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Research Article

Acaricidal Activity of Essential Oil and Aqueous Extract of *Eucalyptus Globulus L.* Against *Tetranychus Urticae* Koch (*Acarina: Tetranychidae*) on Eggplant

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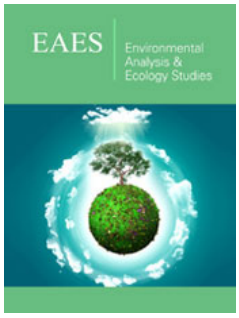

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Abstract

The red mite, *Tetranychus urticae* Koch *(Acarina: Tetranychidae)*, poses a threat to the food security of populations in several countries because it damages several crops such tomatoes, eggplant, cucumber, melon, strawberries, ornamental plants and fruits. As an alternative to chemical acaricides, biological control using plant extracts is one of the most promising sustainable approaches adopted to limit damages caused by *T. urticae*. A survey of the population of mites on eggplant in greenhouse was done. The acaricidal activity of aqueous extract (10, 20, 30 and 40%) and essential oil (1, 2, 3 and 4%) of *Eucalyptus globulus L.* against *T.urticae* on eggplant was studied in a laboratory and greenhouse. The first infestation off eggplant by *T. urticae* was registered at February 2021, maximum infestation rate (58.8±6.11%) was at June/2021, maximum number of *T. urticae* egg (5.56±0.36/cm^2 eggplant leaf) and maximum number of mobile stage (4.22±0.20/cm^2 on eggplant leaf) were at June/2021. The Correct efficacy decreases significantly with decreasing concentrations of *E. globulus* aqueous extract and essential oil. The highest correct efficacies (67.02±3.09% in the laboratory) and (63.72±5.70% in the greenhouse) were obtained with the higher concentration (40%) of aqueous extract of *E. globulus*. Similarly, the highest correct efficacies (69.98±3.79%) in the laboratory and (67.66±4.94) in the greenhouse were registered with a higher concentration (4%) of *E. globulus* essential oil. Both the aqueous extract and essential oil of *E. globulus* are effective in controlling *T. urticae* and can be a natural alternative to hazardous synthetic acaricides in any IPM program against the two-spotted red mite.

Keywords: Two-spotted red mite; *Eucalyptus globulus*; Aqueous extract; Essential oil; Efficacy

Abbreviations: mL: Millimeter; ha: Hectare; cm: Centimeter; L: Light; D: Dark; DM: Digital microscope; SPSS: Statistical Package for Social Science; ANOVA: Analysis of Variance

Introduction

Eggplant or brinjal (*Solanum melongena L.*) is among the most consumed vegetables worldwide and offers to human hepatoprotection and cardio protection due to its phenolic, antioxidant and anti-microbial substances [1]. China, India, Indonesia, Egypt, Turkey and Iraq were the most eggplant productive countries [2]. In Saudi Arabia, eggplant is among the most produced vegetables, along with tomatoes, peppers, cucumbers and zucchini [3]. It is cultivated in an open field and greenhouse. Nevertheless, eggplant suffers from several species of pests like aphids, whiteflies and mites which reduce the quality and quantity of yield. Among mites, the two-spotted spider mite *T. urticae* Koch *(Acarina: Tetranychidae)* is one of the most serious pests, infesting more than 1200 plant species, including crops such as tomato, eggplant, cucumber, melon, strawberry, ornamental plants, and fruits [4,5]. Drawing out the sap of plants using their piercing and sucking mouthparts, the spider mites were responsible for serious damage. Spider mites' motile stages sucking sap principally

from leaves epidermis, which causes yellowing and discoloration of leaves, chlorophyll, and water content lowering, induces fungi, bacteria, and viruses infestations, exerts a negative impact on productivity and economic returns for farmers [2].

The control of the two-spotted mite is particularly problematic due to its short life cycle, high fecundity enforced by life-history characteristics to produce successive generations, and rapid development of resistance to acaricides [6,7]. For several years, the use of chemical acaricides has been the main form of control for mites. However, the continual use of conventional acaricides induces harmful impacts on human health and the environment, as well as the elimination of natural enemies and pollinating insects [4]. Hence, finding other approaches that capitalize on safe natural products is an obligation and a real need. In this case, and in order to reduce synthetic acaricides negative effects, natural-based pesticides have been considered good alternatives and introduced as a prospective choice for arthropod suppression [8]. Indeed, natural-based pesticides have lethal or sublethal effects such as repellent, miticidal, and oviposition deterrent activities on numerous pests and cause fewer effects on the environment, natural enemies and pollinators [2,9].

Furthermore, botanical pesticides have numerous active molecules that exert diverse modes of action and are possibly capable of efficiently averting the appearance of resistant pest races. Then, many plant compounds (alkaloids, terpinoids, etc.) have now been known to affect the growth, development, reproduction of pests, principally insects and mites [10]. In recent years, the miticidal and repellent activities of natural-based pesticides (Aqueous and ethanol extracts, essential and fixed oils) have been actively explored [2,11-13]. Studies have demonstrated that natural extracts and oils are safe, precise in action, and potentially appropriate for utilizing in mite management programs [14-16]. The acaricidal repellent activity of aqueous extracts of *Origanum vulagre* L. and *Azadirachta indica* A. has been evaluated and demonstrated to reduce the reproductive capacity of mites and the survival of the progeny of treated females [10]. Also, the acaricidal activity of essential oils of *Chrysanthemum roseum*, *Nicotina tabacum*, *Derris elliptica*, *azadirachta indica*, *Melia azaderach*, *Xanthium strumarium* has been tested and demonstrate to reduce mite populations [13,17,18]. Although not much work has been done on blue gum *(Eucalyptus globulus)* aqueous extract and essential oil against the two-spotted spider mite, however, studies on the repellent and insecticide activities of blue gum essential oil for numerous insect species indicated that it can be used to control insects [19-21]. Therefore, the current study aimed to evaluate the acaricidal activity of blue gum aqueous extract and essential oil against the two-spotted spider mite using several concentrations.

Material and Methods

A randomized complete design with three replications of nine treatments was done during the 2020/2021 season in a laboratory and a controlled greenhouse at the National Organic Agriculture Center, Uniazah (26.085478 º N43.9768123 ºE), Saudi Arabia. The nine treatments consist of eucalyptus leaf extract in four doses (10, 20,30, and 40mL/100mL) and essential oil (1, 2, 3 and 4mL/100mL) and water as a control.

Plant materials

In October 2020, the greenhouse soils were plugged and divided into eight lines, each 20 meters long and spaced 100cm apart. Before planting, compost as organic fertilizer was added at 5 tons/ha. The eggplant seedlings (three-leaf stage) were transplanted 50cm apart on 14 November, 2020, under a drip irrigation system. Commercial fertilizer (Neutral 7-5-4) and foliar organic fertilizer (multi-mineral) were added during the experiment at recommended doses. In a laboratory at a temperature of 25±2 ºC, relative humidity of 60±10%, and photoperiod of 14 L:10 D, 72 eggplant seedlings (three-leaf stage) were transplanted into pots (capacity of 2 Liters) containing a substrate consisting of 1/3 sand and 2/3 peat and watered each 48 hours. No pesticides were used during the experiments.

Eggplant infestation and evolution the two-spotted spider mite population in the green house

In a controlled greenhouse, eggplants were naturally infested by the two-spotted spider *(T. urticae)* in February 2021. During the February-June/2021 period, the number of infested plants was weekly counted and, the temperature was registered daily at 13 o'clock. In March 2021, 20 infested plants were randomly selected, three leaves of each were marked, and a square of 4cm^2 was drawn. A total of 60 squares were drawn. Of them, 20 squares were selected randomly each week, and their pictures were taken by GSM Mobile using a DM Wi-Fi Microscope (1000×magnification). Thereafter, the numbers of eggs and *T. urticae* observed on each square picture were counted and recorded.

Used compounds

Fresh leaves of *E. globulus* were collected from Al-Qassim Province, Saudi Arabia. Collected leaves were washed with water, dried, and well-ventilated in the shade for two weeks [22]. Thereafter, dried leaves were cut and ground to a fine powder using an electrical grinder. The obtained mixture was stirred thoroughly with repeated agitation at 3 hour intervals for 24 hours, and 4 concentrations (10, 20, 30 and 40%) were prepared from the final extracts [23]. Essential oil was extracted according to Aslan et al. [24] and Cosimi et al. [25] methods. During the experiment, 4 concentrations (1, 2, 3 and 4%) of *E. globulus* essential oil were used.

Acaricidal bioassay

To determine the acaricidal efficacy of the aqueous extract and essential oil of *Eucalyptus*, greenhouse was divided into two parts separated by four lines. The first part was for aqueous extract treatment and the second was for essential oil. In each part, 36 plants were selected and divided into nine groups. Each group consists of four plants corresponding to one treatment, and each plant is a replication. On selected plants, three leaves were marked, and the number of *T. urticae* was counted one hour before treatments using a similar method to that used in the survey of population evolution.

All test procedures were performed under the same conditions using a hand-held sprayer. In the laboratory, 72 total eggplants were used in the experiment, infected in April 2021 using infested leaves collected from eggplant in the greenhouse and divided into two parts (36 plants per part). Similar procedures were followed in the greenhouse. In order to determine efficacy of aqueous extract and essential oil of *Eucalyptus* at different concentrations in greenhouses and laboratories, the number of *T. urticae* was counted one hour before treatment and after 24, 72 and 124 hours of treatment time. The correct efficacy was calculated according to Henderson & Tilton's (1955) equation [26].

Correct efficacy (%) = [(Reduction rate % in treated plant-Reduction rate % in control plant population)/ (100-Reduction rate % in control plant population)] x 100.

Reduction rate % in control plants = [(Population in control plants after treatment-population in control plants before treatment)/Population in control plants before treatment] x 100.

Reduction rate % in treated plants = [(Population in treated plants after treatment-population in treated plants before treatment)/Population in treated plant before treatment] x 100.

Data analysis

Experimental data were statistically analyzed using SPSS (Statistical Package for Social Sciences 15.0). Significance of the results was obtained by a randomized one-way ANOVA at a 95% confidence level. The means were separated using Duncan's multiple range test at P≤0.05.

Results

Population evolution of the two-spotted spider mite population on eggplant

The first eggplant infested by the two-spotted spider mite was observed on February 6, 2021. Obtained results demonstrated that, when the monthly temperature ranges between 20.73±2.91ºC at February 2021 and 39.66±2.91 ºC at June 2021 (Table 1), the infestation rate is of 8.75±0.82% at February and 58.8±6.11 at June 2021 (Figure 1). A Significant difference (0.05) in the infestation rate of eggplant by *T. urticae* was demonstrated between months. In addition, results demonstrated that the minimum infested rate per week of eggplant (1.33 plants) by *T. urticae* was registered in February and the maximum (10.50.86) was in May 2021. Obtained data from the *T. urticae* population survey during the March-June 2021 period show that the number of *T. urticae* eggs per cm^2 of eggplant leaf is 2.580.47 at February and the maximum number (5.560.36) is registered at June. The number of mobile stages of *T. urticae* per cm^2 of eggplant leaf increases with the number of months, and the maximum is 4.220.20 registered at June (Figure 2).

Table 1: Monthly Temperature (Mean±SD) in greenhouse during February-June Period.

Temperature (ºC) (Mean±SD)	February	March	April	May	June
	20.73±2.91	24.65±2.11	28.95±1.52	34.80±2.25	39.66±2.91

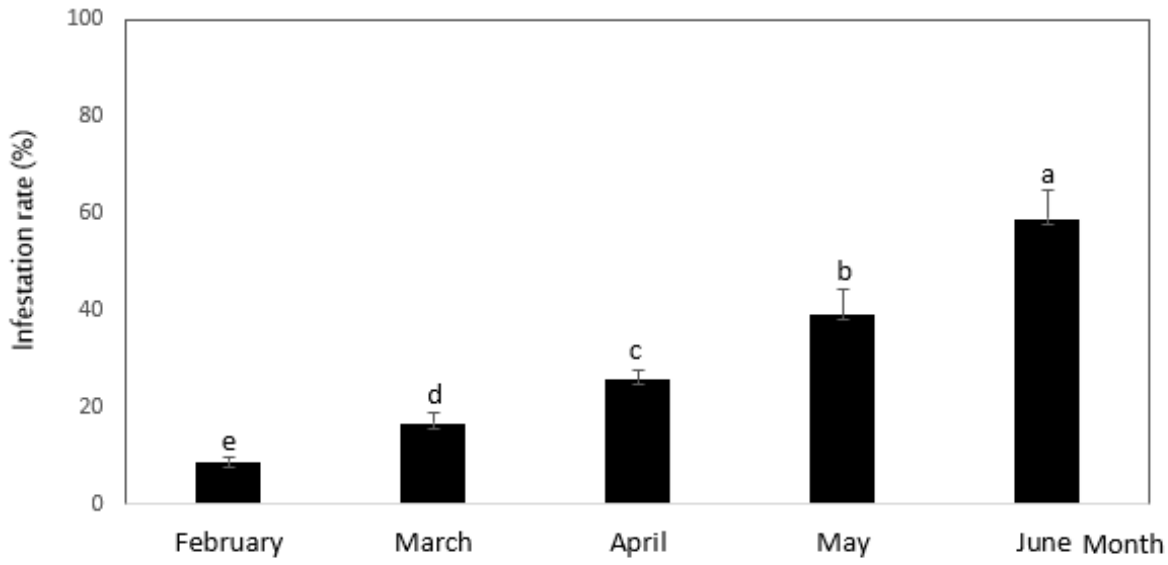

Figure 1: Infestation rate of eggplant by *T. urticae* during February – June/2021 period.
Means followed by the same letters are not significantly different (P≤0.05; Duncan's Multiple Range Test).

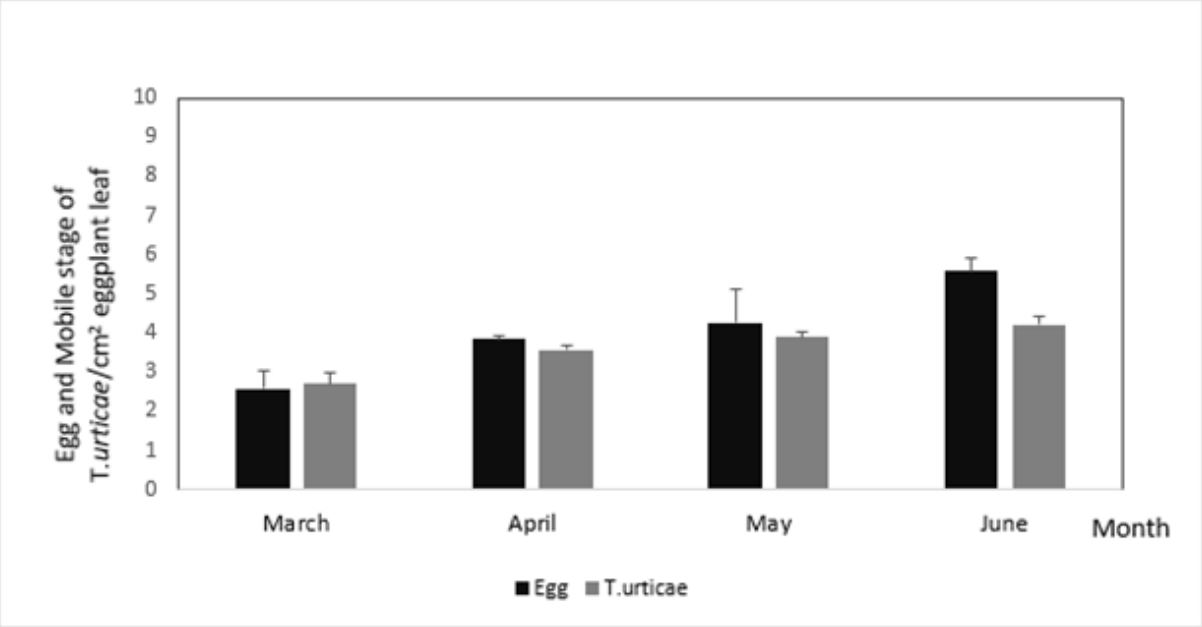

Figure 2: Number of egg and mobile stage of *T. urticae*/cm² of eggplant leaf.

Acaricidal efficacy of aqueous extract and essential oil of *Eucalyptus globulus* in laboratory

Data showing the acaricidal efficacy of *E. globulus* aqueous extract on the mobile stage of *T. urticae* on eggplant in the laboratory are given in Table 2. Four levels of concentration (10, 20, 30 and 40%) were tested for contact toxicity against *T. urticae*. The results demonstrated that the reduction rates of *T. urticae* mobile stage/cm² of eggplant leaf were 38.91±1.20, 53.36±4.11, 62.46±6.85, and 70.42±2.88 by spraying the different concentrations (10, 20, 30 and 40%) respectively. The correct efficacy of corresponding concentrations of *E. globulus* were 31.26 ±6.92, 46.50±3.83, 58.24±7.13, and 67.02±3.09 respectively. These results demonstrated that all tested concentrations of *E. globulus* aqueous extract has toxicity against *T. urticae* compared to control treatment and acaricidal efficacy increased according to concentration increases. The higher correct efficacy (67.02±3.09) was obtained with the higher concentration (40%) of *E. globulus* aqueous extract. However, the lowest correct efficacy (31.26±6.92) was registered with the lowest concentration (10%). A significant difference was demonstrated in reduction rates ($F=49.002$, $df=4$, $P\leq 0.05$) and correct efficacy ($F=15.57$, $df=3$, $P\leq 0.05$) of *T. urticae* population on eggplant leaves after foliar spray with tested concentrations of *E. globulus* aqueous extracts and treatment control.

Table 2: Reduction rate and Correct efficacy obtained across treatments with four concentrations of Eucalyptus globulus aqueous extract in laboratory.

Means followed by the same letters are not significantly different (P≤0.05; Duncan's Multiple Range Test).

Concentration (%)	Eucalyptus Globulus Aqueous Extract	
	Reduction rate (%)	Correct efficacy (%)
Control (Water)	09.73±1.18^{d}	-
10	34.33±4.98^{c}	28.08±5.29^{c}
20	47.66±4.49^{b}	41.92±5.75^{b}
30	61.33±7.03^{a}	57.07±7.94^{a}
40	67.33±4.78^{a}	63.72±5.70^{a}

For *E. globulus* essential oil, like aqueous extract, the obtained results demonstrated that all tested concentrations have a toxicity against *T. urticae* compared to the control treatment and became more effective with increase in the concentration (Table 3). The highest reduction rate (73.06±3.21%) and correct efficacy (69.98±3.79%) were registered with a concentration of 4%. While the least reduction rate (46.82±5.53%) and correct efficacy (40.81±5.69%) were obtained with concentration of 1%. A significant difference was demonstrated in reduction rates ($F=72.461$, $df=4$, $P\leq 0.05$) and correct efficacy ($F=15.19$, $df=3$, $P\leq 0.05$) of the *T. urticae* population on eggplant leaves after foliar spray of *E. globulus* essential oil at tested concentrations and treatment control.

Table 3: Reduction rate and Correct efficacy obtained across treatments with four concentrations of Eucalyptus globulus essential oil in laboratory.

Means followed by the same letters are not significantly different (P≤0.05; Duncan's Multiple Range Test).

Concentration (%)	Eucalyptus Globulus Aqueous Extract	
	Reduction rate (%)	Correct efficacy (%)
Control (Water)	10.26±1.20^{e}	-
10	38.91±6.31^{d}	31.26±6.92^{d}
20	53.36±4.11^{c}	46.50±3.83^{c}
30	62.46±6.85^{b}	58.24±7.13^{b}
40	70.42±2.88^{a}	67.02±3.09^{a}

Acaricidal efficacy of aqueous extract and essential oil of *Eucalyptus globulus* in green house

Different concentrations (10, 20, 30 and 40%) of aqueous extracts and (1, 2, 3 and 4%) of essential oil *E. globulus* were tested to evaluate their toxic effect against *T. urticae* on eggplant, and the obtained results have been summarized in Tables 4 & 5. The results demonstrated that all tested concentrations of aqueous extract and essential oil of *E. globulus* have a toxicity against *T. urticae* compared to control, and efficacy became more effective with increase in concentration. The reduction rate with tested concentrations of aqueous extract against *T. urticae* ranges between 34.33±4.98 and 67.33±4.78. The highest correct efficacy (63.72±5.70) was registered with the higher concentration (40%). However, the lowest correct efficacy (28.08±5.29) was registered with the lowest concentration (10%). A significant difference in reduction rates of *T. urticae* population (F=44.62, df=4, P≤0.05) and correct efficacy (F=12.95, df=3, P≤0.05) was demonstrated between effects of concentrations of *E. globulus* aqueous extract (Table 4). The same trends of reduction rate and correct efficacy were observed after testing the toxicity of essential oil of *E. globulus* (Table 5). The highest reduction rate (69.33±4.92%) and correct efficacy (67.66±4.94%) were registered with the highest concentration (4%). While the least reduction rate (46.33±4.64%) and correct efficacy (43.15±4.36%) were obtained with a concentration of 1%, a significant difference was demonstrated in reduction rates (F=89.50, df=4, P≤0.05) and correct efficacy (F=10.62, df=3, P≤0.05) of the *T. urticae* population on eggplant leaves after foliar spray of *E. globulus* essential oil at tested concentrations and treatment control.

Table 4: Reduction rate and Correct efficacy obtained across treatments with four concentrations of Eucalyptus globulus aqueous extract in greenhouse.

Means followed by the same letters are not significantly different (P≤0.05; Duncan's Multiple Range Test).

Concentration (%)	Eucalyptus Globulus Essential Oil	
	Reduction rate (%)	Correct efficacy (%)
Control (Water)	10.13±2.05^{e}	-
1	46.82±5.53^{d}	40.81±5.69^{d}
2	59.46±4.89^{c}	54.98±4.51^{c}
3	65.12±3.88^{b}	61.24±3.49^{b}
4	73.06±3.21^{a}	69.98±3.79^{a}

Table 5: Reduction rate and Correct efficacy obtained across treatments with four concentrations of Eucalyptus globulus essential oil in greenhouse.

Means followed by the same letters are not significantly different (P≤0.05; Duncan's Multiple Range Test).

Concentration (%)	Eucalyptus Globulus Essential Oil	
	Reduction rate (5)	Correct efficacy (%)
Control (Water)	05.46±1.42^{d}	-
1	46.33±3.29^{c}	43.15±4.36^{d}
2	56.66±4.64b^{c}	54.21±4.58^{c}
3	63.66±3.68a^{b}	61.54±4.01^{b}
4	69.33±4.92^{a}	67.66±4.94^{a}

Discussion

Biological activities of natural products, such as Eucalyptus, have been the focus of attention of many researchers, thanks to the active components it contains [27,28]. The two-spotted spider mite *(T. urticae)* is one of the most serious pests of eggplant and causes high fruit losses due to its feeding on chlorophyll and

pigments of eggplant leaves. The follow-up of *T. urticae* infestations on eggplant in greenhouses demonstrated that the first infestation was observed in February, maximum infestation rate and maximum number of eggs and mobile stages were registered in May and June. The increases in eggplant infestation rate and population (egg/mobile stage) during May and June were related to climatic factors such as temperature. Indeed, several works demonstrates that immature developmental time of *T. urticae* was 25.8 days at 17 °C, 13.75 days at 25 °C and 7.5 days at 27 °C [29-31]. The control of *T. urticae* is notoriously difficult, and conventional acaricides are the primary control method. However, the continual use of conventional acaricides induces harmful impacts in laborers in the field, the environment, and various natural enemies and pollinating insects [4]. Unfortunately, *T. urticae* is among the most resistant pests to several conventional acaricides. Therefore, it is necessary to find safer synthetic pesticide substitutes that can reduce *T. urticae* damage. Botanicals have been considered good substitutes because of their lethal or sublethal effects, such as repellent, low mammalian toxicity, act on the nervous system of the pest, causing physiological and behavioral responses [32].

The current results demonstrated that both the aqueous extract and essential oil of *Eucalyptus globulus* were potent and exhibited acaricidal activity against *T. urticae* and efficacy was obtained by having an acaricidal effect against *T. urticae*. Efficacy became more effective with an increase in the concentrations. A few reports demonstrated that the aqueous extract and essential oil of *E. globulus* can reduce the population of *T. urticae* and substitute synthetic acaricides in integrated pest management program [33,34]. The toxicity of essential oil of *E. globulus* against *T. urticae* was evaluated, and results demonstrate that at a dose of 2%, mortality is of 63.29% for adults and 63.33% for larvae [35]. Indeed, other studies confirmed the toxicity of *E. globulus* against pests [12]. The toxicity of *E. globulus* against *Dermanyssus gallinae* (another species of mite) was also demonstrated [36]. The toxicity of aqueous extracts or essential oils of *E. globulus* is due to them contain of several bioactive compounds which can exert a regulatory effect on *T. urticae* life processes. Acaricidal proprieties of *E. globulus* aqueous extract or essential oil were due to the oxygenated monoterpene 1.8-cineole which is the characteristic compound [37]. Other works demonstrated that the monoterpenoid hydrocarbons 1,8-cineol and α-pinene were the key contributors to toxicity of the essential oil of *Eucalyptus genus* [20]. These authors proved that plant essential oils may have multiple target sites that make them suitable for pest control and pests have a little chance of resistance. In this context, studies demonstrate that the essential oil of *E. globulus* against *T. urticae*, may perhaps interact with the tyramine and octopamine receptors, the GABA system, which modifies the ionic channels, with diverse enzymes like phosphatases and glutathione-S-transferase [2]. This interaction justifies the rapid action of essential oils against some pests. Additionally, the spray of essential oil raised the percentage of N, P and K in plant tissue, which reflects an improvement of plant development and plant resistance to pests [2].

Conclusion

This study indicates that *T. urticae* is among the most serious pests of eggplant, their population increases when the temperature increases, all tested concentrations of aqueous extract and essential oil isolated from *E. globulus* leaves can markedly reduce the population of *T. urticae* on eggplant, especially the highest concentrations. These results demonstrated that both the aqueous extract and essential oil of *E. globulus* can be a natural alternative to hazardous synthetic acaricides. For the application of *E. globulus*, principally the essential oil, as acaricides, further research, such as their effect on natural enemies, especially the predaceous mites (*Phytosilus persimilis* and *Amblysius swirski*), the encapsulation in the frame of controlled release techniques, is proposed to improve their stability and effectiveness.

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