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Field evaluation of water plant extracts on sucking insect pests and their associated predators in transgenic *Bt* cotton

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Abstract

Several plant species and their compounds are well-known to have some pesticidal properties against a wide range of insect pests. Potential of two water plant extracts, *Azadirachta indica* A. Juss and *Melia azedarach* L., in comparison with the synthetic pyrethroid bifenthrin against sucking insect pests and their associated predators, as well on the yield in *Bt* cotton, was evaluated. The population of the sucking insect pests was found lowest in the positive control (only bifenthrin application). The *A. indica* extract reduced the pest population equivalent to the positive control, but the highest populations were observed in both negative controls (only water and 0.1% soap with water application). The *M. azedarach* did not show any harmful effect on the insect population. The predators' abundance was higher in the plots where botanicals were applied, while the lowest population was observed in the bifenthrin treatment. In comparison to the negative control (only water application), (63.4%) cotton yield was increased by the application of *A. indica* and (58.8%) by the application of the synthetic insecticide. Using plant extracts of *A. indica* to control sucking insect pests of cotton can be as effective as synthetic insecticides in terms of crop yield beside they are safer for natural enemies in the field.

Keywords: Biopesticides, *Azadirachta indica*, *Melia azedarach*, Predators, *Bt* cotton, Field evaluation

Background

The genetically modified crops have been cultivated and adopted by the farmers worldwide including Pakistan. The cotton containing *Bacillus thuringiensis* genes known as *Bt* cotton is being cultivated in more than half of the cotton-growing countries (Ali et al. 2010). In Pakistan, it was introduced in 2005 to mitigate the resistant strains of some lepidopterous insect pests (Sabir et al. 2011) with minimizing the insecticidal applications. Reducing the synthetic insecticides use against lepidopterans led to the increasing populations of sucking insect pests (Naranjo 2011). In Pakistan, the economic sucking insect pest complex in cotton include the whitefly, *Bemisia tabaci* Gennadius; the aphid, *Aphis gossypii* Glover; the jassid, *Amrasca devastans* Distant; the thrips, *Thrips tabaci* Lindeman; the red cotton bug, *Dysdercus koenigii* Walk; and the dusky cotton bug, *Oxycarenus hyalinipennis* Costa (Majeed et al. 2016).

The cotton growers mostly rely on synthetic insecticides to manage the sucking insect pests. The insecticide bifenthrin (Talstar) is a synthetic pyrethroid having contact and stomach toxicity (Mukherjee et al. 2010). Previously, it has been reported that this insecticide is very effective against different insect pests in cotton, vegetables, fruits, and in public health for the control of mosquitoes (Reddy and Rao 2002; Gupta et al. 2009). However, the presence of residue in fruits, vegetables, and green leaves above the maximum limit is a major concern about human health (Codex, Alimentarius Commission MRL of Pesticides 2009). Alternatively, insecticidal materials extracted from the plants are more readily biodegradable, may retard the resistance development in insects, and are safer for humans and the environment (Isman 2006). Among these, neem *Azadirachta*

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indica A. Juss (Family Meliaceae) has been emerged and served as a potent biopesticide in the last decade in the subcontinent (Ahmed et al. 2001) and still considered a highly effective compound against soft-bodied insects (Chaudhary et al. 2017). Azadirachtin, the major active ingredient of neem (Alzohairy 2016), responsible for insecticidal activity has gotten much attention in recent years (Chaudhary et al. 2017). Pest control using different plant extracts may help in preventing the resistance development in insects due to the presence of various bioactive compounds. These pesticidal plants have low persistence in the environment and are cheaper mainly for small-scale farmers (Isman 2008). Similarly, *M. azedarach,* having the active compounds triterpenoids, is well known to inhibit the feeding of herbivorous insects (Abdelghany et al. 2012).

Contrarily, natural enemies have immense importance in managing the population of insect pests in global agriculture (Chidawanyika et al. 2012). Unfortunately, the population of natural enemies is being affected negatively through excessive use of synthetic insecticides, but use of the botanical biopesticides have showed less effect (Potts et al. 2016).

The objective of this study was evaluating the effectiveness of two plant extracts in comparison to a synthetic chemical insecticide against sucking insect pests, their predators, and the yield in *Bt* cotton field.

Materials and methods

Study area

A transgenic *Bt* cotton (*Bt* FH-113) field, planted on 15 May 2018 under semi-arid climatic conditions in the farmland (29° 35′ 25.7″ N 71° 10′ 35.0″ E), located at Jalalpur city of southern Punjab, Pakistan, was selected for the study. All the standard cultural practices were followed as recommended for the growing of cotton. The experiment was conducted under a randomized complete block design (RCBD), and the treatments were replicated in 3 blocks.

Collection and processing of plant extracts

The seeds of neem, *Azadirachta indica* A. Juss, and the white cedar, *Melia azedarach* L. (Family: Meliaceae), were collected from different locations around the study area. The seeds were washed and sun-dried for 10 days. The seeds were crushed and powdered by using an electrical blender. To make 10% *w*/*v* extracts, a total of 1 kg of powder was mixed in 10-L water and kept at 25 ± 5 °C for 48 h. Further, 0.1% soap as a detergent was also added to the solution (Belmain et al. 2012). The extracts were stored in a 10-L buckets and kept in the shade. Before application, the extracts were filtered twice through a fine cloth to remove the extra plant materials for the safety of sprayer.

Treatment layout

The two botanicals and the synthetic pyrethroid pesticide bifenthrin (Talstar® 10EC, Jaffer Group of Companies, Pakistan) were tested in this study. The bifenthrin was applied as per the manufacturers' instructions (250 ml/acre). The positive control was bifenthrin insecticide (control—B), and 2 negative controls were made: water + 0.1% soap (control—ws) and water only (control—w). The treatments applications were performed when the population of pests reached at the economic threshold level (ETL). The ETL for sucking insect pests is 5 adults or nymphs/leaf for whitefly, a single adult or nymph/leaf for jassid, and 10 adults or nymph/leaf for aphid (Asif et al. 2016). The application was carried out, using a 15-L knapsack sprayer. The sprayer was cleaned after application of each treatment.

Sampling for the presence of sucking insect pests and their associated predators

From each block, 3 inner rows were selected for sampling. To record the data of a number of insect pests and predators, total 5 plants from the selected rows were visually examined after 3, 7, and 10 days of treatment applications. The main target insect pests were *A. gossypii*, *B. tabaci*, and *A. devastans*. The target predators were the lady beetles (adults) (Coleoptera: Coccinellidae) and green lacewing, *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae). In the case of insect pests and *C. carnea*, both immature and adult populations were considered, while only adults were recorded in case of coccinellids.

Data analysis

Data of the population of insect pests and predators, their abundance, and cotton yield were assessed, using analysis of variance (ANOVA) to check the significance among treatments. Means were separated by Tukey's post hoc Honestly Significant Difference (HSD) test at the 95% confidence interval. All the analyses were performed through Minitab 17.0 software.

Results and discussion

The results showed that the botanicals and bifenthrin insecticide had significant effect on the population of whitefly ($F = 206.6$), jassid ($F = 30.2$), and aphid ($F = 15.06$) at ($P < 0.05$). Only bifenthrin had greater effects, and low populations of all insect pests—whitefly (2.48 individuals/plant), jassid (1.45 individuals/plant), and aphid (1.87 individuals/ plant)—were recorded. The *A. indica* extract also reduced the pests' population, showing similar effects as the positive control of the chemical pesticide. The highest population of all insect pests was observed in both negative controls (only water and 0.1%

soap and water application). The *M. azedarach* was not efficient in suppressing the insect population (Fig. 1).

The population of all three insect pests was found lower by the application of synthetic insecticide; however, the results were comparable in case of *A. indica* treatment. Overall, there was a little difference in the abundance of pest population in *A. indica* and bifenthrin treatment. Bifenthrin was more effective against sucking insect pests of cotton in the present study but had a negative effect on the predators. The treatments had significant ($F = 2.65$, $P < 0.05$ for coccinellids, and $F = 2.47$, $P < 0.05$ for *C. carnea*) effect on the predator's populations. Interestingly, the population of coccinellids was found higher (1.07 individuals/plant) in the plot where *A. indica* was applied, followed by *M. azedarach* (0.93 individuals/plant). The population of coccinellids was lower (0.53 individuals/plant) and had insignificant difference ($P > 0.05$) in positive and negative controls. *C. carnea* was found higher (1.13 individuals/plant) in the plots, where *M. azedarach* was applied, followed by *A. indica* (0.87 individuals/plant) and negative controls (0.87 individuals/plant). In case of bifenthrin, the population of *C. carnea* was very low (0.40 individuals/plant) (Fig. 2). Overall, pest abundance was low in bifenthrin (1.93 individuals/plant) and *A. indica* treatment (2.46 individuals/plant). Pest abundance was higher in both negative controls and *M. azedarach* treatment. The predators' abundance was higher in plots, where botanicals were applied, while the lowest abundance was observed in positive control treatment where bifenthrin insecticide was applied (Table 1).

Previously, many researchers have reported the effectiveness of neem plant extracts against different insect pests (Kunbhar et al. 2018). Furthermore, *A. indica* has various excellent attributes including systemic action in plants, rapid degradation in the environment, and minimal effects on biological control agents (Mordue and Nisbet 2000). *A. indica* was acutely safe for the predators because the predator abundance was higher in both botanicals treatment than in the chemical pesticide. *M. azedarach* did not show significant toxicity against insect pests. Obtained results are in accordance with Kraiss and Cullen (2008) who reported that azadirachtin was much safer for the adults of ladybird beetle *Harmonia axyridis*. Patel et al. (2009) reported a higher activity of coccinellid beetles in mustard crop, using neem oil-based formulation. Similarly, Smitha and Giraddi (2006) stated that botanical pesticides are quite safer to the predatory coccinellids. The results have also been supported by Ma et al. (2000) who tested some biorational pesticides and synthetic insecticides against *Helicoverpa armigera* and *H. punctigera* (Lepidoptera: Noctuidae) and reported bifenthrin and azadirachtin were effective against insect pests and safer for coccinellids and *C. carnea*. In contrast, chemicals were found highly toxic to both predators. Similarly, Qi et al. (2001) reported that azadirachtin at 50–200 ppm concentration did not affect the predation rates of coccinellids beetle. Previously, researchers have studied the effect of botanical insecticides on non-target natural enemies under laboratory and field conditions (Chaudhary et al. 2017; Kunbhar et al. 2018). The harmful effect of azadirachtin compound on the

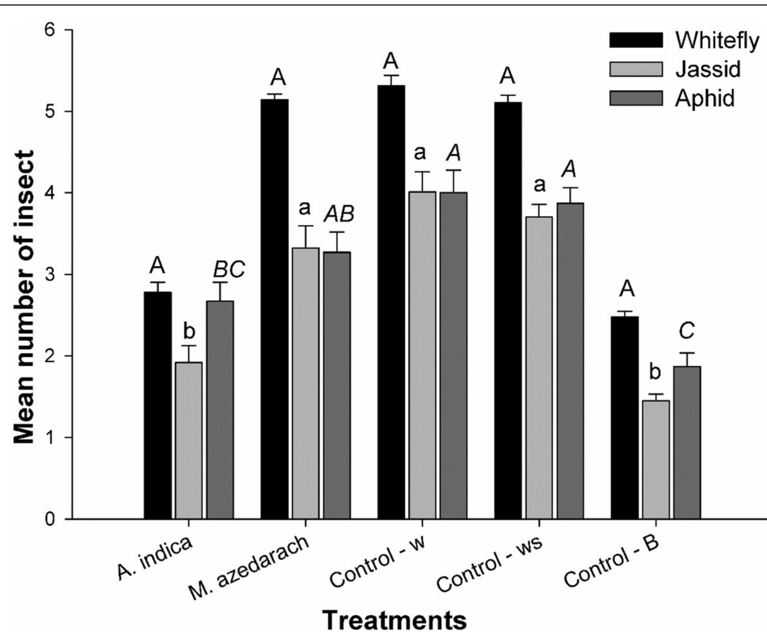

Fig. 1 Effect of plant extracts on the population (means ± SE) of sucking insect pests (average number of aphids, jassid, and whitefly per plant). Means sharing similar letters within treatments for each insect are not significantly different at $P > 0.05$

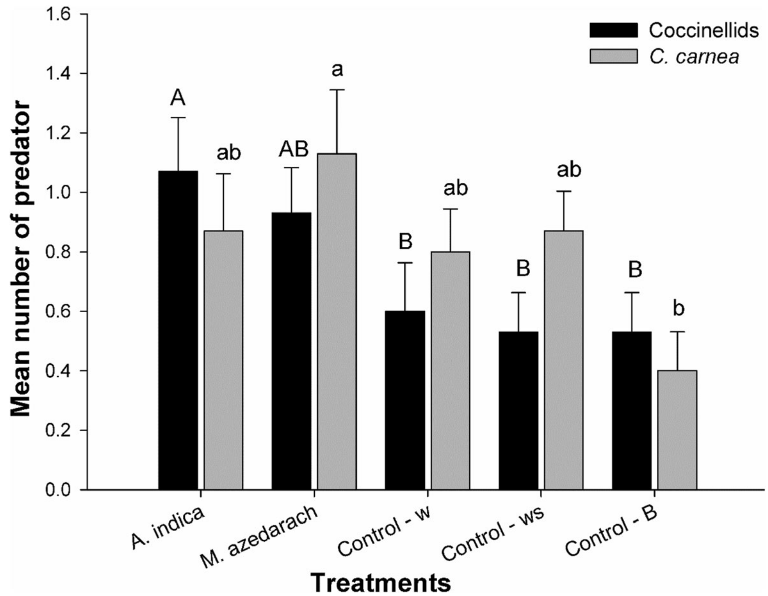

Fig. 2 Effect of plant extracts on the population (means ± SE) of predators (average number of coccinellids and *C. carnea* per plant). Means sharing similar letters within treatments for each insect are not significantly different at $P > 0.05$

immature stage of predators may be expected because it acts as a growth regulator and suppresses the ecdysteroid hormone that regulates the process of molting and development of immature insects (Gontijo et al. 2015).

The cotton yield was higher (2800.0 kg/ha) in case of *A. indica* treatment and (2058.5 kg/ha) with the use of *M. azedarach*. The yield was also comparable with the positive control (2487.6 kg/ha). In both negative control treatments, the yield of cotton was low (1024.6–1111.5 kg/ha) (Fig. 3). In comparison to the negative control (only water application), 63.4% yield was increased by the application of *A. indica*, 50.2% by *M. azedarach* application, and 58.8% by the application of synthetic insecticide (Table 1). Despite the lower number of pests' populations in bifenthrin treatment, the yield of cotton was comparable in the pesticidal plant treatment, *A. indica*. It could be due to the reduction of pests through their associated predators. Two species of coccinellids were recorded in the cotton field: *Coccinella septempunctata* L. and *Menochilus sexmaculatus* Fab. However, the population of *C. septempunctata* was higher compared to the second one. The visual presence of *C. septempunctata* matches with other studies which documented the role of this coccinellid species as major predator responsible for the reduction of cotton insect pests (Arshad et al. 2017). The other possible reason of higher yield could be due to the ability of plants to tolerate the damage and to compensate physiologically to maintain overall yield (Brown 2005). Usually, the compensation requires the good health of plants with sufficient nutrients and water (Tardieu and Tuberosa 2010).

Table 1 Effect of different treatments on the abundance of insect pests, associated predators, and percentage cotton yield

Treatments	Means ± SE		Yield increase (%)
	Pest abundance	Predator abundance	
A. indica	2.46 ± 0.123b	0.97 ± 0.131a	63.41
M. azedarach	3.91 ± 0.179a	1.03 ± 0.132a	50.23
Control—w	4.44 ± 0.157a	0.70 ± 0.108ab	0
Control—ws	4.23 ± 0.127a	0.70 ± 0.097ab	07.83
Control—B	1.93 ± 0.089b	0.46 ± 0.092b	58.81

Abundance values are the average number of pest and predators per plant recorded at 3, 7, and 10 days after application. Key pests are whitefly, aphids, and jassid, while key predators are coccinellid beetles and *Chrysoperla carnea*. Control—w is the application of water only, control—B is the application of the synthetic pesticide Bifenthrin, and control—ws is the application of water containing 0.1% liquid soap only, means sharing similar letters within columns are not significantly different at $P > 0.05$

The use of plant extracts has no greater level of risk to human health as does the use of synthetic insecticides. The neem seed extract offered the cost-effective plant protection alternatives to pyrethroid bifenthrin. Smallholder farmers in Pakistan have accessibility to this plant material and have the labor availability as well. Generally, the botanicals are considered safer to users, animals, and the environment because of their non-persistent nature (Buss and Park-Brown 2002).

Conclusion

The present study showed that the water extract of the tested plant seeds *A. indica* locally available

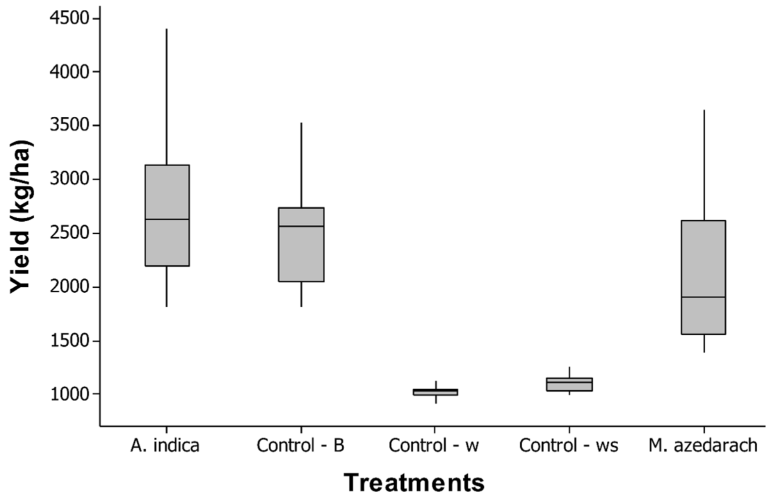

Fig. 3 Effect of plant extracts on crop yield (kg/ha) of cotton. Boxes represent mean and 95% confidence intervals, vertical line across the box is the max. and min. value, and horizontal line in the box is the median value

could be a less expensive and favorable alternative to mitigate the sucking insect pests and to reduce the negative impacts of insecticides. Further studies should be conducted at a larger scale and on the efficacy against different insect pests at different climatic conditions.

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Authors' contributions
MA, MIU, and NSÇ planned and designed the project and experimental layout. MA and AA performed the experiment. RRK and FD performed the statistical analysis. The manuscript was prepared by MA and CK and reviewed by MIU and RRK. All authors read and approved the final manuscript.

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Competing interests
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References

Abdelghany AM, Farag M, Yousef H, Ahmed MH (2012) Insecticidal activity of *Melia azedarach* L. triterpeniods against *Spodoptera littoralis* (Boisd.). J Am Sci 8(3):661–667

Ahmed I, Ali RR, Tabassum R, Azmi MA, Naqvi SNH, Khan MF (2001) Toxicity determination of two pyrethroids Karate (cyhalothrin and deltamethrin) as compared to neem extract (NA) on *Tribolium castaneum* (PARC strain). J Exp Zool 4:169–173

Ali S, Hameed S, Masood S, Ali GM, Zafar Y (2010) Status of Bt cotton cultivation in major growing areas of Pakistan. Pak J Bot 42:1583–1594

Alzohairy MA (2016) Therapeutics role of *Azadirachta indica* (neem) and their active constituents in diseases prevention and treatment. J Evid Based Complementary Altern Med 2016:7382506

Arshad M, Khan HAA, Hafeez F, Sherazi R, Iqbal N (2017) Predatory potential of *Coccinella septempunctata* L. against four aphid species. Pak J Zool 49(2):623–627

Asif MU, Muhammad R, Akbar W, Tofique M (2016) Relative efficacy of some insecticides against the sucking insect pest complex of cotton. Nucleus 53(2): 140–146

Belmain SR, Amoah BA, Nyirenda SP, Kamanula JF, Stevenson PC (2012) Highly variable insect control efficacy of *Tephrosia vogelii* chemotypes. J Agric Food Chem 60:10055–10063. https://doi.org/10.1021/jf3032217

Brown PR (2005) The effect of simulated house mouse damage to wheat in Australia. Crop Prot 24:101–109. https://doi.org/10.1016/j.cropro.2004.06.012

Buss EA, Park-Brown SG (2002) Natural products for insect pest management. UF/IFAS, Florida, USA, Publication ENY-350 Retrieved from https://www.purdue.edu/hla/sites/master-gardener/mglinks/natural-products-for-insect-pest-management/

Chaudhary S, Kanwar RK, Sehgal A, Cahill DM, Barrow CJ, Sehgal R, Kanwar JR (2017) Progress on *Azadirachta indica* based biopesticides in replacing synthetic toxic pesticides. Front Plant Sci 8:610

Chidawanyika F, Mudavanhu P, Nyamukondiwa C (2012) Biologically based methods for pest management in agriculture under changing climates: challenges and future directions. Insects 3(4):1171–1189

Codex, Alimentarius Commission MRL of Pesticides (2009) EFSA Scientific report (2008) Conclusion on peer review of bifenthrin 186;1–109 https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2009.186r

Gontijo LM, Celestino D, Queiroz OS, Guedes RNC, Picanço MC (2015) Impacts of azadirachtin and chlorantraniliprole on the developmental stages of pirate bug predators (Hemiptera: Anthocoridae) of the tomato pinworm *Tuta absoluta* (Lepidoptera: Gelechiidae). Fla Entomol 98:59–64 http://www.jstor.org/stable/24364174

Gupta S, Sharma RK, Gupta RK, Sinha SR, Singh R, Gajbhiye VT (2009) Persistence of new insecticides and their efficacy against insect pests of okra. Bull Environ Contam Toxicol 82:243–247
Isman MB (2006) Botanical insecticides, deterrents and repellents in modern agricultural and an increasingly regulated world. Annu Rev Entomol 51:45–56
Isman MB (2008) Botanical insecticides: for richer, for poorer. Pest Manag Sci 64: 8–11. https://doi.org/10.1002/ps.1470
Kraiss H, Cullen EM (2008) Insect growth regulator effects of azadirachtin and neem oil on survivorship, development and fecundity of *Aphis glycines* (Homoptera: Aphididae) and its predator, *Harmonia axyridis* (Coleoptera: Coccinellidae). Pest Manag Sci 64(6):660–668
Kunbhar S, Rajput LB, Gilal AA, Channa GA, Sahito JGM (2018) Impact of botanical pesticides against sucking insect pests and their insect predators in brinjal crop. J Entomol Zool Stud 6(2):83–87
Ma DL, Gordh G, Zalucki MP (2000) Biological effects of azadirachtin on *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) fed on cotton and artificial diet. Aust J Entomol 39:301–304
Majeed MZ, Javed M, Riaz MA, Afzal M (2016) Population dynamics of sucking pest complex on some advanced genotypes of cotton under unsprayed conditions. Pak J Zool 48(2):475–480
Mordue AJL, Nisbet AJ (2000) Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. An Soc Entomol Bras 29:615–632
Mukherjee I, Singh R, Govil JN (2010) Risk assessment of a synthetic pyrethroid, bifenthrin on pulses. Bull Environ Contam Toxicol 84(3):294–300
Naranjo SE (2011) Impacts of Bt. transgenic cotton on integrated pest management. J Agric Fd Chem 59:5842–5851
Patel BS, Patel IS, Patel GM (2009) Evaluation of different eco-friendly modules for the management of mustard aphid, *Lipaphis erysimi* (Kalt.) in North Gujarat. J Oilseed Res 26:679–680
Potts SG, Imperatriz-Fonseca V, Ngo HT, Aizen MA, Biesmeijer JC, Breeze TD, Dicks LV, Garibaldi LA, Hill R, Settele J, Vanbergen AJ (2016) Safeguarding pollinators and their values to human well-being. Nature 540:220–229. https://doi.org/10.1038/nature20588
Qi B, Gordon G, Gimme W (2001) Effects of neem-fed prey on the predacious insects *Harmonia conformis* (Boisduval) (Coleoptera: Coccinellidae) and *Mallada signatus* (Schneider) (Neuroptera: Chrysopidae). Biol Control 22:185–190
Reddy DJ, Rao BN (2002) Efficacy of selected insecticides against pests of grapevine. Pesticide Res J 14:92–99
Sabir HM, Tahir SH, Khan MB (2011) Bt cotton and its impact on cropping pattern in Punjab. Pak J Soc Sci 31:127–134
Smitha MS, Giraddi RS (2006) Safety of pesticidal sprays to natural enemies in Chilli (*Capsicum annum* L.). J Biol Control 20:7–12
Tardieu F, Tuberosa R (2010) Dissection and modelling of abiotic stress tolerance in plants. Curr Opin Plant Biol 13:206–212. https://doi.org/10.1016/j.pbi.2009.12.012

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