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Toxic and Antifeedant Effects of Different Pesticidal Plant Extracts against Beet Armyworm (*Spodoptera exigua*)

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ABSTRACT

The beet armyworm (BAW), *Spodoptera exigua* (Lepidoptera: Noctuidae) is a highly destructive pest of vegetables and field crops. Management of beet armyworm primarily relies on synthetic pesticides, which is threatening the beneficial community and environment. Most importantly, the BAW developed resistance to synthetic pesticides with making it difficult to manage. Therefore, alternative and environment-friendly pest management tactics are urgently required. The use of pesticidal plant extracts provides an effective way for a sustainable pest management program. To evaluate the use of pesticidal plant extracts against BAW, we selected six plant species (*Lantana camara*, *Aloe vera*, *Azadirachta indica*, *Cymbopogon citratus*, *Nicotiana tabacum*, and *Ocimum basilicum*) for initial screening experiment. Four out of six plant species such as *A. indica*, *N. tabacum*, *C. citratus* and *O. basilicum* showed promising mortality of more than 50%. Therefore, we selected these four plant extracts for the subsequent experiments. Through contact bioassay, *A. indica* showed high mortality 66.63%, followed by the *N. tabacum* 53.33%, at 10% w/v concentration. Similarly, *N. tabacum* showed the highest mortality rate, 66% at 10% w/v concentration, followed by the *A. indica* 46% through feeding bioassay. Furthermore, the feeding deterrence assay showed that *C. citratus* had a high antifeedant index (-50) followed by *A. indica* (-39), and *N. tabacum* (-28). In living plant assay, the *N. tabacum* extract showed a low mean damage score 3.6 on living cotton plant followed by *C. citratus* 4.5 and *A. indica* 5.5. Hence, extracts of three plant species provided promising results against the BAW, which can minimize the use of synthetic chemicals, particularly for small landholding farmers. Further studies are also required to evaluate the effects of these plant extract against BAW on cotton plants under field conditions to optimize the further use.

KEYWORDS

Bioassay methods; feeding deterrence; botanical pesticide; pest management; sustainable agriculture



1 Introduction

Agricultural researchers and producers have always struggled with insect pest management. Controlling insect pest infestations will become more difficult as insecticide resistance grows and more toxic pesticides are phased out [1]. Farmers will be challenged to produce high-quality, pest-free crops on a budget without jeopardizing the environment or endangering worker safety [2,3]. In response to this struggle, more research is being done into alternative control methods that are both cost-effective and environmentally friendly.

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (BAW) is a polyphagous insect pest that has been established all over the world, including the Indian sub-continent [4]. In Pakistan, BAW is one of the major pests of horticultural and field crops (cotton, maize), especially on vegetables (tomato, cotton, cauliflower, spinach) and cereal (chickpea, lentils) crops, respectively [5]. This insect is more likely to persist throughout the year due to the availability of a wide range of hosts [6]. Its larvae feed mainly on foliage and fruit [7]. Young larvae graze and skeletonize leaves. Larvae become solitary as they mature and chew irregular crevices in the foliage. BAW is a heavy foliage feeder, leading to a 100% loss of cereal crops [8].

Huge amounts of synthetic insecticides are being used to bring down the infestation of BAW in Pakistan [9]. The BAW has developed resistance to the widespread use of these synthetic insecticides, especially the organophosphates, carbamates and pyrethroids on which most Pakistani farmers rely [5,10,11]. As sub-continent farmers especially, Pakistan has a long history of using plants with pesticidal properties as an integrated approach to developing more eco-friendly and effective control of different crop pests [12]. Therefore, pesticidal plants would be an alternative and eco-friendly approach to managing the BAW.

Plants are a rich source of bioactive chemical compounds, making botanical insecticides an appealing alternative to synthetic chemical insecticides [13]. Aside from their insecticidal potential, they are reported to be less harmful to the environment and human health than synthetic pesticides [13]. Plant chemicals with pesticidal properties are target-specific, biodegradable to non-toxic products, and potentially suitable for use in an integrated pest management (IPM) program [14]. Many recent studies evaluated the use of botanical biopesticides in small-scale farming, which provides comparable yields to commercial synthetic pesticides by avoiding severe environmental damage. The leaf extract of *Phyllanthus acidus* showed 100% larvicidal effect *Artemia salina* after 24 h of spraying [15]. Similarly, the leaves extract of *Lippia javanica* and *Nicotiana tabacum* have shown promising results in controlling the fall *S. frugiperda* [16].

Keeping the above facts in mind, we conducted a study to evaluate some of the more encouraging pesticidal plant species to develop new, effective, and agro-ecologically sustainable methods for controlling BAW based on previously available knowledge of their abundance, phytochemistry, and safe use. The ultimate goal of the study presented here was to assess the potential effects of pesticidal plants on larval stages, including direct toxicity and feeding deterrence. Finally, the most promising plant extracts were applied to cotton plants to evaluate the foliar damage caused by BAW larvae under cropping conditions to see whether these plant extracts reduced the foliar damage.

2 Materials and Methods

2.1 Experimental Site, Collection and Rearing of Beet Armyworm

The current study was performed at the Eco-Toxicological Laboratory, Department of Entomology, University of Agriculture Faisalabad, Punjab, Pakistan. The fourth and fifth instar larvae of BAW were collected from different host plants in different districts of Punjab province in Pakistan. The BAW larvae were initially reared on young cotton leaves collected from the cotton field to develop a large population. However, the larvae were reared on a semi-synthetic diet after establishing a large population. The diet contained cotton leaf powder (300 g), methyl-4-hydroxybenzoate (3 g), flour (300 g), ascorbic acid

(4.7 g), corn oil (12 mL), sorbic acid (1.5 g), vitamin mixture (10 mL), streptomycin (1.5 g), agar (17 g), yeast extract (48 g). The agar and yeast were mixed with 750 mL boiling water, and added all other components [5]. The diet was placed in plastic cups at room temperature for one hour to become a solid paste.

The newly born larvae were placed in a 450 mL plastic cup and fed on young cotton leaves until the 2nd instar. The cotton leaves were replaced with new ones daily. After the 2nd instar, the larvae were transferred into a prepared diet until the pupal stage. The pupae were collected and transferred to the adult mating cage. The adults were fed on a 10% honey solution. The honey-soaked cotton wool was placed in mating cages. Additionally, the filter paper was placed into the cage for egg collection. The filter paper, along with eggs, was collected every day and placed into the hatching box.

2.2 Collection of Plant Materials and Extract Preparation

We initially selected six plant species: *L. camara*, *A. vera*, *A. indica*, *C. citratus*, *N. tabacum* and *O. basilicum*. These plant species were selected based on previous studies about the phytochemical and efficacy. The leaves of all plant species were collected from the different known locations of Punjab province in Pakistan. These collected leaves were dried and ground into the powder. The powder form of these leaves was kept at cool places until further use. For extract preparation, we used 100 g of fine powder of each plant in 1 L water and kept it at room temperature for 24 h. Subsequently, the prepared extract was filtered and used for bioassay. The two control treatments (positive and negative) were used during this experiment. Positive control included the field-recommended dose of chlorpyrifos, and negative control had the water.

Furthermore, we selected four (*A. indica*, *C. citratus*, *N. tabacum*, and *O. basilicum*) out of six plant species for subsequent experiments. The extract was prepared by dissolving the 150 g powder into 1 L methanol and kept at room temperature for 24 h to improve the extract efficacy. To prepare the five concentrations (0.1%, 1%, 5%, 10% w/v), we weighed and resolubilized the dried residues of extract in acetone. The two control treatments (positive and negative) were used during this experiment. Positive control included the field-recommended dose of synthetic insecticide chlorpyrifos, and negative control included the acetone.

2.3 Bioassay Methods

2.3.1 Contact Bioassay

Contact bioassay is a topical application of plant extracts directly to the body of BAW larvae. We applied 15 μ L of each plant extract directly to the bodies of 2nd instar BAW with the help of a 100 μ L pipette. The treated larvae were then placed into the Petri plates containing the prepared semi-synthetic diet. Two control treatments were used during this bioassay method. The field-recommended dose of chlorpyrifos 40% EC was used as positive control, while the distilled water was used as a negative control. A completely randomized design with eight treatments and three replications was used. Each replication contained five 2nd instar larvae of BAW. Finally, the dead larvae were counted after seven days of treatment and calculated the mortality percentage.

2.3.2 Feeding Bioassay

Feeding bioassay is also known as a leaf-dip bioassay method. Cotton leaf disks with 5 cm diameter were prepared, dipped into different plant extracts for a few seconds and then allowed to dry on tissue paper for 1 h. Each treated leaf disk was placed separately in Petri dishes, and five 2nd instar larvae were released on treated leaves. Eight treatments with three replicates of five larvae were used for each concentration. The field-recommended dose of chlorpyrifos 40% EC was used as a positive control, while the distilled water was used as a negative control. Similarly, the dead larvae were counted after seven days of treatment and calculated the mortality percentage.

2.3.3 Feeding Deterrence Assay

A feeding deterrence assay was performed to evaluate the antifeedant activity of four plant extracts against the BAW larvae by leaf disc no-choice method [17]. Fresh cotton leaves were used to prepare the leaf discs of 3-cm diameter. The prepared leaf discs were dipped into 10% w/v solution of four plant extracts. The leaf disc treated with water was used as a control treatment. The treated leaves were placed into the Petri dishes. The leaf discs were weighed using the analytical balance before the treatment application. Five 2nd instar larvae of BAW were released in each Petri plate. Three replications of each treatment were used. The leaf consumption by BAW larvae after in control and treated leaf discs was evaluated. The remaining uneaten leaf area in control and treated discs were weighed after 72 h. The percentage of the antifeedant index was calculated using the following formula [18]:

$$\text{Antifeedant Index} = \frac{C - T}{C + T} \times 100 \quad (1)$$

where C represents the leaf weight in control leaf discs and T represents the leaf weight in treated leaf discs.

2.3.4 Living Plant Assay

A living plant assay was used to evaluate the foliar damage of BAW on living cotton plants. The non-BT cotton variety FH-1000 was planted in pots. Five cotton seeds were planted in each pot and later were thinned into two after successful germination. The recommended dose of fertilizers (120:60:60, N:P:K) was applied through basal and top dressing. Standard agronomic practices, including hand weeding and watering, were used in all cotton plants. Cotton plants were infested with five larvae after 30 days of plant emergence. To avoid harsh conditions, larvae were placed on the cotton plant early in the morning. After the artificial infestation, cotton plants were covered with a cage to avoid the free movement of larvae. This experiment was carried out with a randomized complete block design with six treatments, including positive control (chlorpyrifos) and negative control (water). Six replications for each treatment were used in this experiment. The plant extract with 10% w/v concentration was applied uniformly to all plants through a handheld plastic sprayer. The first treatment applications were made after 48 h of larval infestation, and subsequent application was used after seven days. The damage data was collected after seven days of treatment applications upto 9 weeks. The foliar damage of each plant was recorded through the Williams' 0–9 scale for the whole plant damage method [19]. According to this scale: 0 = no visible damage, 1 = Pin hole damage, 2 = both shot hole and pinhole lesions, 3 = small 1cm holes on few leaves, 4 = many leaves with 1–3 cm elongated holes, 5 = Large, elongated lesions or tiny pieces chewed on several leaves, 6 = Large, elongated lesions and large pieces chewed on several leaves, 7 = On around half of the leaves, there are numerous holes of all sizes and forms. 8 = the 70% leaf eaten, 9 = almost completely destroyed the leaves [20].

2.4 Statistical Analysis

A randomized complete block design was used during the experiments. Two-way ANOVA was used to analyze the effects of treatments and interactions with bioassay methods and concentrations. Treatments and interactions mean were compared through Tukey's (HSD) test at the 95% confidence interval. All statistical analyses were performed using the GRAPHPAD 8.02 (GraphPad Software, La Jolla, San Diego, CA, USA). The graphs were also prepared with GRAPHPAD 8.02.

3 Results

3.1 Initial Toxicity Screening of Plant Extracts

Six plant extracts were used for contact and feeding bioassays during initial screening experiments. Water extracts (10% w/v) were prepared and used against BAW, showing different mortality ranges (Fig. 1). *L. camara* exhibited the lowest mortality percentage (6.33%) in contact bioassay, geared up to 20% in feeding bioassay. Similarly, *A. vera* plant extract showed low mortality in contact bioassay (13.34%) compared to feeding bioassay (26.67%).

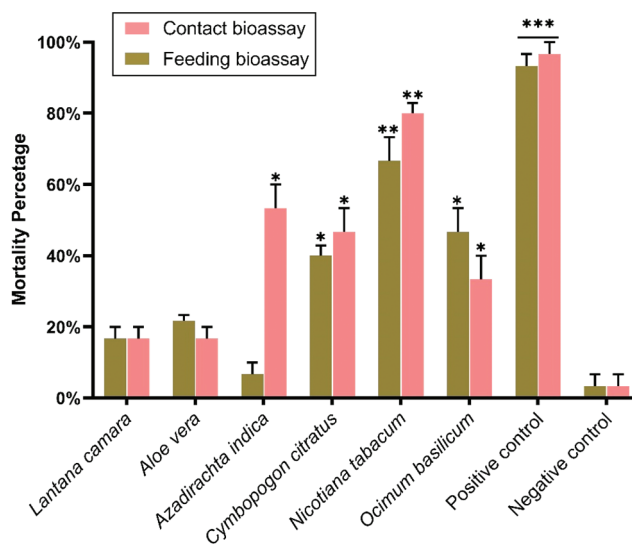


Figure 1: Mortality percentage of BAW larvae after application of different plant extracts through contact and feeding bioassays. A significant difference between the treatments with untreated control is highlighted with *. *** indicates the highly significant ($p < 0.001$), ** indicates the significant difference at $p < 0.005$, * indicates the significant difference at $p < 0.05$, and ns represents the non-significant difference between treatments and control

The most effective plant extracts against BAW larvae were *N. tabacum*, with a high mortality rate of 60%–73.34% in both (contact and feeding) bioassays, followed by *A. indica* 6.53%–53.33% (feeding and contact bioassay), *C. citratus* 40%–46%, and *O. basilicum* 26.66%–40%. The highest mortality percentage was observed in *N. tabacum*, 73.34%. Overall, these results showed that four plant species are most effective against the BAW larvae and demonstrated the effectiveness of different bioassay methods, which is confirmed by statistical analysis. Based on the initial screening results, we selected four (*N. tabacum*, *A. indica*, *C. citratus* and *O. basilicum*) out of six plant species for subsequent experiments.

3.2 Toxicity of Four Selected Plant Extracts against Baw through Two Different Bioassay Methods

Different concentrations of four selected plant extracts were topically applied to BAW larvae, with considerable mortality percentages, except the *O. basilicum* (Table 1). High mortality percentage of BAW larvae was observed at 10% w/v concentrations rather than the other concentrations. At 10% (w/v) concentration, *A. indica* showed high mortality (66.63%), followed by the *N. tabacum* (53.33%), *C. citratus* (46.67%), and displayed a highly significant difference from untreated control ($p < 0.05$). *O. basilicum* showed a low mortality percentage at all concentrations, which was not significantly different from the negative control. The positive control treatment (chlorpyrifos) showed nearly 100% mortality which is superior to all plant extracts.

For feeding bioassay, the cotton leaves were treated with different plant extracts and offered to BAW larvae which showed a significant mortality rate compared to untreated control (Table 2). *N. tabacum* showed the highest mortality (66%) at 10% w/v concentration, followed by the *A. indica* (46%) and *C. citratus* (40%), with a high significant difference from untreated control. The lowest mortality percentage (35%) was observed in *O. basilicum*. Although the mortality percentage in *O. basilicum* treated larvae showed a significant difference from untreated but was comparatively lower than other treatments. Some plant extracts showed better toxicity in one bioassay method, such as *A. indica* (66.67%) through contact bioassay.

Table 1: Mean mortality percentage of plant extracts through contact bioassay methods at different concentrations. Treatments were subjected by two-way ANOVA and means compared with Tukey's test at the 95% confidence interval

Treatments	Mortality percentage at different concentrations				
	0.1%	1%	3%	5%	10%
C.K +	95.3 a	95.2 a	100.0 a	100.0 a	100.0 a
<i>A. indica</i>	13.3 efg	20.0 defg	26.6 cdefg	46.6 bcd	66.6 b
<i>N. tabacum</i>	13.3 efg	20.0 defg	26.6 cdefg	40.0 bcde	53.3 bc
<i>C. citratus</i>	13.3 efg	20.0 defg	26.6 cdefg	33.3 cdef	46.6 bcd
<i>O. basilicum</i>	6.667 fg	13.3 efg	16.6 efg	16.6 efg	20.0 defg
C.K –	0 g	0 g	1.0 g	1.5 g	0 g
F treatments	236.87***				
F concentrations	21.21**				
F interaction ^(treat*conc)	3.25*				

Note: *** Indicates the $p < 0.001$, ** Indicates the $p < 0.005$, * Indicates the $p < 0.05$.

Table 2: Mean mortality percentage of plant extracts through feeding bioassay at different concentrations. Treatments were subjected by two-way ANOVA and means compared with Tukey's test at the 95% confidence interval

Treatments	Mortality percentage at different concentrations				
	0.1%	1%	3%	5%	10%
C.K +	90.4 a	95.3 a	100.0 a	100.0 a	100.0 a
<i>A. indica</i>	11.3 de	15.7 de	26.6 cde	26.7 cde	46.7 bc
<i>N. tabacum</i>	33.4 cd	40.0 bcd	40.0 bcd	53.4 bc	66.7 b
<i>C. citratus</i>	10.3 de	11.0 de	33.3 cd	33.4 cd	40.0 bcd
<i>O. basilicum</i>	10.3 de	11.0 de	33.3 cd	33.4 cd	40.0 bcd
C.K –	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e
F treatments	202.78***				
F concentrations	15.94**				
F interaction ^(treat*conc)	2.04*				

Note: *** Indicates the $p < 0.001$, ** Indicates the $p < 0.005$, * Indicates the $p < 0.05$.

3.3 Antifeedant Activity of Selected Plant Extracts

Four plant extracts were used to determine the antifeedant activity against the BAW larvae. The results of this trial indicated that all four plant extracts exhibited some degree of feeding deterrence when sprayed with 10% w/v concentrations (Fig. 2). *C. citratus* showed a high antifeedant index (–50) followed by *A. indica* (–39), *N. tabacum* (–28). The lowest antifeedant index was observed in *O. basilicum* (–9). Overall results showed that *C. citratus* and *A. indica* plant extracts had the best antifeedant effects against the BAW larvae compared to other plant extracts.

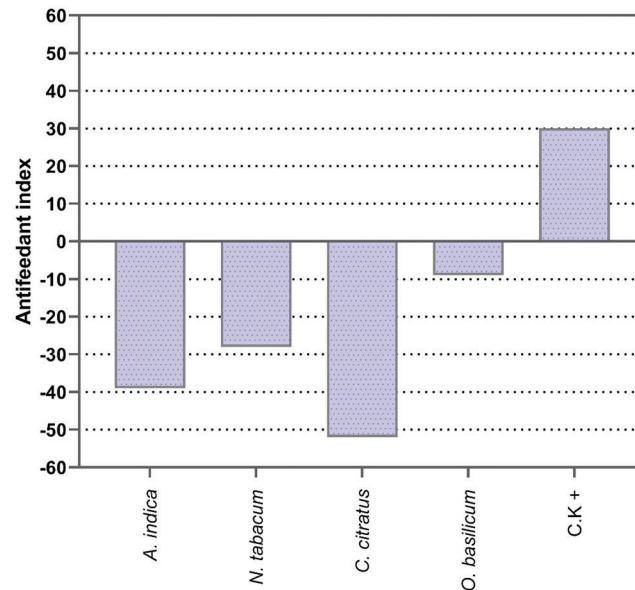


Figure 2: Antifeedant effects of four different pesticidal plant extract against the BAW larvae after 72 h of exposure. Bars represent the percentage of feeding deterrence

3.4 Damage Count of BAW Larvae on Living Cotton Plant

The four most active pesticidal plant extracts were applied to living cotton plants and BAW larvae were allowed to feed on treated plants. The ability of damage caused by the BAW larvae on cotton plants was observed, which showed a significant difference at $p < 0.05$ among the treatments compared to control treatments. The lowest mean damage score was recorded in the positive control (chlorpyrifos) treated plants, and the highest mean damage score was recorded in negative control (water) treated plants. Among the pesticidal plant extracts, *N. tabacum* showed a low mean damage score of 3.6, followed by *C. citratus* 4.5 and *A. indica* 5.7 (Fig. 3). A high mean damage score was observed in *O. basilicum* (6.5) and negative control (7.5). The lowest foliar damage score of 1.3 was observed in chlorpyrifos-treated plants.

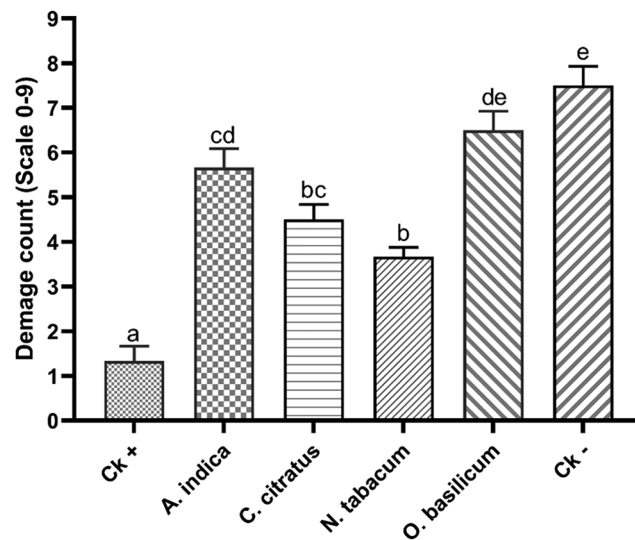


Figure 3: Beet armyworm damage score on cotton after the application of different treatments. The bars indicate the mean \pm SEM of different treatments. Different letters highlight the significant differences among the treatments at $p < 0.05$, and the treatments share similar letters are not significantly different from each

4 Discussion

The extensive use of synthetic pesticides to manage pest infestation in developing countries has some negative impact on human health and the ecosystem [21]. The use of pesticidal plant extracts is an alternative and biocontrol option for sustainable pest management programmes for developing countries, and our results showed that the pesticidal plant extracts can effectively control and reduce the damage caused by BAW larvae which would be an eco-friendly and sustainable pest management approach for smallholder farmers [22].

The *L. camara* and *A. vera* showed comparatively lower mortality percentages during the initial toxicity screening trial than other treatments. These results are surprising because previous studies showed that *L. camara* had high mortality percentage against a wide range of insect species [23–26]. In the current experiment, we used the same conditions and extraction methods for *L. camara* as previously described [23,26]. However, the mortality percentage in our case is lower than the previous studies. Similarly, *A. vera* plant extract also showed low mortality in contact bioassay compared to feeding bioassay. These results differ slightly from previous studies in which the *A. vera* plant extract showed considerable mortality at 10% w/v concentration against housefly and maize storage pest (*Sitotroga cerealella* and *Sitophilus oryzae*) [27–29]. *A. vera* plant extract was also used to manage the aphid population under field conditions [30].

The most effective plant extracts against BAW larvae were *N. tabacum*, with a high mortality rate in both contact and feeding bioassays, followed by *A. indica*, *C. citratus*, and *O. basilicum*. In previous studies, *N. tabacum*, *A. indica*, *C. citratus* and *O. basilicum* showed at least 50% larval mortality with one application method, but in our case, it showed different mortality percentages with respect to different application methods [31–34]. Overall, these results showed that four plant species are most effective against the BAW larvae and demonstrated the effectiveness of different bioassay methods.

Further, we tested different concentrations of four selected pesticidal plant extract against BAW with two bioassay methods. High mortality percentage was observed in *N. tabacum* at (10% w/v) through both bioassay methods. Overall, *N. tabacum* and *A. indica* extracts showed a more significant mortality percentage of BAW larvae. These results are similar to previous studies in which the *N. tabacum* and *A. indica* showed high mortality against different insect species, including lepidopteran insects and also provided the eco-friendly control of harmful pest species [35–39]. *A. indica* showed high mortality percentage at (10% w/v) concentration through the topical bioassay method. These results are consistent with previous studies in which the *A. indica* showed better mortality through topical application [40–42]. Furthermore, some differences were observed in mortality rates in plant extracts prepared with water and methanol. The mortality rate between extracts prepared in methanol and water was almost similar except the *O. basilicum*, which exhibited a lower mortality rate in methanol extract than water extract. The reason behind the low mortality rate is unclear; however, the difference in mortality rate might be due to different extraction efficiency in different solvents (water and methanol). The variations in mortality rate and lack of an obvious dosage impact between methanol and water trials might be caused by differences in feeding rates of BAW by feeding deterrent behaviour [16].

The antifeedant activity of four selected plant extracts was evaluated in which *C. citratus* and *A. indica* plant extracts had the best antifeedant effects against the BAW larvae compared to other plant extracts. Although, these two plant extracts caused relatively low mortality rates against BAW. But, these two plants may have some feeding deterrent compounds which help to minimize the damage to cotton plants. In accordance with our results, the previous studies also showed antifeedant effects of different plant extracts and essential oils against different insect species. Six plant extracts, including *A. indica* and *N. tabacum* were tested against *Demotisca neivai*, which exhibited strong antifeedant effects [43]. The extracts from *C. citratus* showed high antifeedant effects against *Dinoderus porcellus* [44],

Crocidolomia binotalis [45], *S. litura* [46], *Tribolium castaneum* [47,48], and *S. oryzae* [48]. The antifeedant effects of *A. indica* against various insects, especially for lepidopteran pests, are also well demonstrated in previous studies [49–54].

Furthermore, the mean damage score was calculated on living cotton plants treated with four most active plant extracts. The lowest mean damage score was observed in *N. tabacum* and *C. citratus*-treated cotton plants. The reduction in foliar damage of cotton crops might be attained due to the antifeedant, toxicity, and repellent effects of these four pesticidal plants, consistent with previous studies. The foliar damage reduction caused by the antifeedant, toxicity, and repellent effects of specific synthetic and biopesticides against different crop pests has been demonstrated in previous studies [18,33,45,46,55,56]. Synthetic pesticides reduce the plant damage caused by BAW more than pesticidal plant extracts, as shown in chlorpyrifos-treated plants. However, some additional studies showed that almost equal damage and the mortality rate was observed when pesticidal plants extract compared with synthetic chemicals [22,57–59]. Further, additional studies must be performed to know if these pesticidal plant extracts can maintain a similar yield to synthetic pesticides under field conditions.

Our study suggests that the pesticidal plant species are providing eco-friendly sustainable pest management of BAW. Out of all tested species, three pesticidal plant extracts (*N. tabacum*, *A. indica* and *C. citratus*) showed a significant mortality rate and antifeedant effects against BAW larvae. Furthermore, these plant extracts also showed low foliar damage caused by BAW in living cotton plants. However, the *O. basilicum* extract failed to show the expected results, although it somehow showed mortality rate and antifeedant activity.

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