

The present study was undertaken in two independent lines, Bt (Variety: PAU Bt 1 containing cry 1 Ac gene) and non-Bt cotton (variety: F2228) (American cotton, *Gossypium hirsutum*: Family Malvaceae) grown in experimental plots (0.4 ha area each), at the campus of Punjab Agricultural University (PAU), Ludhiana located at an intersection of 30°55' N parallel of latitude and 75°54'E line of longitude. The crops were sown during mid-May and harvested in mid-November using agronomic practices as recommended by PAU, Ludhiana. The data was recorded in two seasons. To record the spider diversity in Bt and non-Bt cotton crops, a plot of 10m x 20m was selected in the center of each experimental crop of the Department of Entomology, PAU, Ludhiana. In each plot, spiders were searched on the plants and the soil directly under the plants and collected using a combination of hand-picking and suction methods at 15-day intervals (Ludy and Lang 2004). Large spiders were gently tapped into the collection vials, while the small spiders were collected by sucking with a homemade aspirator and then blown into a collection vial for further identification.

Immediately after collection, the spiders were counted and their various morphological features were recorded and photographed by observing them under the stereoscopic binocular microscope located in the Electron Microscopy and Nanotechnology Laboratory, PAU, Ludhiana. The spiders were then preserved in 70% ethyl alcohol for later identification. Besides, various parts of some spider specimens were also removed and placed in 10% Potassium hydroxide solution overnight to dissolve the softer parts. They were washed in water, dehydrated, and mounted on slides for detailed examination. Male spiders were identified based on swollen palpus. The male genitalia was studied in an unexpanded condition. The specimens were properly identified as per the diagnostic characteristics described (Tikader and Biswas 1981, Barrion and Litsinger 1995, Sebastian and Pete 2017). To record the abundance of spiders in Bt and non-Bt cotton crops, four plots each having an area of 10m x 20m were selected from all four geographical sides. In each plot of the crop, five plants, one each from all four geographical sides and the center of the plot were selected and marked. Visual searches were made to locate the spiders on the whole plant, from bottom to top, on both the upper and lower sides of the leaves and the soil directly under the plant for the presence of spiders. The total number of spiders present on five plants of a plot was

counted to determine the average number of spiders per plot. Data was recorded at 15-day intervals on the selected plots from July to October for two seasons. Spiders found preying on insects in the crop fields were also photographed to record their predatory activity. Additionally, the data for different meteorological parameters such as mean temperature (°C), relative humidity (%), rainfall (mm), and wind velocity (km/h) were obtained from the Department of Climate Change and Agrometeorology, PAU, Ludhiana and their correlation with spider abundance was determined.

The data was analyzed using a factorial completely randomized design in SAS (Statistical Analysis System) 9.3 software. The effect of weather parameters on spider abundance was determined using a stepwise regression technique. Values were considered significant at a 5% level of significance. Shannon-Weiner index and species evenness index for spiders representing different families were calculated as per the formulae described by Nagrare et al. (2015) and are given below:

$$\text{Shannon-Weiner index (H)} = \sum[(\text{pi}) \times \ln(\text{pi})]$$

Where, pi = proportion of total sample represented by species i

$$\text{Species evenness (E)} = H / \ln(S)$$

Where, H = Shannon-Weiner index, and

S = Total number of species

### RESULTS AND DISCUSSION

Spiders of 11 species representing nine genera and six families were recorded from both Bt and non-Bt cotton crops (Table 1). A total of 523 and 451 spiders were collected from different parts of plants as well as the soil below the plant from Bt and non-Bt cotton crops, respectively. These spider species in decreasing order of their occurrence belonged to families Araneidae (37.68%) followed by Oxyopidae, Lycosidae, Thomisidae, Salticidae, and Pisauridae with maximum species richness observed of family Araneidae (4) followed by family Lycosidae (3), and families Oxyopidae, Thomisidae, Salticidae and Pisauridae (1 each) in both Bt and non-Bt crops (Table 1). The relative occurrence of spiders of different families in Bt and non-Bt cotton crops was almost similar. Spider diversity was similar in both types of cotton crops with predominance of families Araneidae, Lycosidae, and Oxyopidae which together constituted 85.21% of the total spider collection. The Shannon-Weiner index and species evenness index were 1.42 and 0.59, respectively in both the crops indicating rich spider diversity but less equitable distribution. The characteristic features of these species were used for their identification (Figs. 1-4). Characteristic X-shape webs of *Neoscona theis* (family Araneidae) were also observed in the present study (Fig 1B).

Spiders were also found preying on insects in the crop fields (Fig. 5) indicating their potential as biocontrol agents.

The record of seasonal abundance of spiders in Bt cotton grown in both seasons I and II, showed a low incidence (per 200m<sup>2</sup> plot) of spiders in July-August (3.00-10.70) during the growth stage, followed by an increase in September-October (16.00-18.50) near boll opening stage of the crop (Table 2). Seasonal records of the spider abundance in the non-Bt cotton crop grown in the two seasons also showed the same trend with a low average population in July-August and the first fortnight of September (0.00-2.25) followed by an increase from the second fortnight of September to October (3.00-7.50) when the crop was at the boll opening stage (Table 2). Overall, spider abundance was significantly higher in Bt crops as compared to non-Bt crops indicating no adverse effect of Bt toxin on the spider population.

The stepwise regression analysis between spider abundance as the dependent variable and weather parameters, viz. mean temperature, relative humidity,

rainfall, and wind velocity as independent variables revealed that spider abundance was positively correlated with the mean temperature, while relative humidity, rainfall, and wind velocity had a negative correlation with spider abundance. Further, there was a significant effect of mean temperature and rainfall on spider abundance.

In the present study, spiders of families Araneidae, Oxyopidae, and Lycosidae were most abundant on cotton crops. Bukhari et al. (2012) found spiders of the family Lycosidae (57.39%) as most abundant in the cotton crop in Gujranwala, Pakistan. Nagrare et al., (2015) observed spider diversity in transgenic and non-transgenic cotton in the rainfed agroecosystem of India and reported that the family Araneidae contributed one-third spider population (34.56%) followed by Oxyopidae (27%) and Thomisidae (24.53%). The Spiders of the family Salticidae (jumping spiders) which hunt sucking bugs like *Nezara viridula* and other hemipterans, were more common in Bt-cotton (Whitehouse et al., 2014). Wolf spiders of the family Lycosidae are abundant and

**Table 1.** Percent occurrence of different spiders in Bt and non-Bt cotton crops

Family	Species richness	Bt-cotton		Non-Bt cotton		Overall percent occurrence (974 total spiders)
		Number of spiders collected	Percent occurrence (%)	Number of spiders collected	Percent occurrence (%)	
Araneidae	4	205	39.20	162	35.92	37.68
Oxyopidae	1	136	26.00	145	32.15	28.85
Lycosidae	3	112	21.41	70	15.52	18.68
Thomisidae	1	57	10.90	46	10.20	10.57
Salticidae	1	10	1.91	27	5.99	3.80
Pisauridae	1	3	0.57	1	0.22	0.41
Total	11	523	--	451	--	--
H-index	--	--	1.42	--	1.42	--
Evenness	--	--	0.59	--	0.59	-

**Table 2.** Abundance of spider population in Bt and non-Bt cotton crops

Date	Number of spiders (n = 4 plots each)		Number of spiders (n = 4 plots each)	
	Season I		Season II	
	Bt crop	Non-Bt crop	Bt crop	Non-Bt crop
12 <sup>th</sup> July	3.00±1.58 <sup>A</sup>	0.75±0.83 <sup>B</sup>	6.50±2.61 <sup>A</sup>	1.25±0.43 <sup>B</sup>
27 <sup>th</sup> July	5.75±4.21 <sup>A</sup>	1.75±1.30 <sup>B</sup>	7.00±1.41 <sup>A</sup>	1.00±1.22 <sup>B</sup>
12 <sup>th</sup> August	4.25±2.38 <sup>A</sup>	0.00±0.00 <sup>B</sup>	10.70±3.34 <sup>A</sup>	1.00±1.73 <sup>B</sup>
27 <sup>th</sup> August	6.75±1.48 <sup>A</sup>	1.25±0.43 <sup>B</sup>	9.00±3.93 <sup>A</sup>	1.75±1.30 <sup>B</sup>
12 <sup>th</sup> September	16.25±2.05 <sup>A</sup>	2.25±1.64 <sup>B</sup>	18.50±1.50 <sup>A</sup>	1.50±1.12 <sup>B</sup>
27 <sup>th</sup> September	16.00±2.83 <sup>A</sup>	4.75±2.80 <sup>B</sup>	16.70±3.70 <sup>A</sup>	3.00±2.54 <sup>B</sup>
12 <sup>th</sup> October	16.50±1.91 <sup>A</sup>	7.25±3.96 <sup>B</sup>	10.70±3.27 <sup>A</sup>	4.25±2.38 <sup>B</sup>
27 <sup>th</sup> October	17.50±2.52 <sup>A</sup>	7.50±3.64 <sup>B</sup>	8.50±2.17 <sup>A</sup>	3.75±1.48 <sup>B</sup>

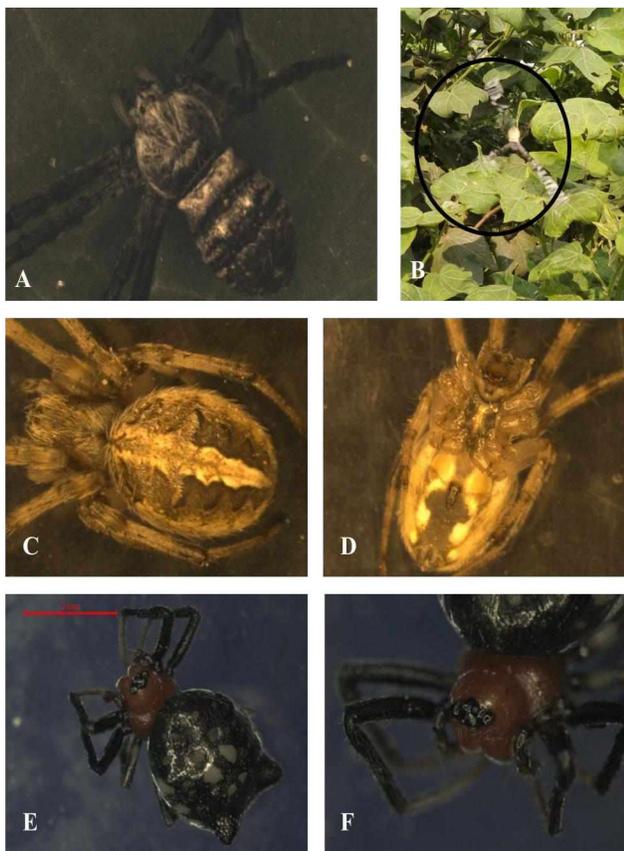
Values are Mean±SD, Values with superscripts (A,B) in a row for two seasons, separately indicate significant differences at P<0.05

rapacious predators at the soil-plant interface in cotton crops. They prey on late-instar larvae of economically important cotton bollworm *Helicoverpa* spp., that pupate in the soil (Rendon et al., 2019).

In the present study, the Shannon-Weiner index (1.42) and species evenness index (0.59) were low in both Bt and non-Bt cotton crops. Bukhari et al. (2012) have reported a higher Shannon-Weiner diversity index (2.80) and species evenness (0.92) for spider species collected from cotton crops in Pakistan. Nagrare et al. (2015) reported that the Shannon-Weiner index was 1.64 and the evenness index was 0.92 for spiders in transgenic and non-transgenic cotton crops in Central India. In the present study in both Bt and non-Bt cotton crops, spider abundance was generally low during the growth period of the crops i.e. in July-August and high near boll bearing and boll opening stages in September-October which are the times when most of the insects visit the crop and cause damage to the bolls and the plant has maximum height and foliage. This may be the reason for the buildup in the number of spiders and other natural enemies

(Rajendran et al., 2018). Bt cotton also acts as a refuge for spiders and other insect predators which get sufficient food available in the field from these non-lepidopteran insects (Mehboob-ur-Rahman and Zafar 2018). This may be the reason for the significantly higher spider abundance observed in Bt crops in the present study.

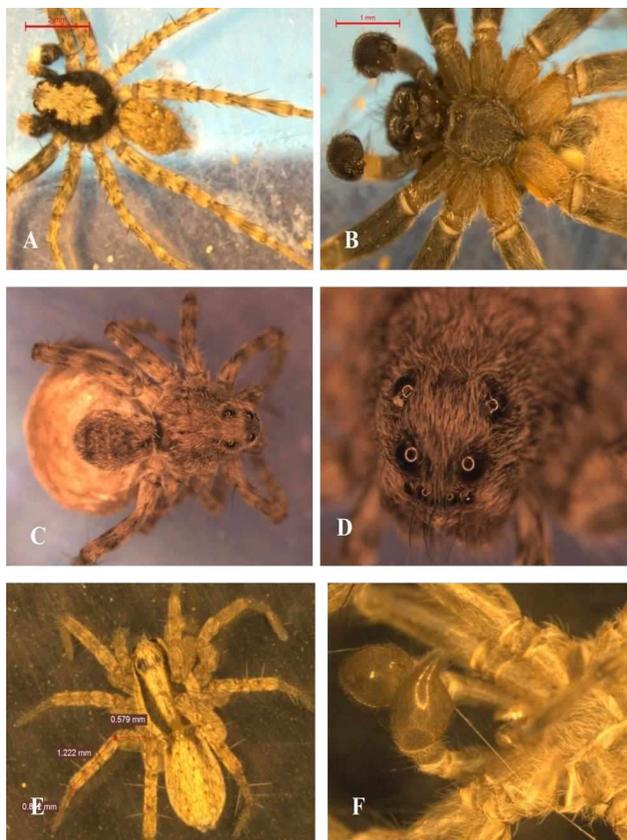
Bt cotton has no adverse impact on spider abundance and distribution of natural insect predators under field conditions (Dhillon et al., 2012, Arshad et al., 2015) although community composition could differ due to differences in the pest complex (Whitehouse et al., 2014). In the present study also, a higher number of spiders were collected from Bt crops (523) as compared to non-Bt crops (451). Contrary to the present results, Kiranmai and Sammaiah (2018) found a higher abundance of arthropods (including spiders) in non-Bt cotton compared to Bt cotton fields. The lower spider population in July-August during the present study may also be due to heavy rainfall. A significant negative effect of rainfall was observed on spider abundance in the present study. The study showed that spider population was positively



**Fig. 1.** *Argiope aemula*, A) Dorsal view and B) Characteristic X-shapes web; *Neoscona theis*, C) Dorsal view and D) Ventral view showing epigynum; *Chorizopes bengalensis*, E) Dorsal view, and F) Front view showing eye pattern



**Fig. 2.** *Neoscona mukerji*, A) Dorsal view and B) Ventral view; *Plexippus paykulli*, C) Dorsal view and D) Front view showing eye pattern; *Thomisus lobosus*, E) Dorsal view, and F) Ventral view.



**Fig. 3.** *Lycosa mackenziei*, A) Dorsal view and B) Ventral view showing sternum and shape of male palps; *Pardosa pseudoannulata*, C) Dorsal view with egg case attached to abdomen and D) Front view showing eye pattern; *Pardosa sumatrana*, E) Dorsal view, and F) Anterior view showing shape of male palps

correlated with mean temperature, while relative humidity, rainfall, and wind velocity were negatively correlated. Similar to our results, Bukhari et al. (2012) reported that increasing rainfall and high relative humidity suppressed spider populations in July and August in the cotton crop of District Faisalabad, Pakistan. Khuhro et al. (2012) reported a negative effect of both temperature and relative humidity on the spider population in cotton crops, while, Muchhadiya et al. (2014) reported a significant positive association of spiders with rainfall.

### CONCLUSIONS

There was no significant effect of Bt toxin on spider diversity and abundance in cotton crops. The abundance of spider communities, was, however, affected by climatic factors and the stage of the crop. There was a rich spider fauna in Bt and non-Bt cotton crops, and evidence of spiders predated on insects suggests utilization of their full potential in the Integrated Pest Management program.



**Fig. 4.** *Oxyopes birmanicus*, A) Dorsal view and B) Front view showing eye pattern; *Perenethis venusta*, C) Dorsal view of abdomen, D) Dorsal view of cephalothorax, E) Ventral view of abdomen showing epigynum, and F) Chelicerae showing arrangement of fangs and teeth



**Fig. 5.** Spiders found predated on different insects in cotton fields, 1) Honey bee, *Apis mellifera*, 2) Scoliid wasp, *Scolia erythrogyga*, 3) lady bird beetle, *Coccinella septempunctata*, and 4) Cotton grey weevil, *Chorthippus albomarginatus*

## REFERENCES

- Arshad M, Khan HAA, Rehman MAU and Saeed NA 2015. Incidence of insect predators and parasitoids on transgenic Bt cotton in comparison to non-Bt cotton varieties. *Pakistan Journal of Zoology* **47**(3): 823-829.
- Barrion AT and Litsinger JA 1995. *Riceland Spiders of South and Southeast Asia*. International Rice Research Institute, Philippines and CAB International, Wallingford, Oxon, Part 2 of 4. pp 180-411.
- Bukhari M, Naeem MM, Rehman KU and Andleeb S 2012. Occurrence and distribution of araneid fauna trapped from cotton fields of district Faisalabad, Pakistan. *World Applied Sciences Journal* **19**: 714-718.
- Caleb JTD and Sankaran PM 2023. *Araneae of India*. Version 2023, Online at <http://www.indianspiders.in>
- Choudhary B and Gaur K 2010. *Bt cotton in India: A country profile*. ISAAA series of biotech crop profiles. ISAAA, Ithaca.
- Cotton Advisory Board 2024. *Cotton*. Ministry of Textiles, Govt. of India. [https://www.ministryoftextiles.gov.in/sites/default/files/Annexure-VII-Note%20on%20Cotton%20Sector\\_0.pdf](https://www.ministryoftextiles.gov.in/sites/default/files/Annexure-VII-Note%20on%20Cotton%20Sector_0.pdf)
- Dhillon MK, Pampapathy G, Wadaskar RM and Sharma HC 2012. Impact of Bt-transgenic cottons and insecticides on target and non-target insect pests, natural enemies and seed cotton yield in India. *The Indian Journal of Agricultural Sciences* **82**(3): 248-254.
- Head G, Brown CR, Groth ME and Duan JJ 2001. Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. *Entomologia Experimentalis et Applicata* **99**: 37-45.
- Kuhro R, Ghafoor A, Mahmood A, Khan MS, Andleeb S, Bukhari M, Maqsood I, Shahjahan MM and Baloch NA 2012. Impact of temperature and relative humidity on population abundance of predatory spiders in cotton fields. *Journal of Animal and Plant Science* **22**: 649-652.
- Kiranmai K and Sammaiah CH 2018. Diversity of soil arthropod community in Bt and non-Bt cotton fields of Karimnagar district Telangana. *IOSR Journal of Agriculture and Veterinary Science* **11**: 01-04.
- Ludy C and Lang A 2004. How to catch foliage-dwelling spiders (Araneae) in maize fields and their margins: a comparison of two sampling methods. *Journal of Applied Entomology* **128**: 501-509.
- Mahalakshmi R and Jeyaparvathi S 2014. Diversity of spider fauna in the cotton field of Thailakulam, Virudhunagar district, Tamil Nadu, India. *Journal of Zoological Studies* **1**: 12-18.
- Malles M and Sravanthy CH 2021. Seasonal diversity and abundance of predatory arthropods (insect predators and spiders) in bt and non-bt cotton fields. *Uttar Pradesh Journal of Zoology* **42**(23): 29-37.
- Mehboob-ur-Rahman ZY and Zafar Y 2018. Past, Present and Future Trends in Cotton Breeding, pp 155-74. In: Arshad M, Khan RR, Aslam Anand Akbar W (eds). *Transgenic Bt Cotton: Effects on Target and Non-target Insect Diversity*. Intech Open, London.
- Meissle M and Lang A 2005. Comparing methods to evaluate the effects of BT maize and insecticides on spider assemblages. *Agriculture Ecosystem and Environment* **107**: 359-370.
- Muchhadiya DV, Saradava DA and Kabaria BB 2014. Population dynamics of insect pests and some of their natural enemies and their correlation with weather parameters on Bt cotton. The *Indian Journal of Agricultural Sciences* **84**(5): 572-578.
- Nagrare V, Vrushi D, Naiwadi BV and Paresh V 2015. Spider diversity in transgenic and non-transgenic cotton in rainfed agro-ecosystem of central India. *Research Journal of Biotechnology* **10**(12): 76-84.
- Rajendran TP, Birah A and Burange PS 2018. Insect Pests of Cotton, pp 360-411. In: Omkar (ed). *Pests and their Management*. Springer Nature, Singapore. [https://doi.org/10.1007/978-981-10-8687-8\\_11](https://doi.org/10.1007/978-981-10-8687-8_11)
- Rajeswaran J, Duraimurugan P and Shanmugam PS 2005. Role of spiders in agriculture and horticulture ecosystem. *Journal of Food Agriculture and Environment* **19**: 147-152.
- Rendon D, Taylor PW, Wilder SM and Whitehouse MEA 2019. Does prey encounter and nutrient content affect prey selection in wolf spiders inhabiting Bt cotton fields? *PLOS ONE* **14**(1). e0210296.
- Sebastian PA and Peter KV 2017. *Spiders of India*. University Press (India) Pvt. Ltd., Hyderabad, Telangana, p 614.
- Tikader BK and Biswas B 1981. *Records of the Zoological Survey of India. Spider fauna of Calcutta and vicinity Part-I*. Miscellaneous Publication, Occasional Paper No. 30, Zoological Survey of India, Calcutta, p 118.
- Van den Berg AM, Dippenaar-Schoeman AS and Schoonbee HJ 1990. The effect of two pesticides on spiders in South African cotton fields. *Phytophylactica* **22**: 435-441.
- Whitehouse MEA, Wilson LJ, Davies AP, Cross D, Goldsmith P, Thompson A, Harden S and Baker G 2014. Target and non-target effects of novel "Triple-Stacked" Bt-transgenic cotton 1: Canopy arthropod communities. *Environment and Entomology* **43**(1): 218-241.
- World Spider Catalog 2024. *World Spider Catalog*. Version 25.5. Natural History Museum Bern, <http://wsc.nmbe.ch>, doi: 10.24436/2