Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v29i04.4661

INSECTICIDAL AND REPELLENT ACTIVITIES OF FOUR PLANT EXTRACTS AGAINST COTTON APHID APHIS GOSSYPII GLOVER (HOMOPTERA: APHIDIDAE) UNDER LABORATORY CONDITIONS

Naimah Asid Alanazi[1]*

[1*]Department of Biology, Faculty of Science, University of Ha'il, P.O. Box 659, Ha'il 81421, Saudi Arabia.

***Corresponding author:** Naimah Asid Alanazi
*E-mail: n.alenezy@uoh.edu.sa

Abstract.
In this study, four chosen wildlife plants (*Caralluma tuberculata*, *Capparis spinosa*, *Marrubium vulares*, and *Argemone ochroleuca*) were extracted by ethanol and tested for their biological and toxicological properties against the cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), in a lab setting. The outcomes showed that the toxicities of different plant extracts against the cotton aphid *A. gossypii* varied. After 24 hours, the cotton aphid *A. gossipyii* displayed the highest mortality rate (91.67%) when treated with at 500 ppm concentration of *Caralluma* extract. These results were followed by 88.33, 83.33, and 75.83% mortalities when aphids were treated with the extracts of *Capparis*, *Marrubium*, and *Argemone*, respectively at the same concentration and observed time. When *Caralluma* (66.46 and 53.35 ppm) and *Capparis* (97.18 and 69.28 ppm) were treated after 24 and 48 hours, respectively, the LC_{50} values of cotton aphid *A. gossipyii* were significantly reduced. Also, the plant extract from *Argemone* demonstrated moderate repellent activity, as evidenced by the repellency rate of 73.33 at 500 ppm, while the plant extract from *Caralluma* had the highest repellent percentage of 97.78% at 500 ppm, followed by *Capparis* (93.33%) and *Marrubium* (86.67%) at the same concentration. In conclusion, the ethanolic extracts of *C. tuberculate* and *C. spinosa* demonstrate their potential uses as promising natural insecticides for aphid control.

Keywords: Cotton Aphid, Plant extracts, *Caralluma tuberculata*, *Capparis spinose*, Toxicity, Repellency rate

Introduction
Cotton aphid *Aphis gossypii* Glover (Homoptera: Aphididae), is a significant pest in greenhouse crops and horticulture in open fields (Campolo et. al., 2014; Sarwar et. al., 2014; Zhang et. al., 2022). As a result, it can effectively support a wide variety of host plants (Fuller et al., 1999; Sarwar et. al., 2014). According to Blackman & Eastop (1985), there are over 320 plant species from 46 families that are suitable hosts for *A. gossypii* worldwide. Among these, cotton (*Gossypium hirsutum*) is one of the most important cash crops and is highly susceptible to a variety of arthropod pests, including cotton aphids (Sarwar et. al., 2014, Zhang et. al., 2022). Currently, the only method

of controlling the cotton aphid *A. gossypii*, is the application of synthetic pesticides, which are harmful to both humans and the environment.
Although botanical pesticides have been used for thousands of years to control insect pests, there has been a rise in interest in using them as an alternative to synthetic pesticides in recent years (Asiry, 2015). The well-known properties of plant-based insecticides, or botanicals, include lower environmental toxicity and insecticidal and repellent properties (Koul and Walia, 2009). Alkaloids, terpenoids, cucurbitacin, glycosides, flavonoids, and other compounds that have been employed as toxins against a variety of insect pests that harm commercial crops are abundant in plants and constitute a valuable source of chemicals (Koul and Walia, 2009).

The succulent and perennial herb, *Caralluma tuberculata* N. E. Brown grows wild in Pakistan, India (Andhra Pradesh), Saudi Arabia, the United Arab Emirates, south-east Egypt, Iran, and Nigeria (Bibi et. al., 2015; Baig et. al., 2021; Iftikhar et. al., 2022). The medicinal properties of this plant are attributed to a variety of bioactive secondary metabolites, including tannins, reducing sugars, terpenoids, steroids, beta cyanin, and amino acids. Furthermore, the Caper (*Capparis spinosa* L.), a perennial spiny shrub belonging to the Capparaceae family, has large, white to pinkish-white flowers and fleshy leaves (Annaz et. al., 2022). Originating in the Mediterranean region, this plant is now found throughout Southern Europe, Northern and Eastern Africa, including Madagascar, Southwestern and Central Asia, Indonesia, Australia, Papua New Guinea, and Oceania (Chedraoui et. al., 2017; Annaz et. al., 2022). Certain subspecies and varieties are found in particular geographic areas.

Distribution of *C. spinosa* subsp. spinosa includes the Arabic Peninsula, China, Southern Europe, and northern Africa, including the Sahara (Chedraoui et. al., 2017; Annaz et. al., 2022). Researchers have been interested in the phytochemical perspective of plants belonging to the genus Capparis (Family: Capparaceae), specifically because of their high glucosinolate content (El-Shershaby, 2010). Traditionally, *Marrubium vulgare* (Family: Lamiaceae) was used as a cholagogue, purgative, diuretic, bitter tonic, carminative, appetizer, and to treat pulmonary infections, cough, rheumatoid arthritis, night blindness, and dyspeptic complaints (El-Shershaby, 2010). Alkaloids, sterols, steroids, terpenoids (diterpene), saponins, flavonoids, catecholic tannins, anthocyanins, phenolic compounds, and numerous other bioactive components were found in *M. vulgare*, according to a phytochemical analysis of the plant (Al-Snafi et. al., 2021). Known as "chicalote," *Argemone ochroleuca* Sweet (Papaveraceae) is a native North American weed that has extensively spread from the southern United States to central Mexico (Martinez, 1996). People in Tepotzotlán, State of Mexico, use it in traditional medicine. According to information gathered from local healers, eye infections can be treated with infusions of the aerial portion of *A. ochroleuca* (Neri, 1995). In Saudi Arabia, *A. ochroleuca* Sweet. was found to be a common and invasive plant, occurring in a variety of habitats, including disturbed areas and roadsides (Thomas et. al., 2016).

All above plant species have been tested and showed insecticidal properties against a variety of insect pests including mealybug, *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Pseudococcidae) (Sardar et. al., 2018), maize weevil *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) (Mamoon-Ur-Rashid et. al, 2019), Saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Sivanidae) (Alqurashi and Bakhashwain 2010), red flour beetle, *Tribolium castanium* Herbst (Coleoptera:Tenebrionidae) (Bakhashwain and Alqurashi 2010), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Martínez et al., 2017) and potato tuber moth *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) (Allahverdizadeh and Mohammadi 2016).

Nevertheless, no research has been done to look into the biological and toxicological effects of plant extracts from these plants on the cotton aphid *A. gossypii*. In order to evaluate the biological and toxicological properties of plant extracts from *C. tuberculata*, *C. spinosa*, *M. vulares*, and *M. vulares* as a safe method against the cotton aphid *A. gossypii*, this study was conducted.

Material and Methods

Collection and Preparation of Tested Plants

The following plants were collected in their fresh leaves from different parts of the Kingdom of Saudi Arabia: *Caralluma tuberculata*, *Capparis spinosa*, *Marrubium vulares*, and *Argemone ochroleuca*. Water was running over them to wash them. After air drying in the shade, the plant parts were baked to preserve their weight. The dried leaves were ground in a grinder to produce powdered ingredients. The dust was sealed in bags until it was needed for the extraction procedure. Each material's sample weighed 50 grams was put in a 500 ml beaker and mixed with 100 ml of ethanol separately. The liquid was stirred for 30 minutes with a magnetic stirrer and then allowed to stand for the next 24 hours. The mixture was then filtered again using a fine cloth and Whatman No. 1 filter paper. After that, the filtrate was concentrated in a water bath at 55°C using a rotary vacuum evaporator. Next, using a Labconco Freeze Dryer-18 type 75018, the extracts were freeze-dried for 48–72 hours. A stock solution was made from the lyophilized residue of each plant extract.

Collection of Tested Insect

Cotton aphids *A. gossipyii* were taken from an infected organic cucumber farm in Al-Shanan village, in the West of the Hail region of Saudi Arabia. Aphids were bred on squash plants grown in pots and housed in cages measuring 50 cm in length, 50 cm in width, and 90 cm in height, with a 0.5 mm mesh covering. Plants that occasionally harbored aphid infestations were fed. Using the identification key (Blackman & Eastop 2000), the cotton aphids *A. gossipyii* were identified.

Toxicity Test

At the Laboratory of Entomology, the Department of Biology, University of Ha'il, Saudi Arabia, stock solutions of lyophilized pure plant extracts were prepared in distilled water (0.5 g/100 ml). Four different concentrations of 200, 300, 400, and 500 parts per ml were prepared from the stock solutions of the different plant extracts used in this study. One ml of each concentration was applied to filter paper with a Whatman No. 9 diameter. Filter papers were placed in the bottom of each Petri dish (90 mm) after it had dried, and then 40 wingless aphids and a squash crop leaf were added. Aphid mortality %s was computed following a 24- and 48-hour treatment period. The negative control treatment was given just 1 ml of water. As a positive control, 5% concentration of the insecticide Malathion, which is composed of organophosphates, was also used; this was the amount specified on the bottle. For every treatment, three replications were conducted.

Repellency Test

The repellency test was conducted using the methodology described by Talukder and Howse (1994). These investigations were carried out in 90 mm-diameter petri dishes. Filter sheets (No. 40) with treated and fresh portions without treatment were divided into two sections. One side of the filter paper was coated with a 1 ml solution of each plant extract solution. After joining the treated and untreated halves of the filter paper, allowing it to air dry, the mixture was placed in a petri dish. Thirty newly hatched adult aphids without wings were released and covered in the center of each Petri plate. Three replications were used for each dose. The number of insects on each section of the Petri plate was counted every two hours. As per Jilani et al.'s (1988) methodology, the data were expressed as a percentage of repulsion (PR).

Data Analyses

Repeated-measure ANOVA was used to determine the percentages of aphid mortality. This analysis was used to assess the effects of time (a repeated factor with two observations), the concentrations of the four plants (18 experimental units with controls), and their interactions. Fisher's least significant difference (LSD) tests were used to ascertain differences in treatment means when significant treatment differences ($P \leq 0.05$) were observed. Furthermore, the LC_{50} values were calculated using Finney's (1971) methodology. Nonetheless, Abbott's method (1952) was used to correct the statistics to take control mortality into consideration. To find out which of the four plants was most successful at keeping aphid adults that had emerged from their eggs at bay, the percentages of repellency tests were analyzed using a one-way ANOVA. A covariate factor called replicate blocking was included in the model. Fisher's least significant difference (LSD) tests were repeated to find differences in treatment means when significant treatment differences ($P \leq 0.05$) were noted.

All of the aforementioned data analyses were performed with SPSS version 26.0.

Results

Toxicity of Plant Extracts Against Cotton Aphid A. gossipyii

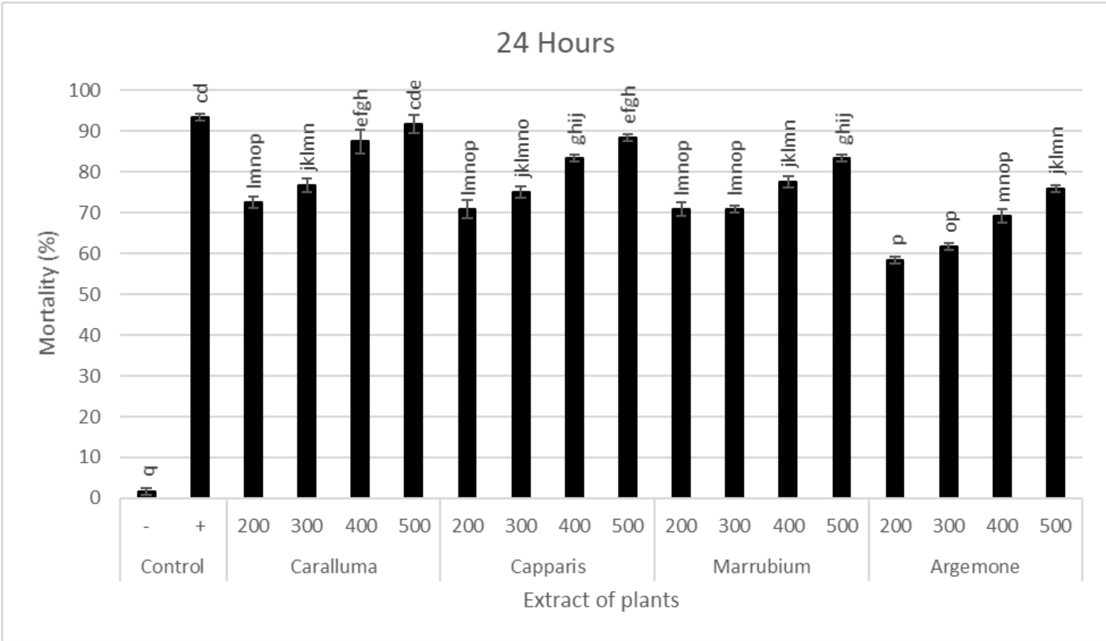
This study demonstrates that the mortality of the cotton aphid *A. gossipyii* over time was more significantly influenced by the plants that were used, with varying concentrations for each observation period (Table 1 and Figure 1). After 24 hours, the cotton aphid *A. gossipyii* with the highest mortality rate (91.67%) was treated with a 500ppm concentration of *Caralluma* extract. Aphid mortality varied from 72.5 to 91.66.2% and from 70.83 to 88.33% at 24 hours, respectively, when aphids were treated with extracts of *Capparis*, *Marrubium*, and *Argemone*, respectively, at the same concentration and observed time. Aphid mortality showed 88.33, 83.33, and 75.83% for *Capparis*, *Marrubium*, and *Argemone*, respectively, at the same concentration (Figure 1).

In comparison with the positive control (malathion), which had the second highest percent mortality 96.67% after the extract of *Caralluma*, the results also showed the same patterns with increasing time. After 48 hours, the cotton aphid *A. gossipyii* treated with the extract of *Caralluma* at a concentration of 500 ppm showed a higher percentage mortality (98.33%). This was followed by 94.17, 89.17, and 82.5% mortalities for the extracts of *Capparis*, *Marrubium*, and A*rgemone*, respectively, at the same concentration and observed time (Figure 1).

The toxicity results displayed in Tables 2 and 3 indicate that the LC_{50} values of cotton aphid *A. gossipyii* were significantly reduced when *Caralluma* (66.46 and 53.35 ppm) and *Capparis* (97.18 and 69.28 ppm) were treated after 24 and 48 hours, respectively, compared to other utilized plant extracts. The LC_{50} values for the other plant extracts, such as *Argemone* and *Marrubium*, were 94.19 ppm and 74.92 ppm, respectively, after 48 hours of exposure. The plant extracts from *Caralluma*, *Capparis*, and *Marrubium* outperformed the plant extract from *Argemone* when the LC_{50} values were compared (Tables 2 and 3).

Table (1): Result of repeated-measures ANOVA for the overall effects of the solvents' concentrations (C), observed time (T), their interaction (C x T) on the mortality of cotton aphids *Aphis gossypii.*

Factor	DF	Mean Square	F Value	Pr > F
Concentrations of solvents (C)	17	0.304	35.0	0.0001
Time (T)	1	2.07	163	0.0001
C × T	17	0.068	5.35	0.0001
Error	36	0.013		



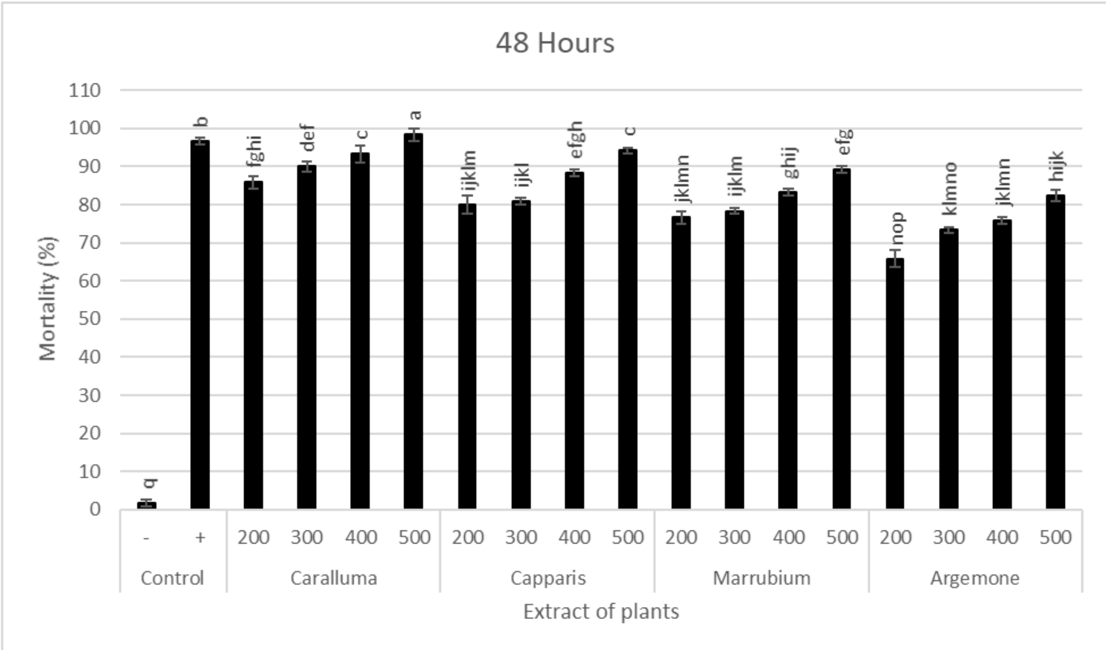


Figure 1. Means and S.E. of (%) mortalities of the cotton aphid *A. gossypii* across four plant extracts after two observed times (24 and 48 hours). Means with the same letter in each separate observed time are not significantly different ($P > 0.05$), according to the LSD test.

Table 2. LC_{50} values, slope, toxicity index and 95% lower & upper confidence limits of four plant extracts against cotton aphid, *Aphis gossypii* after 24 hrs exposure.

Treatments	Slope± SE	LC_{50}	95% Confidence limits		Toxicity Index*	χ^2	Sig.
			Lower	Upper			
Caralluma	1.01±**0.46**	66.46	48.98	79.69	100.00	1.326	0.515
Capparis	1.58±**0.48**	97.18	86.68	110.25	68.39	0.868	0.648
Marrubium	1.96±**0.50**	107.31	95.17	127.94	61.93	1.62	0.444
Argemone	1.20±**0.44**	146.56	131.53	198.81	45.35	0.737	0.692

*Toxicity index = [(LC_{50} of the most toxic tested compound/LC_{50} of the tested compound)x 100]

Table 3. LC_{50} values, slope, toxicity index and 95% lower & upper confidence limits of four plant extracts against the cotton aphid, *Aphis gossypii* after 48 hrs exposure.

Treatments	Slope± SE	LC_{50}	95% Confidence limits		Toxicity Index*	χ^2	Sig.
			Lower	Upper			
Caralluma	1.17±**0.49**	53.35	38.17	79.62	100.00	1.104	0.576
Capparis	1.63±**0.53**	69.28	53.41	82.71	77.01	2.550	0.279
Marrubium	2.06±**0.64**	74.92	49.92	89.51	71.21	1.812	0.404
Argemone	1.22±**0.46**	94.19	71.79	117.41	56.64	0.354	0.838

*Toxicity index = [(LC_{50} of the most toxic tested compound/LC_{50} of the tested compound)x 100]

Repellency Rate of Plant Extracts Against Cotton Aphid A. gossipyii

When tested plant extracts applied to the cotton aphid, *A. gossypii*, the one-way ANOVA showed a more notable variation in the plant extracts' repellency rate (Table 4). *Caralluma* possesses the most amazing resistance to aphids, according to statistical analysis (Figure 2). At 500 ppm, the repellent percentage of the plant extracts from *Caralluma* was 96.78%, followed by that of *Capparis* (93.33%) and *Marrubium* (86.67%) at the same concentration (Figure 2). However, the repellency rate of 73.33 at 500 ppm (Figure 2) suggested that the plant extract from *Argemone* had moderate repellent action. The rate at which repellency grew and the dose level were directly correlated, according to the data. At 500 ppm, the maximum level of repellency was seen in all studied plant extracts.

Table (4): Result of one-way ANOVA displays the effects of different concentrations of four plants on a repellency of cotton aphids *Aphis gossypii.*

Factor	DF	Mean Square	F Value	Pr > F
Concentrations of solvents	17	12.874	76.083	0.000
Block	2	0.271	1.600	0.217
Error	34	0.169		
Total	53			

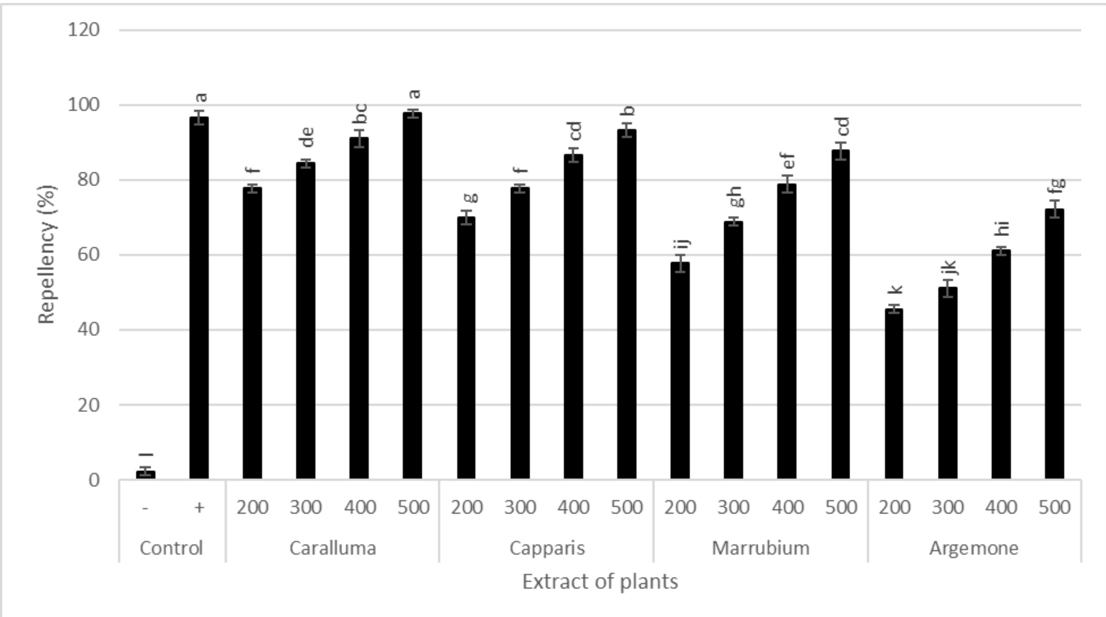


Figure 2. Percentage means and S.E. of repellency of the cotton aphid *A. gossypii* after exposure to the five used plants' ethanolic extract. Standards with the same letter are not significantly different (P > 0.05), according to the LSD test.

Discussion

Outbreaks of the cotton aphid, *A. gossypii*, can negatively affect output and result in financial losses. It is an important worldwide pest of horticulture and fiber crops. Therefore, to manage aphids, broad-spectrum pesticides are frequently applied in large quantities. The environment and people suffer when synthetic pesticides are used extensively (Asiry 2015). This has prompted scientists and legislators to look for alternative sustainable practices, like using natural goods made from plants found in nature (Asiry, 2015). Numerous investigations have demonstrated the insecticidal effects of wildlife plants on the cotton aphid [i.e. Neem (Santos et. al., 2004); *Hyoscyamus muticus, Verbascum sinuatum* and *Rumex dentatus* (Soliman et. al., 2005); Neem leaf extract and Moringa leaf extract (Ali et. al., 2022)]. However, no research has looked into how plant extracts from *C. tuberculata*, *C. spinosa*, *M. vulares*, and *M. vulares* affect the cotton aphid *A. gossypii*. The toxicity and repellency of *C. tuberculata*, *C. spinosa*, *M. vulares*, and *M. vulares* against the cotton aphid *A. gossypii*, are therefore reported for the first time in this work. The current investigation found that the ethanolic extracts of *C. tuberculate* and *C. spinosa* plants have greater insecticidal efficacy against cotton aphids *A. gossipyii*. The findings showed that ethanolic plant extracts from both *C. spinosa* and *C. tuberculate* were more toxic and repellent to cotton aphids *A. gossipyii*. These findings are consistent with earlier research showing *C. tuberculata* extracts exhibited insecticidal effects against a variety of pests, including mealybug, *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Pseudococcidae) (Sardar et. al., 2018), maize weevil *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) (Mamoon-Ur-Rashid et. al, 2019), Saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Sivanidae) (Alqurashi and Bakhashwain 2010) and red flour beetle, *Tribolium castanium* Herbst (Coleoptera:Tenebrionidae) (Bakhashwain and Alqurashi 2010). The current investigation shows that aphid mortality rates were higher when *C. tuberculate* and *C. spinosa* were applied at larger doses than when *M. vulares* and *A. ochroleuca* were applied. The catecholic B-ring found in hazardous compounds such saccharides and glycosides, flavonoids, alkaloids, terpenoids, and volatile oils found in plant extracts appears to be the cause of their toxicant properties against insects (Koul and Walia, 2009). According to reports, flavonoids are a significant class of phytochemicals that make up 5–10% of the secondary metabolites that are known to exist in plants and function as a defense mechanism by poisoning insects (Madanat et al., 2016). The poisonous compounds in the extracts may have had an adverse effect on the neurological system, resulting in the death of *A. gossipyii* cotton aphids. These extracts cause the acetylcholinesterase enzyme to be activated, which results in the production of acetylcholine. Acetylcholine is necessary for transferring impulses that build up at nerve terminals and ultimately result in paralysis and death (Hatem, 2020). Flavonoids, which are found in *C. tuberculata*, are known to contribute to RNA reduction, cytotoxicity, and mutagenicity (Qureshi et al., 1991). Studies on *C. spinosa*'s phytochemicals revealed that it contains a wide range of bioactive substances, including fatty acids, vitamin C, vitamin E, steroids, alkaloids, terpenoids, saccharides, and glycosides (Moufid and Eddouks 2015). This could account for the greater death rate of cotton aphid *A. gossipyii* in the current study when they were treated with *C. spinosa* extract. However, more research on the chemical constituents and long-term effects of these plant extracts on aphid suppression is required.

Conclusion

The study's findings demonstrated that treating the cotton aphid *A. gossipyii* with ethanolic extracts of *C. tuberculate* and *C. spinosa* increased the aphid mortality, suggesting that these natural insecticides have promising uses in the field for managing aphid populations. Extensive field research is necessary to evaluate their efficacy as an insecticide in managing aphid infestations. It would also be worthwhile to look at any potential negative effects these plant extracts may have on beneficial insects. This could, therefore, lead to a decision to suggest its usage to farmers as a long-term solution for managing aphid infestations.

Acknowledgements
The author is incredibly appreciative of the farmers who kindly allow to use their property to collect aphids. Additionally, the author is very grateful to Prof. Dr. Khalid A. Asiry from Department of Agriculture, Faculty of Environmental Sciences, King Abdulaziz University-Jeddah for his insightful feedback on the manuscript.

Conflicts of Interest
The author declares no conflict of interest.

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