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Research Article

Behavioral and Insecticidal Effects of Three Plant Oils on *Bemisia tabaci*

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Abstract

Background and Objective: The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most important economic pests, causes great losses in many economic crops. The present study aimed to control *B. tabaci* through suitable successful Integrated Pest Management programs (IPMs) safe enough to man and environment, by inserting some plant oils as alternatives or synergists for pesticides. **Methodology:** The effects of three plant oils cumin (*Cuminum cyminum*), thyme (*Thymus vulgaris*) and garlic (*Allium sativum*) as repellents or oviposition deterrents against whitefly, *Bemisia tabaci* were tested. The insecticidal effect of these oils and their mixtures with a recommended insecticide (Cetam) against *B. tabaci* was investigated for inserting them within IPM programs. Additionally, the deterrence effects were analyzed using SAS9.2 procedures at probability level of 0.05 and the toxicity was analyzed using probit analysis. **Results:** All three tested oils effectively prevented *B. tabaci* adults either from feeding or laying eggs. The highest repellency and anti-oviposition effects occurred with thyme oil treatment, followed by garlic oil. The greatest effect on immature stages was found with thyme oil, which reduced the survival rate of egg and pupae of *B. tabaci* after egg treatment, while the greatest effect on survival of larvae was observed with garlic oil. *Bemisia tabaci* larvae were more sensitive to plant oil treatments, compared with eggs and pupae. The adult mortality was increased in conjunction with increasing dose of tested oils. All mixtures of cetam with plant oils and mixtures of oils together resulted in additive or potentiating effect. **Conclusion:** It is concluded that the tested oils may use as insecticide alternatives in an integrated pest management program for the subject pest.

Key words: *Bemisia tabaci*, joint toxic, anti-oviposition, thyme, cumin, garlic

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is among the most important economic pests attacking cotton, vegetables and ornamentals^{1,2}. Larval instars and adults of *B. tabaci* directly damage plant leaves by continual sap sucking and by excreting honeydew which serves as a medium for sooty moulds, which severely reduces the productivity of plants by interfering with photosynthesis³. Cotton fiber stickiness due to honeydew is a severe problem in many cotton producing countries as this leads to significant loss of cotton quality and marketability^{4,5}.

At least, 21 aleyrodidae pests have been documented in Egyptian cropping system⁶. Four of them (*B. tabaci*, *B. argentifolii*, *Trialeurodes ricini* and *T. vaporariorum*) are vectors of plant geminiviruses⁶.

Although chemical control is widely used for the management of *B. tabaci*, it has rapidly developed resistance to a number of insecticides. Therefore, the alternative control methods should be developed and new types of pest control agent's advantage with higher activity against the target pests and lower impact on humans and environmental quality should be investigated. The use of biologically based compounds in plant extracts or essential oils may be an alternative to currently used insecticides to control insects. Moreover, essential oils have a broad spectrum of insecticidal activity due to the presence of several modes of action, including repellent and antifeedant activities, inhibition of molting and reduction in growth and fecundity^{7,8}. Plant oils effect directly as repellents or even indirectly as antifeedant compounds or toxins, so, they may help to control *B. tabaci* by expulsion effect and consequently, by reducing virus transmission to the plants⁹. Plant essential oils may also have minimal direct and/or indirect effects on natural enemies^{10,11}. Essential oils and crude extracts of some plants have been evaluated for repellency and insecticidal activity against the sweet potato whitefly (*B. tabaci*) and some of them can be used as an alternative method of controlling this deleterious pest through suitable integrated pest management programs¹².

The objectives of the present study were to test the repellency, anti-oviposition and insecticidal activity of cumin (*C. cyminum*), thyme (*T. vulgaris*) and garlic (*A. sativum*) oils against *B. tabaci* different stages and investigate the insecticidal effect of tested oils in comparison with a recommended insecticide (Cetam) against *B. tabaci* adults. The joint toxic action of cetam with tested plant oils and plant oils together were studied in order to minimize the usage, dose or concentration, of conventional insecticides.

MATERIALS AND METHODS

The experiments of the present study were carried during the summer season of 2016.

Whitefly tomato culture: *Bemisia tabaci* adults from the laboratory tobacco culture have been reared on tomato plants *Lycopersicon esculentum* (Solanaceae) since 2000 till now, in greenhouses at $25 \pm 7^\circ\text{C}$, $65 \pm 5\%$ RH and under natural light conditions.

Tested materials

Plant oils: The oils of cumin (*Cuminum cyminum*), thyme (*Thymus vulgaris*) and garlic (*Allium sativum*) were supplied by the Department of Pharmacology, Faculty of Pharmacy, University of Alexandria, Egypt.

Insecticide: Cetam® 20%, provided by El-Helb Company of Pesticides and Chemicals, New Damietta City-1st Ind. Zone, Egypt.

Chemical name: (E)-N1-[(6-chloro-3-pyridyl) methyl]-N2-cyano-N1-methyl acetamidine.

Repellence activity

Tested concentrations: Basic stock solution of each tested oil was made in distilled water containing 0.05% triton X-100 and 0.1% dimethyl sulfoxide (DMSO) as an emulsifier. The series of concentrations were prepared in distilled water. The effectiveness of 0.05, 0.1, 0.5, 1 and 2% concentrations of each oil on the olfaction response of *B. tabaci* adults was checked.

Choice test: The tested oil was prepared at concentrations of 0.05, 0.1, 0.5, 1 and 2%. Uninfested tomato seedlings were sprayed with five concentrations of the tested oils using sprayer until runoff, three plants in three replications were used for each oil and control. Two control plants were made: The first were sprayed with distilled water and the second were sprayed with 0.05% triton X-100 and 0.1% (DMSO) only. The treated and control tomato seedlings were allowed to dry in shadow for 2 h, the treated plants were put together with control plants into insect cages, The arrangement of plants was completely randomized. Into each cage approximately 250 adults were exposed to seedlings. The adults were left in cages for 48 h, after this time plants were carefully extracted from the cages, in the early morning and the number of adults per plant was counted, then the percent of repellency to each plant was calculated.

Anti-oviposition activity: All procedures were the same as the choice test but the adults were left in cages for 6 days, then plants were taken carefully into the laboratory, all adults were removed from plants and the number of eggs laid on 10 randomly chosen leaves was counted using the binocular. The average number of eggs per leaf was taken for the calculation of percentage of anti-oviposition effect.

Data analysis: Adult repellency and oviposition percentages were calculated according to Pascual-Villalobos and Robledo¹³:

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

where, C is the No. of the adults or eggs in the control and T is the No. of the adults or eggs in the treatment.

Insecticidal activity

Insecticidal activities of the tested oils against different stages of *B. tabaci*: Twenty whitefly adults of mixed sexes were confined on a tomato seedling for 48 h to oviposition. The adults were then removed and tomato seedlings that infested with the eggs were sprayed with the concentrations (25, 50, 100, 500 and 1000 ppm) of each of the tested oils. Control plants were sprayed with 0.01% triton X-100 only. Each treatment and control was replicated three times. The effect of tested materials on the progeny resulting from the treated eggs was determined. The treated and control tomato seedlings were allowed to dry in shadow for 2 h and then were transferred into glasshouse.

Ovicidal effect: Effect on percent of hatchability was measured based on the number of hatched eggs 10 days after treatment, the percent of egg hatchability was calculated to determine the ovicidal effect.

Larvicidal effect: The larvicidal effect was examined on treated and control plants infested with eggs. Mortalities of the larval stage were assessed on the basis of failure to emerge into pupal stage 10 days after egg hatchability, relative to the number of successfully emerged larvae from treated eggs.

Percent of adult emergence or mortality of the pupae: The percent of emerged adults was calculated relative to the total number of pupae which developed from the surviving larvae was recorded.

Statistical analysis: Statistical analysis of the obtained data and all the probable comparison combinations were analyzed in factorial (Two factors) design by using SAS9.2 procedures¹⁴ at probability level of 0.05.

Insecticidal activity of the tested oils and cetam against *B. tabaci* adults: The insecticidal effects of LC₅₀ concentrations of each oil and cetam on *B. tabaci* were checked.

Bioassay test: Uninfested tomato seedlings were sprayed with five concentrations (25, 50, 100, 500 and 1000 ppm) of the mentioned tested oils using sprayer until runoff. Two control plants were made: The first were sprayed with distilled water and the second were sprayed with 0.01% triton X-100 only. The neonicotinoid insecticide (Cetam) was used as a positive control for the tested oils. The treated tomato seedlings were allowed to dry in shadow for 2 h and then 21 adult whiteflies per replicate were exposed to the treated and control seedlings covered with glass cages with muslin in the upper opened. The treated and control tomato plants were kept under a glasshouse conditions. The adult mortality was determined 48 h after inoculation and repeated for three replicates. Concentration-mortality regressions were statically analyzed with probit analysis¹⁵.

Joint toxic action of cetam with plant oils against *B. tabaci* adults: To minimize the usage of conventional insecticides, such as cetam, joint toxic action of it with plant oils was studied, where, LC₂₅ of cetam were mixed with the LC₂₅ of plant oils and LC₂₅. Control plants were sprayed with distilled water or 0.01% triton X-100 only. The treated tomato seedlings were allowed to dry in shadow for 2 h and then twenty adult whiteflies of mixed sexes per replicate were exposed to the treated and control seedlings covered with glass cages with muslin in the upper opened, then kept under a glasshouse conditions. The adult mortality was determined 48 h after inoculation and repeated for three replicates.

The expected mortality was calculated for each tested material in the mixture separately. Therefore, the expected mortality for the mixture of two materials was calculated by adding the mortalities of each material used in the mixture. Co-toxicity factors were calculated according to Mansour *et al.*¹⁶ as follows:

$$\text{Co-toxicity factor} = \frac{\text{Observed mortality (\%)} - \text{Expected mortality (\%)}}{\text{Expected mortality (\%)}} \times 100 \quad (2)$$

This factor was used to categorize the results into three categories as follow: Co-toxicity factors $\geq +20$ meant potentiation; co-toxicity factors < -20 meant antagonism and co-toxicity factors between -20 and $+20$ meant additive effect.

RESULTS

Repellence and anti-oviposition activities: The repellent and anti-oviposition effects of tested oils on *B. tabaci* presented in Fig. 1. Figure 1 shows revealed that *B. tabaci* adults of mixed sexes were significantly influenced by oil type and oil concentration, at probability level of 0.05. Regarding the repellency effect of the tested oils, all tested oils repelled *B. tabaci* adults, the percent of repellency ranged from 37.49% (for 0.05% cumin oil) to 98.36% (for 2% thyme oil). Thyme oil showed the highest repellency effect on greenhouse whitefly (84.01%). While, the lowest repellent effect was recorded in the case of cumin oil (65.12%). Moreover, it was observed from the results that the repulsive responses to the tested oils increased by increasing the oil concentration up to (93.53% when the concentration 2% was used.

The present results suggested that all three tested oils affected ovipositional behavior of *B. tabaci*, the highest anti-oviposition effect was caused by thyme oil 2 and 1% (100 and 97.7%, respectively), followed by garlic oil 2% (95.55%).

When linking the repellent with the anti-oviposition effects, it could be concluded that the most effective oil against oviposition, thyme oil, was the same as most repellent one, this may explain the mechanism that prevent insects from laying eggs. This prediction was confirmed by

Sliva *et al.*¹⁷, who suggested that there are four mechanisms involved in the inhibition of insect oviposition: Repellent effects, locomotor stimulants, suppressive effects and/or deterrents.

So, it was expected that if thyme, garlic or cumin were intercropped with the tomato plants, this might reduce the attraction of tomato to *B. tabaci*, hence, suppressing pest population.

Insecticidal activities of the tested oils against different stages of *B. tabaci*: The effect of the tested oils on the progeny resulting from the treated eggs was determined by development from hatching to adult emergence.

The effect of different tested materials on *B. tabaci* hatchability percent was investigated. The statistical analysis of the obtained data proved the significant differences among all obtained percent of hatchability of *B. tabaci* as response to the tested oils (Fig. 2).

Applications of all tested oils significantly suppressed whitefly egg hatches compared with the control (Fig. 2). The obtained results indicated that the treatment of *B. tabaci* egg with thyme oil resulted in significant reductions of eggs hatchability when compared with the control where, it reduced egg hatchability about 45-60%, followed by garlic oil which reduced egg hatchability about 40-50% when compared with control. On the contrary, cumin oil showed the lowest effect on egg hatchability, cumin oil caused about 10-30% reduction of egg hatchability.

Concerning the effect of tested oils on larval mortality, the statistical analysis of the obtained results shown in Fig. 3 declared that all three tested plant oils significantly reduced development of *B. tabaci* larvae into pupae. Mortalities of

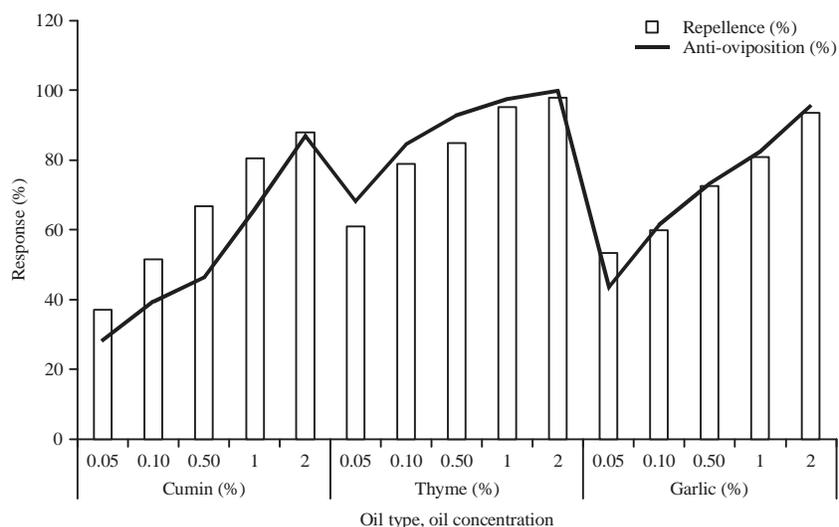


Fig. 1: Repellence and anti-oviposition effects of tested oils on *B. tabaci*

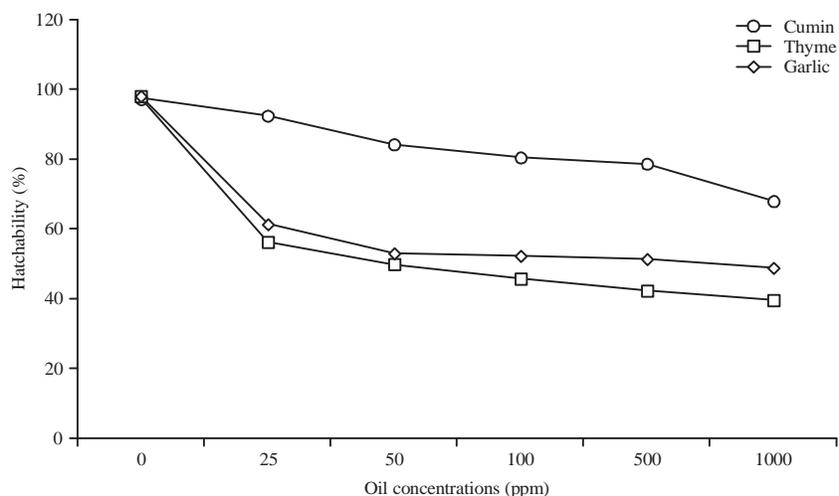


Fig. 2: Mean percentages of egg hatchability of *B. tabaci* on control plants and plants treated with various concentrations of tested oils

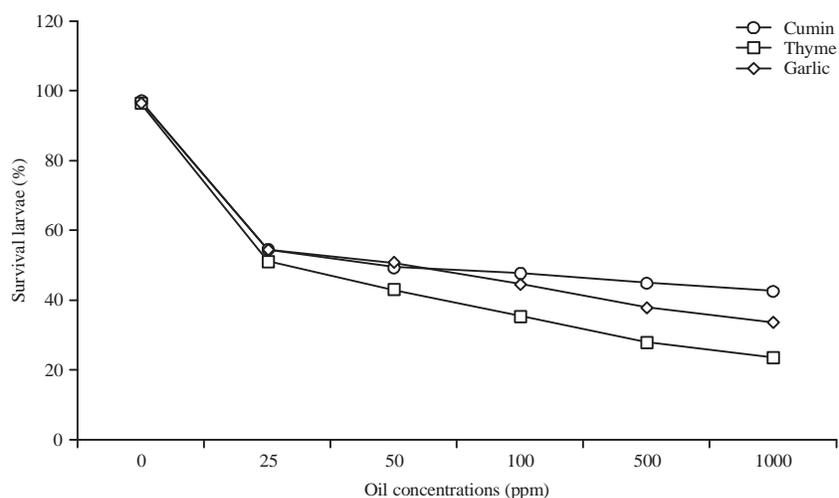


Fig. 3: Mean percentages of *B. tabaci* larvae survival on plants treated with various concentrations of tested oils and control plants

B. tabaci larvae which survived from the treated egg, thyme oil at 1000 and 500 ppm concentrations, resulted in higher mortalities, 76.42 and 72.05%, respectively, as compared with control, followed by garlic oil 1000 ppm with larval mortality 66.43%.

At the lower concentrations, there is no significant difference between cumin and garlic oils.

Adult emergency (or survival pupae) significantly affected with three tested oils (Fig. 4), oil of thyme was the most effective, reducing the emergence of *B. tabaci* adults 58.93% as compared with the control, followed by garlic oil with reduction of percent adult emergence (54.4%). At the lower concentrations, there was no significant

difference between cumin and thyme oils with reduction 35.5 and 35.31%, respectively.

From the present results it was cleared that larvae were the more sensitive stage to the tested oils compared with eggs and pupae. While pupae were the more resistant stage to the different treatments.

Insecticidal activity of the tested oils and cetam against *B. tabaci* adults:

Potency of the tested materials against *B. tabaci* adults was evaluated. The probit analysis of the obtained data illustrated the insecticidal activity of the tested materials as LC₅₀ values (Table 1, Fig. 5). It is clear from the obtained results shown in Table 1 that cetam was the most

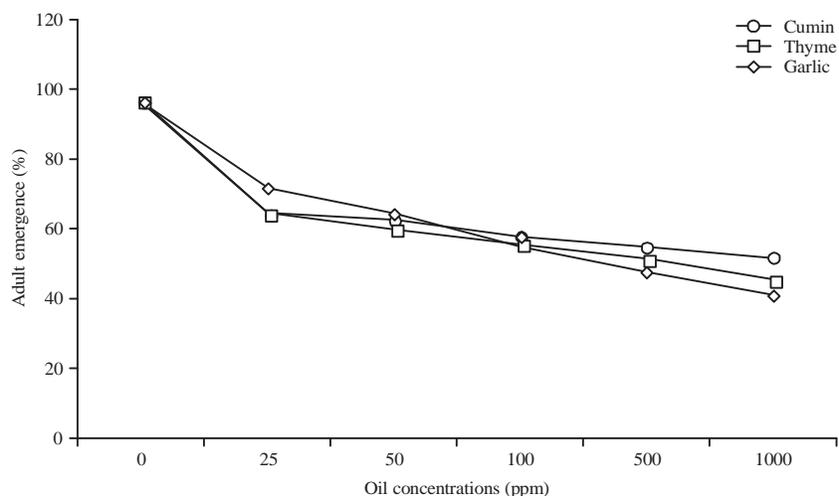


Fig. 4: Mean percentages of *B. tabaci* adult emergency on control plants and plants treated with various concentrations of tested oils

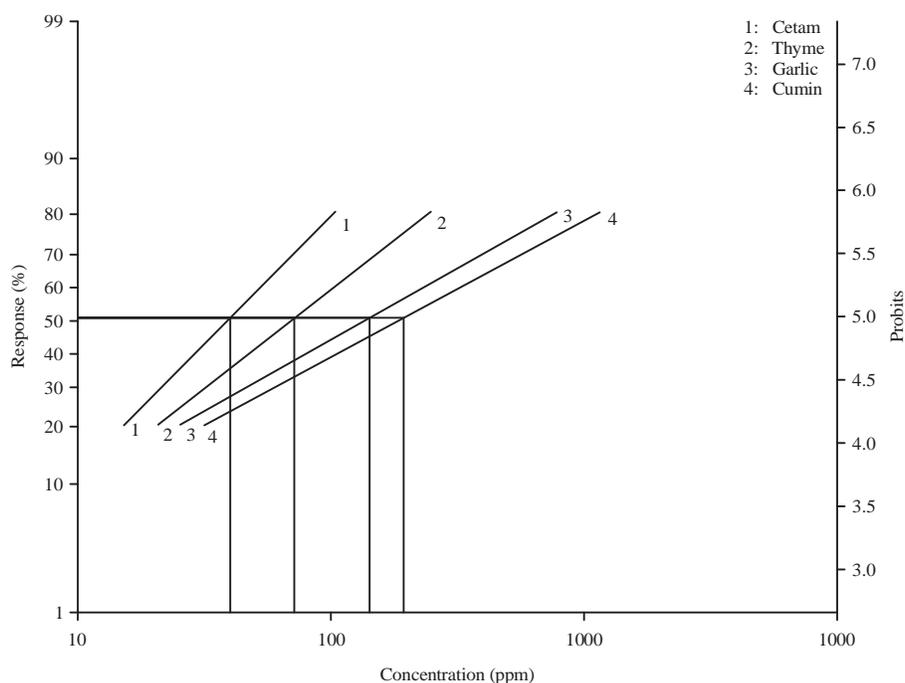


Fig. 5: Insecticidal activity of tested materials against *B. tabaci* adults 48 h after treatment

Table 1: Comparative toxicity of tested materials on *B. tabaci* adults after 48 h from treatment

Tested materials	LC ₂₅	LC ₅₀	Lower limit	Upper limit
Cetam	18.435	39.792	30.33	49.372
Cumin oil	45.491	192.176	138.382	275.186
Thyme oil	26.691	71.756	54.905	91.736
Garlic oil	35.85	141.135	102.436	195.121

potent against whitefly adults with LC₅₀ value of 39.792 ppm, followed by thyme oil and then by garlic oil with LC₅₀ values of

71.756 and 141.135 ppm, respectively. On the contrary, cumin had less toxic effect against *B. tabaci* adults with LC₅₀ 192.176 ppm.

When linking the current results with the previous behavioral results, it could be concluded that there is a positive correlation between the repellency and the toxicity of the tested oils, the most toxic oil, thyme oil, is the most repellent one. This insecticidal effect may be due to the feeding deterrence and antifeeding effect.

Table 2: Joint toxic action of cetam (LC₂₅) with tested plant oils (LC₂₅) against the whitefly *B. tabaci* adults

Mixture	Expected mortality (%)	Observed mortality (%)	Co-toxicity factor
Cetam+cumin	57.00	66.67	16.96
Cetam+thyme	59.00	75.00	27.12
Cetam+garlic	58.33	73.33	25.72
Cumin+thyme	54.66	60.33	10.37
Cumin+garlic	54.00	58.00	7.41
Thyme+garlic	56.00	62.67	11.90

Joint toxic action of cetam with certain plant oils against the whitefly *B. tabaci* adults: The effect of applying mixtures of cetam (LC₂₅) with the plant oils (LC₂₅) was determined against whitefly *B. tabaci* adults, the resulted joint toxic action presented in Table 2.

It is clear that, all mixtures of cetam with all plant oils resulted in additive or potentiating effect with Co-Toxicity Factors (CTFs) ranged between +16.96 to +27.12. While, the mixtures of plant oils together resulted in additive effect, where CTFs ranged from +7.41 to +11.9 (Table 2). Generally, the joint toxic effect of cetam with the tested plant oils was higher than the effect of mixing plant oils together. Present results revealed that the higher potentiating effect, after 48 h of exposure insects, was obtained when cetam was mixed with thyme oil with CTF 27.12, while the lowest additive effect was obtained when cumin oil was mixed with garlic oil with CTF 7.41. These results means that the dosages of cetam can be reduced when they are used in mixtures with these natural products, subsequently, this may reduce the impact of these compounds on the natural enemies in crop fields.

DISCUSSION

The present resulted behavioral effects of the tested oils on whitefly were previously approved by Yang *et al.*¹⁸, who resulted that in choice tests, the mean numbers of eggs laid by *B. tabaci* females on plants treated with *T. vulgaris* oil was 59.0% fewer than numbers of eggs on control plants. Dehghani and Ahmadi¹⁹ also found that the greatest repellence effect 3 and 6 days after whitefly, *Trialeurodes vaporariorum* infestation occurred with water extracts of *Cuminum cyminum* L. and *T. vulgaris* L. treatment, respectively and the lowest anti-oviposition index was recorded for aqueous extract of *C. cyminum*. The study of Sertkaya *et al.*²⁰ showed that essential oils obtained from medicinal plants such as thyme were have a repellency effects to cotton whitefly adults. El-Meniawi *et al.*²¹ studied the olfaction repose of *B. tabaci* adults using olfactometer, they found that among ten plant oils, garlic and thyme oil showed a repellent effect against *B. tabaci*. Deletre *et al.*²² reported

that the cumin mixture and its derivatives, cuminaldehyde were repellent to *B. tabaci*. Where, cuminaldehyde could be responsible for the repellent effect of the cumin mixture. Barkman²³ found that all tested mixtures of cumin and other tested oils or their derivatives had a significant repellency effect on *B. tabaci* in high concentration (1%). Regarding the same effects, Moore *et al.*²⁴ identified *Cuminum cyminum* as one of four highly repellent essential oils among 20 tested essential oils on *B. tabaci*. Legaspi and Simmons²⁵ reported that *Bemisia argentifolii* laid the fewer numbers of eggs on plants sprayed with garlic oil.

The repellent and anti-oviposition effects of the tested oils were reported on different insects by Pavela²⁶ who found that the oil of *T. vulgaris* had most repellent and high anti-oviposition effects against brown house mosquito (*Culex quinquefasciatus* Say). Choi *et al.*²⁷ showed that *T. vulgaris* essential oil and its primary constituents, carvacrol and thymol had repellent activity within the tested materials against *Culex pipiens pallens*. The insecticidal and repellent activities of thyme (*Thymus vulgaris* L.) have been reported against red flour beetle *Tribolium castaneum*²⁸ and Indian meal moth (*Plodia interpunctella* Hübner)²⁹.

Wang *et al.*³⁰ suggested that intercropping of aroma plants, *Perilla frutescens* (L.) and wild mint, *Mentha arvensis* L., with tomato plants, reduced the population of *Trialeurodes vaporariorum* Westwood by 39.1 and 41.5% as compared to the control, respectively.

Present results about high contact toxicity of essential oils are in accordance with those presented by Kim *et al.*³¹, who reported that some essential oils such as thyme and garlic resulted high suppression of *B. tabaci* population. The results of Yang *et al.*¹⁸ revealed that among the three tested oils, essential oil of *T. vulgaris* was the most effective, reducing the survival rate of eggs, nymphs and pupae of *B. tabaci* as compared with the control, also they found that egg hatchability was reduced ~50% with essential oils from *T. vulgaris*.

Many studies confirmed the effects of plant oils against *B. tabaci* immature stages, such as Yarahmadi *et al.*³², who showed that all concentrations of both essential oils *Geranium* and *Artemisia* were significantly suppressed all developmental stages of *B. tabaci*. Himat³³ concluded that neem formulations proved to be effective in reducing the hatchability of the eggs of the whitefly *B. tabaci*.

There are many reports that supported the potency effect of thyme oils, Barkman²³ concluded that essential oils from *T. vulgaris* and its derivatives or their mixtures showed the strongest contact toxicity on adults of *B. tabaci* biotype

Yang *et al.*¹⁸ reported that, among the tested oils, essential oil derived from *T. vulgaris* had the greatest contact toxicity against *B. tabaci*. Aroiee *et al.*³⁴ showed that thyme (*T. vulgaris*) was the most effective essential oil against whitefly, *T. vaporariorum*. Aslan *et al.*³⁵ found that essential oil vapors from *Thymus vulgaris* L. (Lamiaceae) had high toxicities against the adults of *B. tabaci*.

Concerning the toxicity of garlic oil against *B. tabaci*, the present results agreed to a large extent with the findings of Liu *et al.*³⁶, who evaluated the fumigant toxicity of essential oils of Chinese medicinal herbs against *B. tabaci*, they found that the two main constituent compounds of garlic essential oil, diallyl trisulfide and diallyl disulfide exhibited strong fumigant toxicity against the whitefly. Nzanza and Mashela³⁷ showed that fermented plant extracts of neem and wild garlic, alone or in combination, have insecticidal properties to maintain lower population densities of whitefly and aphid.

The toxicity of cumin oil also reported by Deletre *et al.*²² reported that the cumin mixture and its derivatives, cuminaldehyde were toxic against *B. tabaci* and they found that cumin mixture limited the white y net-crossing rate by killing them. Barkman²³ found that, an effect for the 4 h toxicity was only found for the cumin, cinnamon and lemongrass mixtures.

The current results were confirmed by Deletre *et al.*²², who revealed that cumin mixture was more toxic than Cuminaldehyde and-terpinene and the major compounds of cumin, citronella and lemongrass essential oils had synergistic/additive effects. Nzanza and Mashela³⁷ suggested a synergistic effect of fermented plant extracts of neem and wild garlic as a bio-pesticide, where, the mixture of neem and wild garlic was more effective in reducing population densities of whitefly and aphid than either plant extract applied alone.

CONCLUSION

Finally, it could be concluded that the repellent effect and toxicity of the tested oils make them potential materials for use in a comprehensive integrated pest management program for the subject pest.

SIGNIFICANCE STATEMENTS

The present study for the first time clarified the repellent, anti-oviposition and insecticidal effects of the tested oils, cumin, Thyme and Garlic, on *B. tabaci*. Also, the results showed the additive or potentiating effect of these oils when mixed with insecticide (cetam) and used for manage *B. tabaci*.

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