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RESEARCH ARTICLE

Field Evaluation of Indigenous Plant Extracts and Clothianidin against Wheat Aphid, *Rhopalosiphum padi* (Aphididae: Hemiptera)

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ABSTRACT

The experiment was conducted to assess the potential effect of botanical extracts *Capparis aphylla*, *Solanum surrattense*, and *Haloxylon salicornium* alone and in combination with Clothianidin against wheat aphid, *Rhopalosiphum padi* (L.) under field conditions. Data were recorded at 0h, 12h, 24h, 36h, and 48h time intervals. Results revealed that T_6 (*C. aphylla* + Clothianidin) demonstrate higher rates of population reduction, population reduced from 16.33±0.88 to 0.33± 0.33 at 48 hours interval, whereas botanical extracts of treated, *S. surattense* exhibit lower aphid mortality and population reduced from 16.65±0.57 to 3.33±1.45. Overall, the plant extracts and insecticide demonstrate effective control over aphid but it has a negative impact on beneficial insects and their adverse impacts must be taken into account while implementing pest management approaches. Future pest management strategies should emphasize the exploration of alternative formulations, and optimizing application dosages and timing to reduce the detrimental effects on beneficial insects.

Key words: Plant extract, Aphid attack, Wheat, Clothianidin.

INTRODUCTION

Agricultural production is facing a major threat from insect pests (Razaq *et al*., 2019) (Khalid and Amjad, 2018), and they are responsible for 15% of crop losses worldwide (Maxmen, 2013). Aphids are a group of pest species that infest multiple crops including wheat (Arif *et al*., 2012; Simon and Peccoud, 2018). They can infest both the aerial and underground parts of the plants and suck very nutritious sap with high levels of sugar and amino acids. While feeding, they remove a significant number of amino acids and sugars and lead to the depletion of chlorophyll contents (Sytykiewicz *et al*., 2013). It secretes honeydew on the surface of the leaf, which invites sooty molds and interferes with photosynthetic and respirational functions (Hamza et al., 2018; Razzaq et al., 2021; Zafar et al., 2022). It also serves as a vector for plant pathogenic viruses (Douglas, 1993; Harris and Maramorosch, 2014; Jakobs *et al*., 2019). *Schizaphis graminum*, *Rhopalosiphum padi*, and *Sitobion avenae* are significant wheat yield-reducing species of aphid and reduce yield by 61% (Shah *et al*., 2017) (Razzaq et al., 2021).

Monocultural practices are dependent on inputs like the use of fertilizer and pesticides for pest management (Shah *et al*., 2020; Naeem *et al*., 2021). Insecticides are used for pest management of various pests and cause problems like environmental pollution, which threatens human health (Silva *et al*., 2005). Ingredients of synthetic pesticides are associated with chronic disorders in humans due to their contact or consumption (Damalas and Koutroubas, 2016). They are not biodegradable and thus bioaccumulate in the environment and become responsible for ozone layer depletion, soil pollution, and groundwater pollution (Sande *et al*., 2011).

Neonicotinoids are extensively used for aphid management (Shah *et al*., 2017). The residual effects of insecticides in food crops lead to the prevention of their use for aphid management (Mahmood *et al*., 2016). The most important problem of insecticide use is insecticide resistance development in the pest population (Nenaah *et al*., 2015) (Shah *et al*., 2023). The drawbacks associated with the misuse and overuse of synthetic pesticides lead to the requirement for alternative pest management

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(Kamal *et al*., 2019). Biological control is an important alternative approach to chemical control (Kamal et al, 2019).

Prior to the synthetic pesticide development and technological revolution, plant-based botanicals were used for pest management (Mahmood *et al*., 2016). As a result, the use of plant-based natural products is slowly declining until now, when the side effects of synthetic pesticides start threatening human health and safety. Nowadays, there is a trend toward the evaluation of different plant extracts that may have insecticidal potential and can be used as an alternative to synthetic pesticides (Silva *et al*., 2005).

The global drift is towards the utilization of those food products that are produced using safe and sound methods (Zaghum *et al*., 2021). Natural plant protection products are one of the most desirable methods of pest management. Residual detection of dangerous chemical pesticides and the rise in consumer awareness of food safety issues led to the ban on various pesticides in agricultural production, and plant-based biopesticides are capturing increasing demand in organic agriculture (Karaca *et al*., 2017; Mishra *et al*., 2018). Plant-based botanicals may perform like natural pesticides and can play the role of antifeedant repellant against various insect pests, but they are much safer for humans and our environment.

Those plant species are recognized to have diverse bioactive compounds, including flavonoids, steroids, and alkaloids (Silva *et al*., 2005). The significance of botanicals is linked to their success, biodegradability, a different mode of action, less toxicity, and the availability of plenty of source materials (Neeraj *et al*., 2017). Moreover, their re-entry and post-harvest intervals are very short. Food that is produced using plant-based protection materials sells at premium prices (Gupta and Dikshit, 2010). That's why botanicals are of supreme importance in crop production for human utilization now that there is a profitable market among consumers and they are ready to pay more for organically produced food products (Misra, 2014).

Capparis aphylla (Capparacea), locally known as Kari in Punjabi, is a small tree or shrub that is found in the desert areas of the Indian subcontinent, Saudi Arabia, and Africa. It is being studied because it has a very high nutritional profile and good medicinal effects (A *et al*., 2015; Eddouks *et al*., 2017). In the Perso-Arabic traditional medicine system, its shoots are used as an anti-fertility drug in combination with the shoots of *Peganum hermala* (Singh and Singh, 2011). Due to their high nutritional profile and bioactivity, they are also used as food; their flowers are eaten as vegetables, and fruits are used in a pickle (Khalid *et al*., 2021). Different pharmacological properties like insecticidal properties, anti-aging, anti-arthritis, anti-microbial, anti-viral, anti-atherosclerotic, anti-inflammatory, analgesic, and nociceptive action are associated with different parts of *C. aphylla* (Nazar *et al*., 2020).

Neonicotinoids are systematic insecticides that are very persistent in plant tissues and can be present in pollen and nectar. Hence, it is potentially harmful to non-target organisms like bees (Simon-Delso *et al*., 2015). Clothianidin, a neonicotinoid insecticide, acts on the nervous system of insect arthropods by binding to the nicotinic acetylcholine receptors (nACHRs). It is extensively used in agriculture and found to be very effective against a number of insect pests like aphids, thrips, and whiteflies (Elbert *et al*., 2008). Its use is linked with the decline of beneficiary insects and pollinators, which can greatly affect ecosystem services and agricultural production (Goulson *et al*., 2015).

The current study aimed to evaluate the effectiveness of plant extracts of three native plants from Cholistan: *Capparis aphylla*, *Solanum surrattense,* and *Haloxylon salicornium,* with the integration of an insecticide, Clothianidin, against wheat aphids under field conditions.

MATERIALS AND METHODS

Plant Material

Certified wheat seed (*Triticum aestivum)* variety AKBAR-19 FMC Pakistan (pvt) limited, were purchased from the pesticides market. Wheat seed was treated with Imidacloprid 360g/l and tebuconazole 12.5g/l with dose 200ml per 100kg purchased with brand name Hombre Ultra® Cereal Seed Treatment Bayer Pakistan (pvt) limited**.** The plants *S. surattense, H. salicornicum, C. aphylla* were collected from the cholistan between Yazman and fortabbas, Punjab, Pakistan (29.040671°N, 72.132966°E), identified by a taxonomist at the Cholistan Institute of Desert Studies, The Islamia University, Bahawalpur.

Experimental Site and Design

The field experiment was conducted in the Department of Entomology Islamia University of Bahawalpur, Punjab, Pakistan (29.35° N latitude, 71.69° E longitude) on silt-loamy soil. This research site lies in a warm desert climate, a mean annual temperature of about 25 °C and an annual average precipitation of 233mm. The trails were laid out in Randomized Complete Block Design (RCBD) under the factorial arrangement with three replications having a plot size of 9 square meters.

Sowing of Wheat

The soil was ploughed three times to loosen it, and a laser land leveller (Laser land leveller MF375 (75HP) produced by Millat Tractors, pvt, ltd) was used for levelling. The broadcasting method was used to sow wheat on a one-acre field at a seed rate of 40kg/acre. Additionally, 50kg/acre of urea fertilizer, 50kg/acre of DAP, and 50kg/acre of MOP were used during the land preparation of one hectare. The field was irrigated according to its needs, and all necessary agronomic practices were followed to ensure a successful trial.

Preparation of Plant Extract

A method called "Cold Water Extraction." Used for plant extraction using a mixture of plants dry powder as a solute and water as a solvent, the method involves using an electric grinder to make fine powder and a muslin cloth to strain the solution to obtain extract (Daya *et al.* 2011). To prepare the extract, the collected plants were washed with distilled water and dried for two weeks in shade. The dried plants were then ground into a fine powder using an electric grinder (WestPoint Spice Grinder WF-9227) to obtain particle sizes ranging from 100-1000 µm, suitable for insecticide application. The resulting powder was stored in an airtight container at room temperature (37 °C). To prepare the insecticidal solutions, 24g of dried powder from each plant species (*S. surattense*, *H. selicornicum*, and *C. aphylla*) were dissolved in 100ml of distilled water as a solvent to make 124ml of each solution.

Insecticide

Clothianidine 200g/L(17.70w/w) brand name Ceedo® 20%EC (Smart Agro Chemicals (pvt) limited) is an enhanced 3rd generation neonicotinoid insecticide, inhibits Acetylcholine Receptors, Mode of action is systemic, trans-laminar and mitigate sucking pest in different crops, ineffective against beneficial insects.

Statistical Analysis

To check the effect of treatments on the wheat aphid and different beneficial insects. For data regarding population reduction was taken before spray and after spray and calculated using the following formula:

% population reduction

$$=\frac{\text{population before treatment}-\text{population after treatment}}{\text{population before treatent}}x100$$

Data on the number of aphids per plant were recorded after 12 hours, 24 hours, 36 hours and 48 hours. The data was analyzed by the one-way analysis of variance (ANOVA) in software statistic 8.1 using LSD at 5% significance level.

RESULTS

According to the results T_1 (*Haloxylon salicornicum* extract): the aphid population steadily dropped from 13.11±0.39 at 12 hours to 4.69±0.66 at 48 hours, showing a significant reduction as compared to the initial population (19.33±0.57). T_2 (*Solanum surrattense* extract): the aphid population has shown a similar tendency to T_1, decreasing from 14.50±1.08 at 12 hours to 3.33±1.45 at 48 hours. Similar results were found by (Shah *et al*., 2017). The population decline was significant as compared to the initial population (16.65±0.57). T_3 (*Capparis aphylla* extract): the aphid population also decreased, reaching 2.25±1.37 at 48 hours, much less than the initial population (16.50±0.57). According to the (Zhang *et al*., 2016) the results were same. T_4 (*Haloxylon salicornicum* extract + clothianidin insecticide), T_5 (*Solanum surratense* extract + insecticide), T_6 (*Capparis aphylla* extract + insecticide), and T_7 (insecticide): these treatments all resulted in a significant d in the aphid numbers across the 48 hours, proving the effectiveness of the integrated approach. The populations ranged from 0.33±0.33 to 4.25±0.16 at 48 hours. T_0 (control): the aphid population in the control group remained relatively constant over time, with values ranging from 18.65±0.57 to 22.7±0.16 across the 48 hours. Same finding was found by (Kirkland *et al*., 2018).

According to the results T_1 (*Haloxylon salicornicum* extract) and T_2 (*Solanum surrattense* extract) exhibited a continuous reduction in ladybird beetle populations over the 48 hours. These results were comparable to the (Mzimela, 2017). The population numbers ranged from 1.66±0.66 to 2.66±1.45 individuals. T_3 (*Capparis aphylla* extract) demonstrated a significant decrease in ladybird beetle populations, reaching 0.00±0.00 individuals at all periods. Comparable results were also found by (Mzimela, 2017). The integrated treatments, including T_4, T_5, and T_6, which combined the botanical extracts with clothianidin insecticide, resulted in a notable decline in ladybird beetle populations. The count falls to 0.00±0.00 individuals. T_7 (insecticide alone) showed a similar effect, leading to a decline in ladybird beetle populations; with counts reaching 0.00±0.00 individuals. These results were also resemble with (Veres *et al*., 2020). The control group (T_0) did not exhibit a significant change in ladybird beetle populations over the 48 hours, with counts ranging from 4.33±0.33 to 6.66±0.33 individuals.

According to the results T_1 (*Haloxylon salicornicum* extract) and T_2 (*Solanum surrattense* extract) showed a relatively consistent population of green lacewing insects over the 48 hours, with population counts ranging from 1.33±0.66 to 2.00±0.57 individuals. T_3 (*Capparis aphylla* extract) and T_4 (*Haloxylon salicornicum* extract + clothianidin insecticide) led to a decrease in the green lacewing population, with numbers ranging from 0.00±0.00 to 0.33±0.33 individuals. Same findings were found by (Kirkland *et al*., 2018; Mzimela, 2017). T_5 (*Solanum srrattense* extract + insecticide) showed a similar effect, causing a reduction in the green lacewing population, with numbers ranging from 0.66±0.33 to 1.66±0.33 individuals. T_6 (*Capparis aphylla* extract + insecticide) and T_7 (insecticide alone) had a notable impact on the green lacewing population, causing values to decrease to 0.00±0.00 individuals at all periods. The control group (T_0) demonstrates a constant population of green lacewing insects throughout the 48 hours, with numbers ranging from 2.00±0.57 to 2.33±0.33 individuals. Comparable results were also found by (Veres *et al*., 2020; Zhang *et al*., 2016).

According to the results T_1 (*Haloxylon salicornicum* extract) and T_2 (*Solanum surrattense* extract) showed a slight decline in hoverfly populations over the 48-hour intervals, with counts ranging from 1.00±0.57 to 2.00±0.57 individuals.T_3 (*Capparis aphylla* extract) and T_4 (*Haloxylon salicornicum* extract + clothianidin insecticide)

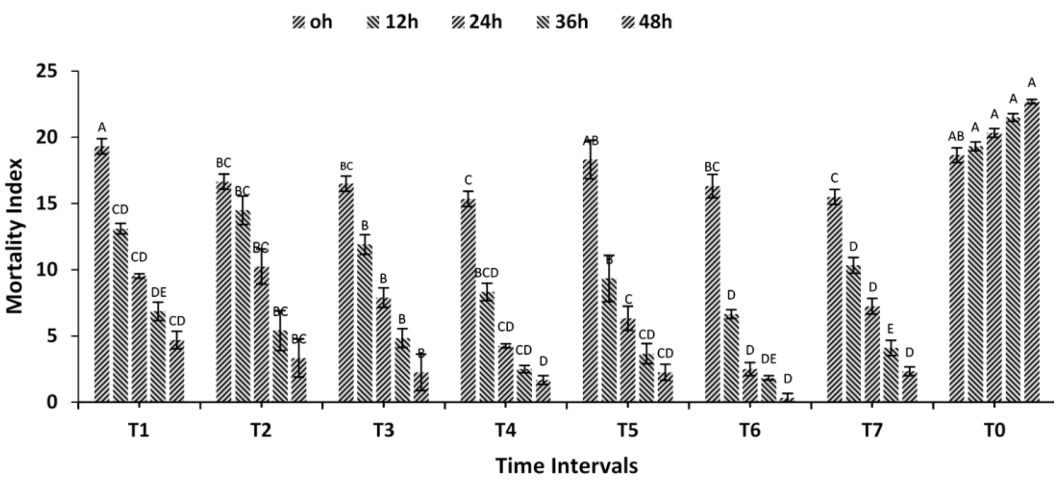

Fig. 1: Mean population (±SE) of Aphid adults assess after applying botanicals alone and in combination with clothianidin at 0h, 12h, 24h, 36h, and 48h. Mean values followed by the same letters on subsequent bars are not of significant differences. T_1= *Haloxylon salicornicum*, T_2 = *Solanum surrattense*, T_3= *Capparis aphylla*, T_4= *H. Salicornicum* + clothianidin, T_5= *S. surrattense* + clothianidin, T_6= *C. aphylla* + Clothiandidin, T_7= Clothianidin, T_8= Control

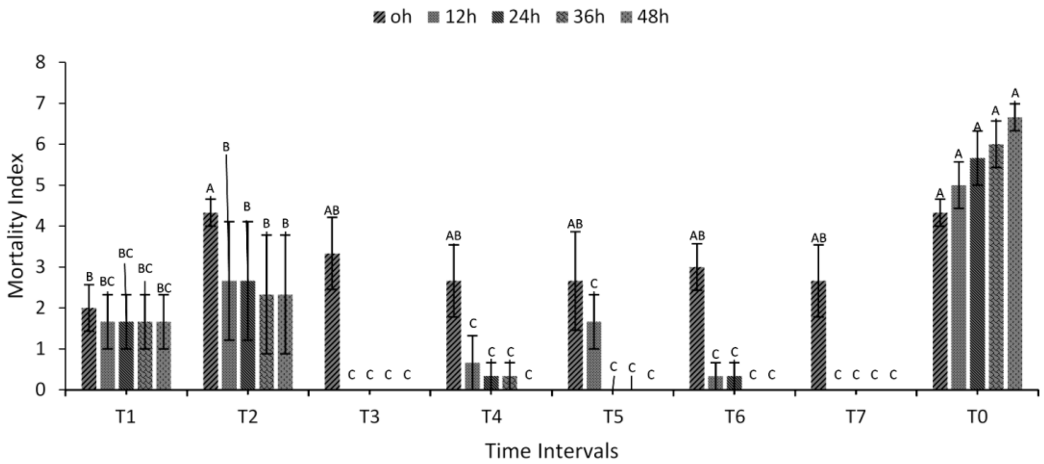

Fig. 2: Mean population (±SE) of Ladybird Beetles adults assess after applying botanicals alone and in combination with clothianidin at 0h, 12h, 24h, 36h, and 48h. Mean values followed by the same letters on subsequent bars are not of significant differences. T_1= *Haloxylon salicornicum*, T_2 = *Solanum surrattense*, T_3= *Capparis aphylla*, T_4= *H. Salicornicum* + clothianidin, T_5= *S. surrattense* + clothianidin, T_6= *C. aphylla* + Clothiandidin, T_7= Clothianidin, T_8= Control

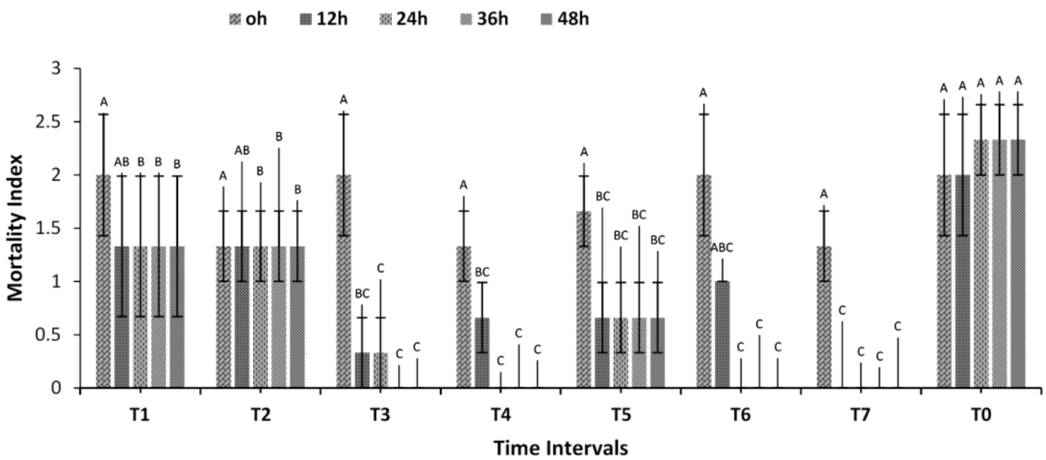

Fig. 3: Mean population (±SE) of Green Lace Wing adults assess after applying botanicals alone and in combination with clothianidin at 0h, 12h, 24h, 36h, and 48h. Mean values followed by the same letters on subsequent bars are not of significant differences. T_1= *Haloxylon salicornicum*, T_2 = *Solanum surrattense*, T_3= *Capparis aphylla*, T_4= *H. Salicornicum* + clothianidin, T_5= *S. surrattense* + clothianidin, T_6= *C. aphylla* + Clothiandidin, T_7= Clothianidin, T_8= Control

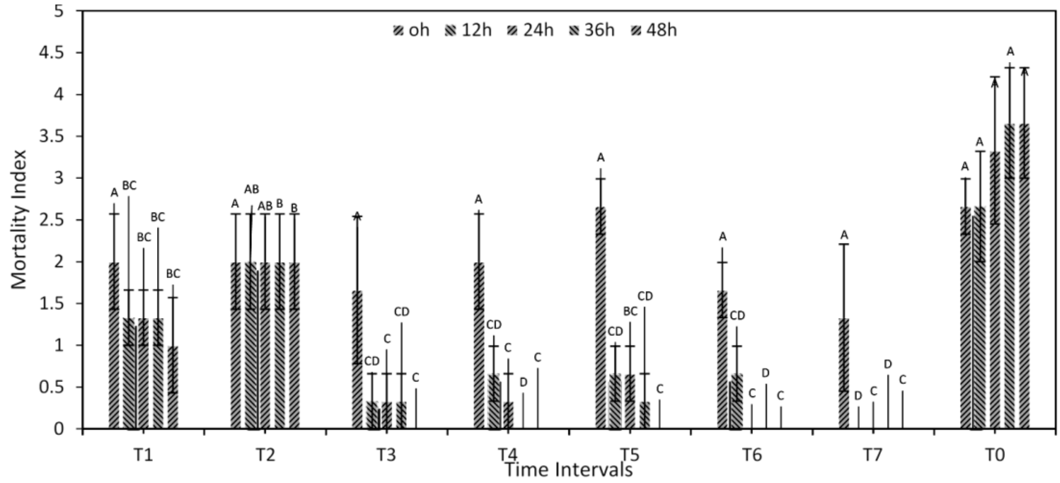

Fig. 4: Mean population (±SE) of Hover Fly adults assess after applying botanicals alone and in combination with clothianidin at 0h, 12h, 24h, 36h, and 48h. Mean values followed by the same letters on subsequent bars are not of significant differences. T_1= *Haloxylon salicornicum*, T_2 = *Solanum surrattense*, T_3= *Capparis aphylla*, T_4= *H. Salicornicum* + clothianidin, T_5= *S. surrattense* + clothianidin, T_6= *C. aphylla* + Clothiandidin, T_7= Clothianidin, T_8= Control

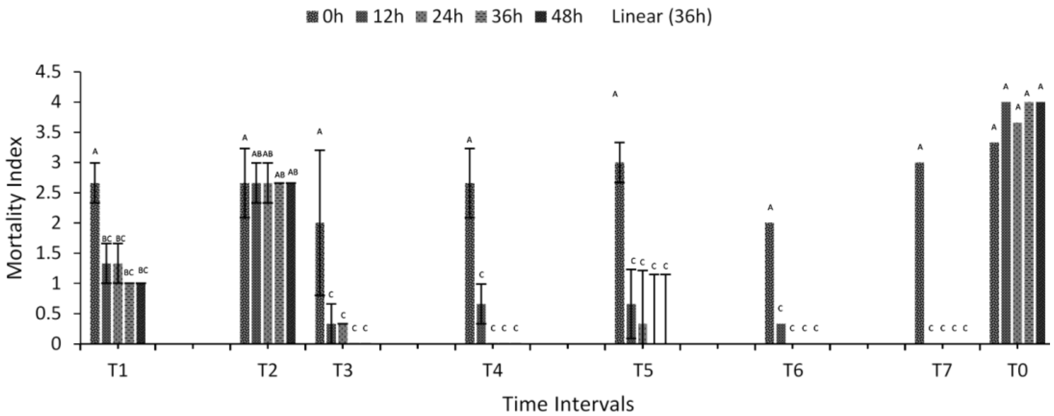

Fig. 5: Mean population (±SE) of Bracon Wasp adults assess after applying botanicals alone and in combination with clothianidin at 0h, 12h, 24h, 36h, and 48h. Mean values followed by the same letters on subsequent bars are not of significant differences. T_1= *Haloxylon salicornicum*, T_2 = *Solanum surrattense*, T_3= *Capparis aphylla*, T_4= *H. Salicornicum* + clothianidin, T_5= *S. surrattense* + clothianidin, T_6= *C. aphylla* + Clothiandidin, T_7= Clothianidin, T_8= Contro

Table 1: Treatments and Integration of Insecticide with Plants Extract

Treatment	Description
T_1	*S. surattense* 240g/kg
T_2	*H. salicornicum* 240g/kg
T_3	*C. aphylla* 240g/kg
T_4	Clothianidine 2ml only
T_5	*S. surattense* + Clauthianidine
T_6	H. selicornicum + Clauthianidine
T_7	C. aphylla + Clauthianidine
T_0	Control group with no treatment received

resulted in a significant decrease in hoverfly populations, with numbers ranging from 0.00±0.00 to 0.33±0.33 individuals.T_5 (*Solanum surrattense* extract + insecticide) and T_6 (*Capparis aphylla* extract + insecticide) also demonstrate a reduction in hoverfly populations, with counts ranging from 0.00±0.00 to 0.66±0.33 individuals.T_7 (insecticide alone) had a notable impact, resulting in hoverfly populations decreasing 0.00±0.00 individuals at all-time intervals. The control group (T_0) maintained a relatively stable hoverfly population throughout the 48 hours, with numbers ranging from 2.66±0.33 to 3.66±0.66 individuals. These results were correlated with (Kirkland *et al*., 2018; Mzimela, 2017; Veres *et al*., 2020).

According to the results T_1 (*Haloxylon salicornicum* extract) showed a slight decrease in the bracon population over the 48 hours, with counts ranging from 1.00±0.00 to 1.33±0.33 individuals.T_2 (*Solanum surrattense* extract) maintained a relatively constant bracon population, with counts ranging from 2.66±1.20 to 2.66±1.20 individuals.T_3 (*Capparis aphylla* extract) and T_4 (*Haloxylon salicornicum* extract + clothianidin insecticide) resulted in a significant decrease in the bracon population, with counts ranging from 0.00±0.00 to 0.33±0.33 individuals.T_5 (*Solanum surrattense* extract + insecticide) and T_6 (*Capparis aphylla* extract + insecticide) also showed a reduction in bracon populations, with counts ranging from 0.00±0.00 to 0.33±0.33 individuals.T_7 (insecticide alone) had a significant impact, causing the bracon population to decrease to 0.00±0.00 individuals at all time intervals. The control group (T_0) sustained a relatively stable bracon population throughout the 48 hours, with counts ranging from 3.66±0.88 to 4.00±1.15 individuals.

DISCUSSION

The aim of this study was to evaluate the efficacy of three botanical extracts, *Haloxylon salicornicum, Solanum surrattense*, and *Capparis aphylla*, both individually and in combination with the insecticide clothianidin, in controlling aphid populations on wheat plants and also to checks their impact on beneficial insect species such as ladybird beetles, green lacewings, hoverflies, and bracon wasps. A control group (T_0) is included for the baseline comparison.

The results obtained from the experiment show different levels of efficacy for each treatment. Treatments T_1 (*Haloxylon salicornicum* extract), T_2 (*Solanum surrattense* extract), and T_3 (*Capparis aphylla* extract) all resulted in a considerable decrease in the aphid population, suggesting these botanicals have the potential to be effective in controlling aphids on wheat plants. Furthermore, the integrated treatments (T_4, T_5, and T_6) combining botanicals with insecticide also significantly reduced aphid populations. However, it is worth noting that the control group (T_0) didn't show any significant change in aphid populations.

Moving towards the impact on beneficial insects, the results showing a variable effect of different treatment on different species (Zafar et al., 2021; Zafar et al., 2020). Treatments T_1, T_2, T_4, T_5, T_6, and T_7 resulted in decline of ladybird beetle populations, indicating that both the botanical extracts and the integrated treatments had a notable effect on ladybird beetle populations. Furthermore, the insecticide alone (T_7) also had a considerable effect. In the case of green lacewing populations, T_3 and T_4 led toward decline, while the integrated treatments (T_5 and T_6) also shown a decrease in green lacewing populations. The control group (T_0) sustained a constant green lacewing population. Likewise trends were observed for hoverfly populations, with T_3, T_4, T_5, T_6, and T_7 negatively impacting their populations. Again, the control group (T_0) maintained a consistent and stable hoverfly population. Additionally, T_3, T_4, T_5, T_6, and T_7 exhibit a considerable decline in bracon wasp populations, while the control group (T_0) maintained a stable population.

Limited literature is available on the evaluation of these botanicals (Khalid, 2022). *Haloxylon salicornicum* is not previously explored for pest management purposes. *Solanum surrattense* extract of different parts exhibits larvicidal activity against 4 vector mosquito species (*Anopheles culifacius, A. stephensi, Aedes aegypti and Culex quinquefasciat*us) (Bansal *et al.*2009). Combination of *Solanum surrattense* extract and Cypermethrin insecticide exhibit a synergistic larvicidal effect against mosquito larva as *S. surrattense* enhances the effect of Cypermethrin (Mohan *et al.*,2007). *Solanum surrattense* extract with a combination of *Withania somnifera* extract is used against mosquitoes and result shows that *Solanum surrattense* extract is more effective than *Withania somnifera* and its efficiency increases as the concentration of *Solanum surrattense* increases and efficiency decreases as the concentration of *Withania somnifera*.(Bansal *et al.*, 2015). *Capparis decidua* has shown aphidicidal activity against three aphid species, *Aphis gossypii* on marwar teak, *Lipaphis erysimi* on mustard, and *Mysus persicae* on cabbage (Tripathi *et al.*, 1999) (Sundararaj, 2012).

Mortality by the application of C. decidua extract against the aphid species *Lipaphis erysimi* in mustard fields increased with the concentration and time of application (Tripathi *et al.*, 1999). Clothianidin seed treatment effectively controls wheat aphids without affecting ladybird beetles (Zhang *et al.*, 2016). Clothianidin effectively reduces sap-sucking pest populations like aphids (Cui *et al.*, 2012). However, neonicotinoids, including clothianidin, have been found to have negative impacts on beneficial insects, leading to a decline in their population (Muth and Leonard, 2019). (Mansour *et al.*, 2011) reported the synergistic larvicidal effect of mixing some botanical extracts (*Piper nigrum, Azadirachta indica*¸*Conyza aegyptiaca, Cichorium intybus* & others) with commercial insecticides (Deltamethrin, Methomyl, Chlorpyrifos and Flufenoxuron) against housefly larva. Synergistic adulticidal activity of same botanicals with integration of same commercial insecticides are also observed against adult of housefly (Mansour *et al.*, 2012). Synergistic effect of seed extract of *Khaya senegalensis* and *Daucus carota* with integration of synthetic insecticides Fenitrothion and Lambda-cyhalothrin were observed against mosquito species *Aedes aegypti* and *Culex annulirostris*. The result shown that botanicals are more effective than insecticides and can be used as resistance management tool because of varied mode of action (Shaalan *et al.*, 2005).

The results of this study align well with previous research and exhibit the synergistic efficacy of botanicals and insecticides in managing aphid populations while negatively effecting beneficiaries, and the mortality rates increased over time.

Conclusion

This study provides evidence that *haloxylon salicornicum, solanum surrattense*, and *capparis aphylla* extracts, both individually and in integration with clothianidin insecticide, have the potential to control aphids on wheat plants effectively. Yet, it is substantial to acknowledge the limitations of this study. Factors such as environmental conditions, application techniques, and variations in aphid susceptibility may determine the efficacy of the treatments. These limitations should be considered while interpreting the results and applying them to practical scenarios.

Additionally, it is important to consider the adverse effects on beneficial insect populations such as ladybird beetles, green lacewings, hoverflies, and bracon wasps. These findings call attention to the importance of taking into account the potential impacts on beneficial insects when applying pest management approaches in agricultural systems. The consolidated approach of combining botanicals with insecticides guaranteed pest management strategies in wheat cultivation. However, further research is warranted to explore the best application methods, dosage considerations, and the long-term effects of these treatments on crop yield and non-target organisms. By addressing these research gaps, we can develop more sustainable and environmentally-friendly pest management approaches in the future.

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