Assessing the Impact of Ovulation Inhibition in Fumaria parviflora Lam and Teucrium polium on Bemisia tabaci Genn

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ABSTRACT

Objective: The purpose of this study was to investigate the effect of the extract of Fumaria parviflora Lam. and Teucrium polium Linn. on repelling and inhibiting the spawning of white cotton boll Bemisia tabaci Genn.
Methods: In this study, the extract of Fumaria parviflora Lam. and Teucrium polium Linn. was obtained and tested for its repellent and inhibitory properties against white cotton boll Bemisia tabaci Genn. The extract was applied to cotton bolls and the behavior of Bemisia tabaci Genn. was observed and recorded.
Results: The results of this study showed that the extract of Fumaria parviflora Lam. and Teucrium polium Linn. exhibited significant repellent properties against white cotton boll Bemisia tabaci Genn. The extract effectively repelled the insects, reducing their presence on the cotton bolls. Additionally, the extract demonstrated inhibitory effects on the spawning of Bemisia tabaci Genn., reducing the number of eggs laid on the cotton bolls.
Conclusions: This study provides valuable insights into the potential use of the extract of Fumaria parviflora Lam. and Teucrium polium Linn. as a natural repellent and inhibitor for controlling white cotton boll Bemisia tabaci Genn. infestations. The findings contribute to the development of environmentally friendly and sustainable pest management strategies in cotton cultivation.

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1- Introduction

Whitefly Bemisia tabaci is a serious pest of agricultural crops and ornamental plants in farms and greenhouses worldwide (Zhang et al., 2004). The adult insects and nymphs feed on the sap of plants, resulting in chlorosis in green plants. They also excrete a significant amount of honeydew, which promotes the growth of sooty mold and disrupts the plant's natural photosynthesis (Chu & Henneberry, 1998). In addition, whitefly is a vector of several plant viruses, particularly geminiviruses such as Tomato yellow leaf curl virus, which causes a 40 to 70 percent reduction in tomato yield (Brown & Czosnek, 2002). Managing this pest becomes challenging after its population increases. Currently, whitefly control heavily relies on synthetic insecticides. However, the indiscriminate use of insecticides not only leads to the development of resistance in pest growth stages but also poses numerous environmental and human safety issues (Palumbo et al., 2001). Furthermore, natural enemies suffer due to repeated pesticide use, and their effectiveness is limited during heavy whitefly infestations (Sugiyama et al., 2011). Therefore, there is a crucial need for an effective alternative for controlling this pest that is environmentally safe for humans and other non-target organisms. Currently, many researchers are focusing on developing new environmentally friendly methods. One of the promising approaches is the use of plant compounds that have a significant impact on pest population control. Plants produce various secondary metabolites that play a role in protecting plants against internal and external factors. They defend plants against herbivores and microorganisms (Pichersky & Gershenzon, 2002; Bakkali et al., 2008). Monoterpenes, sesquiterpenes, and diterpenes are examples of these metabolites (Heywood et al., 1997; Barney et al., 2005). It has been established that plant compounds have a suitable potential as alternatives to chemical insecticides, with advantages such as low toxicity to mammals, rapid degradation, and easy local accessibility (Roger et al., 2012). Plant compounds possess characteristics such as toxicity, repellency, antifeedant, oviposition deterrent, and insect growth regulation (Sharma & Gupta, 2009). Numerous studies have focused on the potential of extracted compounds against whitefly, and several plants have been introduced as alternatives to chemical toxins for whitefly control (Ateyyat et al., 2009; Pavela & Herda, 2007).

Teucrium polium Linn., commonly known as "Kolpoorah" (chickpea plant), is a plant from the Lamiaceae family. The extract of Kolpoorah plant contains alkaloids, glycosides, triterpenes, sterols, flavonoids, terpenoid compounds, tannins, bitter furan derivatives, and saponins (Amir Heidari, 1994). It is an herbaceous plant with a height of 10 to 40 centimeters and a cottony appearance. It is usually found in various regions, including Iran, such as deserts, rocky coasts, and sandy areas in Europe, the Mediterranean region, North Africa, and Southwest Asia (Zargari, 1992).

Mahdavi Arab et al. (2008) studied the insecticidal effects of plant extracts on the cowpea aphid in the laboratory and their impact on the sugar beet leaf miner in greenhouse conditions. The results of this research showed that the methanol extract of Kolpoorah caused 81.6% mortality in cowpea aphids. Irannejad et al. (2012) investigated the side effects of hexaflumuron, pymetrozine, and spirotetramat insecticides and four plant extracts (Kolpoorah, Shatarah, Avishan, and Ovishan) on biological parameters of the green lacewing Chrysoperla carnea. The results showed that the Kolpoorah extract had no negative effect on the growth of the green lacewing.

Fumaria parviflora Lam., commonly known as "Shatarah," is an herbaceous plant belonging to the Fumariaceae family. It has a brittle, short, and branched stem. This plant is native to Europe and Asia. It grows as a weed alongside roads and is mostly exported by Eastern European countries (Zargari, 1992). The aerial parts of the plant contain approximately 1% alkaloids. Most of these alkaloids are derivatives of benzyl isoquinoline. The most important alkaloids include fumarine (protopine), fumariline, and sinactine. Other compounds in Shatarah include flavonoids, plant acids, especially fumaric acid, and mucilage (Zargari, 1992). Sindhu et al. (2012) investigated the acaricidal activity of F. parviflora, Juniperus excelsa Bieb., Operculina turpethum Linn., Acacia nilotica Linn., and Buxus papillosa C.K.Schneid. extracts against the tick Rhipicephalus microplus Canestrini and reported that the F. parviflora extract had higher toxicity compared to the other studied plants. Considering the widespread use of pesticides by greenhouse farmers to control pests, especially whiteflies, and the environmental hazards associated with their use, the insecticidal properties of Kolpoorah and Shatarah extracts were evaluated in this study for their repellent and oviposition deterrent effects on adult whiteflies.

2- Materials and Methods

2-1-Collection, identification, and location of experiments

First, the whitefly-infested leaves were transferred from the educational farm of the Islamic Azad University, Jiroft Branch, to the laboratory. The whiteflies were taxonomically studied, and the population was identified as Bemisia tabaci (Samih et al., 2006). After species confirmation, adult insects were collected from the farm and transferred to the research greenhouse. The cultivation of cotton whitefly and experiments were conducted in the research greenhouse under controlled conditions (temperature of 27±2 degrees Celsius, relative humidity of 55±5%, and a 16:8 light-dark cycle).

2-2-Host plant cultivation

In this study, the tomato variety "Kal-Geen-Tri" was used for cultivating the whitefly and conducting experiments. The plants were grown in disposable plastic pots with a diameter of 15 cm and a height of 20 cm, filled with suitable soil, in the greenhouse. The pots were manually irrigated every two days. NPK nutrient solution was used to improve plant growth. To prevent possible contamination, the pots were covered with 12-mesh silk fabric and transferred to cages with dimensions of 80×60×60 cm.

2-3-Whitefly rearing

Adult whiteflies were reared as the primary source on tomato plants in cages covered with 12-mesh silk fabric, with dimensions of 80×50×60 cm, for 5 generations. Due to the increased pest density after 1 to 2 generations, the previous pots were replaced with new ones every 15 days.

2-4-Preparation of plant extracts

Plant samples with insecticidal effects were selected for extract preparation (Sindhu et al., 2012; Samareh Fekri et al., 2016). The evaluated plants included shoot and root extracts of marigold and chickpea plants. Various plant parts, such as stems, leaves, and flowers of marigold, and stems, flowers, roots, and leaves of chickpea, were collected from different regions of Kerman province in the months of Ordibehesht and Khordad, 1401. The extraction of samples was performed according to the methods described by Su et al. (2009) and Yao et al. (2011). The plants were washed with distilled water, dried in an oven at 45 degrees Celsius for 3 days, powdered using an electric mill, and sieved through a 40-mesh sieve. For each amount of plant sample, 5 times the amount of 95% ethanol was added (1 gram of powder with 5 milliliters of ethanol). The ethanol and plant mixture was kept in darkness at a temperature of 20 to 25 degrees Celsius for 7 days, with proper mixing twice a day. Afterward, the mixture containing the extract was filtered through a filter paper, and the remaining residue was subjected to a second extraction. The ethanol (95%) was added again to the remaining powder (1 gram of powder with 2.5 milliliters of ethanol), and it was kept in darkness for 7 days. The extracts obtained from the first and second extractions were mixed and dried using a rotary evaporator.

2-5-Preparation of experimental concentrations

For the experiments, concentrations of 4000, 6000, and 8000 ppm were used. To prepare the stock solution, 0.05 grams of raw extract was dissolved in 3.0 milliliters of dimethyl sulfoxide and 1% Tween 20. Distilled water was then added by double distillation to reach a volume of 5 milliliters, resulting in a concentration of 10 milligrams per milliliter or 10000 ppm. The concentrations used in the experiments were prepared from the stock solution. For the control, 3.0 milliliters of dimethyl sulfoxide and 1% Tween 20 were mixed, and distilled water was added by double distillation to reach a volume of 5 milliliters.

2-6-Assessment of the contact toxicity of the studied extracts on adult cotton whiteflies

The method described by Al-mazraawi and Ateyat (2009) with slight modifications was used to assess the contact toxicity. Branches with two to four leaves (approximately 10 centimeters long) of tomato variety "Kal-Geen-Tri" were individually dipped in each of the experimental treatments for 5 seconds and then dried in the presence of dry air. The control plants were also dipped in the control solution and dried in the air. Subsequently, the plants were placed in plastic pots with dimensions of 15×10 cm using the hydroponic method. The pots were placed in wooden cages with dimensions of 40×40×35 cm. Adult whiteflies of the same age were kept in the refrigerator for 15 minutes to anesthetize them, and then 50 insects were placed in a Petri dish between the treated and control plants. The distance between the treated and control plants was approximately 25 centimeters. The number of adult insects attracted to each plant was recorded after 24, 48, and 72 hours. The experiment was conducted with 5 replicates for each treatment. The percentage of repellency was calculated using the following formula.

$$RP\% = \left[\frac{C-T}{C+T}\right] \times 100 \qquad \text{(Formula 1)}$$

RP%: Removal percentage
C: The number of insects in the control treatment
T: Number of insects in extract treatment

2-7-Evaluation of the Ovicidal Inhibitory Effect of Studied Extracts on Whitefly Eggs

In this experiment, the inhibitory effect of concentrations of 4000, 6000, and 8000 ppm of shutter and clipore extracts on the ovicidal activity of whitefly eggs was evaluated. For this experiment, 4 to 5 leafed tomato plants were selected.

The plants were submerged in the mentioned concentrations, while the control plants were submerged in the control solution.The plants were placed in disposable cups using hydroponics, with the stem of the plant inserted through the opening of the cup lid, which had a height of 15 cm and a diameter of 10 cm, into the cup solution. The cups were covered with transparent disposable cups with similar dimensions, which were covered with mesh as cup cages. The edges of the cups were connected using glass tape. A small opening was made on the top of each cup to place a glass vial for releasing adult insects. Inside each cup cage, a pair of adult male and female insects, which were 24 hours old, were released, and the number of eggs laid on each plant was counted and recorded after 72 hours under a stereomicroscope. This experiment was conducted in four replicates. The percentage of egg inhibition was calculated using the following formula.

$$OD\% = \left[\frac{Ck-T}{Ck}\right] \times 100 \qquad \text{(Formula 2)}$$

OD%: Percentage of inhibition of spawning
Ck: The number of eggs in the control
T: The number of eggs in the treatment

Experiments were conducted in the form of randomized complete block design and statistical analysis of data by SPSS software.

3-Results

3-1-The effect of different concentrations of Clippore extract on complete whitefly insects after 48, 24, and 72 hours was investigated

The analysis of variance results showed a significant difference in the percentage of repellency among different treatments of Clippore extract at a 1% level of significance ($F_{8,44}$=50.014, p=0.000). The highest repellency was observed at a concentration of 8000 ppm after 24 hours, while the lowest repellency was observed at a concentration of 4000 ppm after 72 hours. There was no significant difference in the percentage of repellency on complete insects between the concentration of 4000 ppm after 24 hours and the concentration of 6000 ppm after 48 hours. Additionally, no significant difference was observed in the effect of Clippore extract on the repellency between the concentration of 4000 ppm after 48 hours and the concentration of 8000 ppm after 72 hours (Table 1).

Table1. The mean of repellency percent of T.polium extract on B. tabaci adults

Plant extract	Concentration(PPM)	Time		
		24(h)	48(h)	72(h)
	4000	28.8±1.49^{d}	20.8±1.49^{c}	9.6±0.97^{a}
Teucrium polium	6000	36±1.78^{e}	27.2±1.49^{d}	16±1.78^{b}
	8000	44.8±1.49^{f}	40±1.26^{e}	22.4±2.4^{c}

In each column, means with common letters are in the same statistical group at 5% probability level (Duncan test).

3-2-The impact of different concentrations of shutter extract on adult whitefly insects was evaluated after 48, 24, and 72 hours

The results of the analysis of variance indicated a significant difference in the percentage of repellency among the different treatments of shutter extract at a significance level of 1% ($F_{8,44}$=83.47, p=0.000). The highest repellency effect was observed at a concentration of 8000 ppm after 24 hours, while the lowest repellency effect was observed at a concentration of 4000 ppm after 72 hours. Significant differences were observed in the repellency effect at a constant concentration after 24 and 48 hours, but no significant difference was observed in the repellency effect at a constant concentration between 48 and 72 hours (Table 2).

Table 2.The mean of repellency percent of F.parviflora extract on B. tabaci adults

Plant extract	Concentration(PPM)	Time		
		24(h)	48(h)	72(h)
	4000	40.8±1.49^{b}	33.6±0.97^{a}	32.8±1.49^{a}
Fumaria parviflora	6000	62.4±2.03^{e}	51.2±1.49^{c}	48.8±1.49^{c}
	8000	72±1.26^{f}	32.8±1.20^{d}	55.6±1.16^{d}

In each column, means with common letters are in the same statistical group at 5% probability level (Duncan test).

3-3-The effect of different concentrations of Shatera and Clapora extracts on adult whitefly was evaluated after 48, 24, and 72 hours

The results of the analysis of variance showed a significant difference in the percentage of repellency between the different treatments of Shatera and Clapora extracts at a 1% level of significance ($F_{17,89}$=121.70, p=0.000). The highest repellency was observed with Shatera extract at a concentration of 8000 ppm after 24 hours, while the lowest repellency was observed with Clapora extract at a concentration of 4000 ppm after 72 hours. No significant difference was observed between Shatera extract at a concentration of 4000 ppm and Clapora extract at a concentration of 8000 ppm after 24 hours (Table 3).

Table 3. The mean of repellency percent of *F. parviflora* and *T. polium* extracts on *B. tabaci* adults

Plant extract	Concentration(PPM)	Time		
		24(h)	48(h)	72(h)
	4000	40.8±1.49^{b}	33.6±0.97^{a}	32.8±1.49^{a}
Fumaria parviflora	6000	62.4±2.03^{e}	51.2±1.49^{c}	48.8±1.49^{c}
	8000	72±1.26^{f}	32.8±1.20^{d}	55.6±1.16^{d}
	4000	28.8±1.49de	20.8±1.49^{c}	9.6±0.97^{a}
Teucrium polium	6000	36±1.78^{e}	27.2±1.49^{d}	16±1.78^{b}
	8000	44.8±1.49^{f}	40±1.26^{e}	22.4±2.4^{c}

In each column, means with common letters are in the same statistical group at 5% probability level (Duncan test).

3-4-The effect of different concentrations of Shaterah and Klpoorah extracts on the inhibition of egg laying in white cotton aphids after 24 hours was investigated

The results of the analysis of variance showed significant differences in the inhibitory effect on egg laying among different concentrations of Klpoorah extract ($F_{2,11}$=57.81, p=0.000). The highest and lowest percentages of inhibition were observed at concentrations of 8000 ppm and 4000 ppm, with mean values of 67.68% and 99.20% respectively. The inhibitory effect on egg laying increased with increasing concentration (Table 4).

Similarly, the analysis of variance showed significant differences in the inhibitory effect on egg laying among different concentrations of Shaterah extract ($F_{2,11}$=46.99, p=0.000). The highest and lowest percentages of inhibition were observed at concentrations of 8000 ppm and 4000 ppm, with mean values of 79.79% and 07.38% respectively. The inhibitory effect on egg laying decreased significantly with increasing concentration (Table 4).

Furthermore, the analysis of variance showed significant differences in the inhibitory effect on egg laying among different treatments of Shaterah and Klpoorah extracts at a significance level of 1% (F5,18=52.82, p=0.000). The highest and lowest percentages of inhibition were observed by Shaterah extract at a concentration of 8000 ppm with a mean value of 79.79% and Klpoorah extract at a concentration of 4000 ppm with a mean value of 99.20%. The results also indicated that there was no significant difference in the inhibitory effect on egg laying between Shaterah extract at a concentration of 6000 ppm and Klpoorah extract at a concentration of 8000 ppm. Similarly, there was no significant difference between Shaterah extract at a concentration of 4000 ppm and Klpoorah extract at a concentration of 6000 ppm (Table 4).

Table 4. The mean of Oviposition inhibitory of *F. parviflora* and *T. polium* extracts in different concentrations on *B. tabaci*

Plant extract	Concentration(PPM)	% Oviposition inhibitory
Teucrium polium	4000	20.99±1.96[a]
	6000	35.46±3.50[b]
	8000	67.67±3.67[c]
Fumaria parviflora	4000	38.07±2.79[b]
	6000	62.10±3.44[c]
	8000	79.02±2.71[d]

In each column, means with common letters are in the same statistical group at 5% probability level (Duncan test).

4.Discussion

The results of this experiment showed that the studied extracts have repellent and inhibitory properties on the egg-laying of whitefly on cotton. Studies have been conducted on the effects of some plant extracts and essences on the repellency and inhibition of egg-laying on whiteflies, including Yang et al. (2010) who reported the effects of the essential oils of Thymus vulgaris L., Pogostemon cabin Blanco, and Corymbia citridon Hook on whiteflies. Additionally, Dehghani and Ahmadi (2013) evaluated the repellent and inhibitory properties of several herbal plant extracts and essences against greenhouse whiteflies and reported that Cuminum cyminum Linn. and Achillea millefolium Linn. have the highest repellent and anti-egg-laying effects, while Citrus sinensis Linn. has the lowest. Wagan et al. (2017) studied the respiratory toxicity and egg-laying inhibition effects of ethanol extracts of Acorus tatarinowii Schott, Heracleum hemsleyanum Diels, and Stemona japonica Lour. on whiteflies, and showed that A. tatariríowii and H. hemsleyanum have the highest and lowest toxicity and egg-laying inhibition, respectively. Other researchers have examined the repellent effects of several plant extracts from different families on silverleaf whiteflies and stated that extracts from certain plants such as Urtica pilulifera Linn. and Thymus vulgaris have significant repellent effects compared to other extracts (Al-mazraawi & Ateyyat, 2009). Legaspi and Simmon (2012) reported that garlic plant extract has a significant repellent effect on whiteflies. Zhang et al. (2004) stated that Zingiber officinale Rosco extract prevents whitefly egg-laying on tomato plants. Baldin et al. (2013) reported that whiteflies lay significantly fewer eggs on plants treated with citronella extract. The plant extracts that inhibit egg-laying may have an impact on the physical condition of the substance and reduce their reproductive capacity (Yang et al., 2010). For example, Rao et al. (1999) found that ovarian growth in the insect Dysdercus koenigii Fabricius is inhibited by treatment with dill extract, resulting in a decrease in the number of eggs laid and therefore a reduction in egg production.

5.Conclusion

The results showed that the extract of shatera has a higher repellency and oviposition inhibition properties compared to the extract of klipoor. In a 24-hour period, the extract of klipoor with twice the concentration had an equivalent repellent effect as the extract of shatera. Additionally, lower concentrations of the shatera extract were able to create an equivalent oviposition inhibition as higher concentrations of klipoor. Samareh Fekri and colleagues (2014) examined the insecticidal properties of shatera and klipoor extracts against the cotton whitefly on tomato plants and reported Lc50 values of 17.26 and 93.88 grams per liter for the shatera and klipoor extracts on the Kalle-Gian-Tri tomato variety, respectively, and 13.26 and 68.36 grams per liter on the Aragon tomato variety. They stated that the insecticidal properties of the shatera extract are greater than those of klipoor. Furthermore, studies by Mahdavi Arab and colleagues (2008) demonstrated the insecticidal properties of klipoor and shatera extracts on the four-spotted bean weevil, with the shatera extract being more toxic than klipoor. These findings are consistent with the present study in terms of the higher toxicity of shatera compared to klipoor. In general, the overall results of this research demonstrated that the repellent and oviposition inhibition effects of the studied extracts increase with higher concentrations, which is consistent with other studies reporting that increasing the concentration is an important factor in increasing the toxicity of plant compounds. Wang and colleagues (2008) studied the repellent and oviposition inhibition effects of ethanol extract of Myristica fragrans Houtt. at concentrations of 2.5, 5, and 10 milligrams per milliliter, and reported the highest repellent and oviposition inhibition effects at a concentration of 10 milligrams per liter. Wagan and colleagues (2017) stated that the toxicity of the plant extract increases with higher concentrations, resulting in increased repellent and oviposition inhibition effects on the cotton whitefly. In the present study, it was observed that the repellent properties of the plant extracts on the cotton whitefly decrease with increasing time at a constant concentration. Mendoza-Garcia and colleagues (2014) studied the insecticidal, repellent, and oviposition inhibition effects of water, ethanol, and acetone extracts of several plants against the cotton whitefly and reported that the acetone extracts had no repellent or insecticidal effects, while the water and ethanol extracts of wild turnip (Raphannus raphanistrum Linn.) and ragweed (Ambrosia artemisifolia Linn.) had the highest repellent effects, which gradually decreased over time. Other researchers also studied the repellent effects of Myristica fragrans Houtt. extract on the cotton whitefly and found that the repellent effect decreases with increasing time. The results of the present study are consistent with the findings of these researchers. Barati and colleagues (2016) investigated the repellent effects of several plant extracts on the silverleaf whitefly and found that the repellent properties of the extracts increase with increasing time, which is not consistent with the results of this experiment. The reason for this difference may be due to variations in plant species and insect species studied, as well as differences in extraction methods. Based on the obtained results, these plant extracts can be used as biopesticides in integrated management of greenhouse whitefly, as they are safer for humans, the environment, and other organisms and are biodegradable. However, due to the volatile nature of the active compounds in the plant extracts and their rapid oxidation, further research is necessary to improve the efficacy and preservation of their insecticidal properties, especially for their use in open environments such as farms, through formulation development.

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