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Original article

# Bioefficacy of essential oils emulsion and predatory mite, *Euseius scutalis* (Athias-Henriot) (Acari: Phytoseiidae) for the management of citrus brown mite, *Eutetranychus orientalis* (Klein) (Acari: Tetranychidae)

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ABSTRACT

The citrus brown mite, *Eutetranychus orientalis* (Klein), is a significant pest that is widely distributed in different agricultural systems. It causes significant damage to field crops and fruit trees. The most common method of control is the use of chemical acaricides. However, the extensive use of such chemicals developed resistance in addition to environmental and health hazards. Plant-derived acaricides might therefore be an environmentally friendly alternative to synthetic ones in order to increase agricultural production efficiency and to protect consumer health. Following this prospective, the present study was aimed to evaluate the toxic effects of anise (*Pimpinella anisum*), rosemary (*Rosmarinus officinalis*), eucalyptus (*Eucalyptus globulus*), clove (*Eugenia caryophyllus*), and garlic (*Allium sativum*) essential oils (EO) against the eggs and adult's females of *E. orentalis* under laboratory conditions. Additionally, *Euseius scutalis*, a predator mite, was investigated as a bioagent to control *E. orientalis* under field conditions. GC-MS was used to assess the chemical components of the tested EOs. The results showed that anethole; 9, 12-Octadecadienoic acid (Z, Z); camphor; octatriacontyl pentafluoropropiona; and eugenol, were the major components for anise, rosemary eucalyptus, garlic, and cloves, respectively. Comparably, anise has been shown to be the most effective EO against *E. orentalis* eggs and adults as it exhibited a high level of efficiency and required the lowest concentration to reach the $LC_{50}$. Additionally, the three *Euseius scutalis* release rates significantly reduced the *Eutetranychus orientalis* population. Ten individuals /seedlings in particular induced the highest reduction (84.95%). Based on our findings, we suggest using *Euseius scutalis* release and anise essential oil as alternative strategies to control of *E. orentalis*.



## 1. Introduction

The citrus brown mite, *Eutetranychus orientalis* (Klein), is the most significant phytophagous species in fields and greenhouses. It is found in tropical regions and threatens a variety of economically important horticultural and ornamental plants (Kamali et al., 2004). In developing countries, chemical control is remaining the most utilized tactic in pest management (Aktar et al., 2009). However, unlimited use of these chemicals against pests has massive effect on the environment, populations of natural enemies, soil and human health (Kumar et al., 2010). In addition, it leads to disrupt the biotic balance, increasing secondary pest outbreaks, pest resistance and resurgence. In order to avoid the downsides of synthetic pesticides, research has been conducted in recent years to identify eco-friendly, economical, and effective alternative approaches, including the use of plants derived pesticides derived, microorganisms, and natural enemies (Miresmailli and Isman, 2006). In this context, numerous studies have been conducted on the use of essential oils and their active ingredients as sustainable methods for the management of phytophagous mites, (Miresmailli and Isman, 2006; Han et al., 2010; Elhalawany and Dewidar, 2017),

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however *Eutetranychus orientalis* was the subject of few of these studies (Elhalawany et al., 2019). Many essential oils from rosemary, garlic, eucalyptus jojoba were proven to have insecticidal properties and generally much safe to the environment and humans (Afify et al., 2012; Hussein et al., 2013). Also, there were many experiments on the use of biocontrol agents such as phytoseiid mite against many tetranychide mite infesting different crops (El-Ghobashy, 2012; Ebrahim, 2016). *Euseius scutalis* (Athias-Henriot) is considered one of the most pollen-feeding phytoseiid mite species. It is widely distributed in cultivated and uncultivated plants (including citrus crops). It feeds and survives on plant tissues without causing economic damage (Adar et al., 2012). In addition, it showed a large scale of environmental tolerances (temperature and humidity). Several previous studies have shown that *E. scutalis* can develop and reproduce feeding on both *T. urticae* and *E. orientalis* (Stathakis et al., 2021). Hence, it could be considered as one of the most suitable and effective bio-control agent for integrated pest management programs against plant feeding mites. The present work was therefore aimed on one hand to evaluate the chemical composition and the effect of some plant essential oils on the eggs and adult females of pest mite *E. orientalis*, and on other hand control of *E. orientalis* on citrus seedling by releasing of predatory mite *E. scutalis*.

## 2. Materials and methods

### *2.1. 1. Preparation of essential oils emulsion*

Essential oils of anise (*Pimpinella anisum*), rosemary *(Rosmarinus officinalis*), eucalyptus (*Eucalyptus globulus*), clove (*Eugenia caryophyllus*), and garlic (*Allium sativum*) were obtained from El-Gabry medical herbs pharmacy in Giza, Egypt. To prepare different concentrations of essential oil emulsions; two drops of 0.05 % Tween-80 as an emulsifier agent were added to the oils and the known volume was completed with distal water then the mixture was stirred for 30 min. Tween-80 (0.05 %) with distal water was used as a control treatment (Koschier et al., 2002).

### *2.2. Chemical composition*

The essential oils chemical constituents were analyzed using GC–MS/FID (Gas chromatography coupled to mass spectrometry/ flame ionization detector) at the laboratory of environmental and food pollutant analysis, Faculty of Agriculture Fayoum University Egypt as recommended by Adams (1995). The identification of the compounds was based on the comparison of their relative retention indexes and mass spectra with those provided in mass spectra library NIST (2007), literature and instrument's databases (Adams Book 07, Nist 98, Xcalibur).

### *2.3. Source of E. orentalis prey mites*

The citrus brown mites, *E. orientalis* were collected from leaves of castor bean (*Ricinus communis*). The adult females of *E. orientalis* were transferred to castor bean disks, which were placed on moistened cotton pads resting on sponge layer in the foam dish (15 × 20 cm). The colonies were maintained at room temperature. The leaves were examined and replaced with fresh ones when becoming yellow and crowding of mites. In addition, water was added to prevent the mites from escaping.

### *2.4. Adult spraying test*

Four concentrations (1 %, 2 %, 3 % and 4 %) of each essential oil were used in five replicates per concentration to calculate the $LC_{50}$ and $LC_{90}$ according to the method proposed by Mailloux and Morrison (1962). Briefly, discs of mulberry leaves (3 cm in diameter) were placed in a foam dish (15 × 20 cm) which was lined with a layer of sponge and cotton pads soaked in water. Ten adult females of mites were transferred to each disc (10 adult female/leaf discs) in five replicates per treatment and repeated in each concentration. 50 adult females in five replicates (10 adult females per leaf disc) were act as control treatment. Each disc of mulberry leaves was sprayed by a glass atomizer in each concentration for each essential oil while the control was sprayed by distal water with two drops of 0.05 % of Tween-80. At 24, 48, and 72 h post treatments, the dead and live adult female mites were counted using a binocular microscope and a fine camel hairbrush. The mean mortality rate, $LC_{50}$ and $LC_{90}$ were calculated according to Abbott's formula (1925).

### *2.5. Egg hatchability test*

Leaf discs of mulberry were used as substrate. For each treatment, five leaf discs were used, and the female mites were transferred to each disc and given 24 h to lay eggs before being removed. Subsequently, five replicates leaf discs, 20 eggs per each replicate were used (100 eggs in five replicate)/ per each concentration (4 %, 3 %, 2 %, and 1 %). Eggs were sprayed by a glass atomizer in each concentration for each essential oil emulsion while, the control was sprayed by distal water with two drops of 0.05 % of Tween-80. Eggs were maintained under laboratory conditions for seven days till hatching. The numbers of hatched and non-hatched eggs were recorded. Corrected mortality was calculated using Abbott's formula (1925) whereas, $LC_{50}$, $LC_{90}$ and slope values were estimated according to Finney (1971).

### *2.6. Euseius scutalis (A-H) releasing*

This experiment was carried out under the field condition to estimate the effect of the predatory mite *Euseius scutalis* against *E. orientalis* at Fayoum Governorate on mandarin seedlings (with age of two years old) with three levels of release (3, 5 and 10 predators/ seedling) from May to October 2021. In brief, forty–mandarin seedlings infested with the mite pest *Eutetranychus orientalis* were prepared and divided into four groups of 10 seedlings each as three levels of release and control. Make mass rearing of predatory mite *Euseius scutalis* using bean plant *phaseolus vulgaris* as a host of pry and predator to obtain a lot of numbers of this mite to use in releasing. The leaf of bean plant with the predatory mite was collected and packed in plastic bags and transferred to the field experiment of seedlings and then distributed on the seedlings with three levels 3, 5 and 10 individuals of predators per seedling. The next samples were collected every 10 days after release the predators and the numbers of motile stage prey and predators were recorded till the end of the experiment. The percent of reduction was calculated the using the equation of Henderson and Tilton (1955) as following:

$$\text{Reduction} = 1 - \frac{\text{N.of motile stage of mite before treatment in control} \times \text{N.of motile stage of mite after treatment}}{\text{N.of motale stage of mite after treatment in control} \times \text{N.of motale stage of mite before treatment}} \times 100$$

One-way analysis of variance (ANOVA) and the Least Significant Difference (LSD) have been utilized to analyze the data using SAS Program version 9.1 (SAS Institute, 2003). The data were subjected to probit analysis (Finney 1971) to determine lethal concentration of essential oil needed to kill 50 % and 90 % of *Eutetranychus orientalis (*$LC_{50}$ and $LC_{90}$), slope, and toxicity index values using Ldp line software (http://www.ehabsoft.com/ldpline/). The reduction percent of *E orientalis* was calculated according to the equation proposed by Henderson and Tilton (1955) to select the suitable level of release, which could be recommended. In full test *F, P* and LSD values were used for comparing the means of reduction ($\alpha \leq 0.05$).

## 3. Results

### 3.1. Chemical components of essential oils

The percentages of identified components and their retention time (R.T.) were given in Table 1. The major constituents of the oil are anethole (26.175 %); 9, 12-Octadecadienoic acid (Z, Z) (30.03 %); octatriacontyl pentafluoropropiona (11.522 %), camphor (14.262 %); and eugenol (32.823 %) in anise, rosemary, garlic, eucalyptus and cloves, respectively.

### 3.2. Efficacy of essential oils emulsion against Eutetranychus orientalis Klein

#### 3.2.1. Adult spraying test

The acaricidal activities of the five EOs against adult females of *E. orientalis* after 72 hrs are presented in Table 2. All treatments induced significant effects on *E. orientalis* when compared to the control. The anise EO showed the highest significant mortality (77.7 %) at a concentration of 4 % ($P \leq 0.05$) as the other four EO induced mortality below 70 % at the same concentration. Also, $LC_{50}$ of anise attained at a concentration of 1.27 % which comparably lowers than the concentrations required to the $LC_{50}$ the other four EO and this evidenced that the strongest acaricidal activity was for the anise EO (Table 4 and Fig. 1).

#### 3.2.2. Egg hatchability test

As shown in Table 3, all the tested EO showed pronounced ovicidial activity in comparison with the control. The rosemary EO showed maximum inhibition of the egg hatchability (93.33 %) followed by the anise EO (84 %) while the lowest inhibition of egg hatchability was induced by eucalyptus and garlic showed (73.33 %). $LC_{50}$ attained at a concentration of 0.593 %, and 0.597 % for rosemary and anise, respectively. The slopes for rosemary, anise, cloves, eucalyptus and garlic are 1.67, 1.16, 0.99, 0.91 and 0.99, respectively. Thus, it is shown that rosemary essential oil became more effective with increase in the concentrations (Table 5 and Fig. 1).

### 3.3. Euseius scutalis (Athias-Henriot) releasing

On the control seedlings leaves, the average number of *E. orientalis* varied throughout time. At the beginning of the trial, means were 100 individuals/20 leaves, and they peaked between July 30 and September 28 with 330 mites/20 leaves during the 2021 season. (Fig. 2). On May 31 (at the beginning of the experiment), the means for 3, 5, and 10 levels of release were 90, 65, and 100, respectively. On October 8, the means for the three levels decline to their lowest densities of 13, 7, and 11. The mean numbers of *E. orientalis* in the treated seedlings changed throughout time. At all inspection dates, their numbers were always lower than that of the control. Each treatment was statistically different from the control.

The maximum reduction in pest mites was observed for the first, second, and third levels of release on September 28 with percent of 94.27 %, 96.27, and 98.48 %, respectively, and this associated with highest numbers of *E. scutalis* (15, 17, and 25 predators per 20 leaves)/330 individuals in the released and unreleased one, respectively (Fig. 2).

In all assessments, the highest mean percentage of reduction (84.95 %) in *Eutetranychus orientalis* was attained at a release rate of 10 predatory/seedlings meanwhile, the lowest one (76.10 %) was occurred at a release rate of 3 predators/ seedling. This decrease did not differ significantly between the release rate of 3 and 5 seedlings/predators (Table 6). *Euseius scutalis* was observed on mandarin seedlings where it was released but not in the control one after the release. In the 3, 5 and 10 release rates, the mean number of predators was significantly different ($p \leq 0.001$) (Table 7).

## 4. Discussion

Using conventional chemical acaricides is the most common method to control the citrus brown mite, E. orientalis (Afify et al., 2012). However, the indiscriminate use of such chemicals induced great problems to the environment in addition to development of resistance to many acaricides (Heikal et al., 2019). To avoid these problems, alternative methods for the control of E. orientalis are being evaluated, including the use of essential oils that proved to be promising agents for the control of agricultural pests (Heikal et al., 2019). Following this perspective, in the present study anise, rosemary, garlic, eucalyptus and cloves oils were tested for their acaricidal effects against E. orientalis. Analysis of these oils revealed that the major constituents were anethole in anise, 9,12-octadecadienoic acid (Z,Z) in rosemary, octatriacontyl pentafluoropropiona in garlic, camphor in eucalyptus and eugenol in cloves similar to these reported by Kuś and Jerković, (2021) and Elazab et al. (2022).

In the present study, anise EO showed the most significant acaricidal efficacy with a mortality rate reaching 77.7 % after 72 h while the cloves EO showed the lowest efficacy. This may be attributed to the presence of anethole as major oils constituents. Anethole was proven to have direct effect on the nervous system due to its inhibitory activity against the AChE enzyme by binding with the active site of AChE by the hydrophobic interactions (El-Sayed et al., 2022). In agreement with our result, Vinicius et al. 2018 found that anise was the most promising extract against *T. urticae* with a mortality rate above 75 %. Also, El-Sayed et al. (2022) confirmed the acaricide activity of anise against *T. urticae*. In addition, anethole phenylpropanoid; the most important component of anise, was very efficient in controlling *Aedes aegypti* and *Culex pipiens* (Knio et al., 2008). The obtained $LC_{50}$ for anise also reflects its high acaricidal activity against E. orientalis.

Regarding egg hatchability, the highest mean number of unhatched eggs was associated with rosemary (93.3 %), followed by anise (84 %), while eucalyptus and garlic showed the lowest mean number (73.33 %). Similarly, Menaceur et al. (2016) found significant reduction in the hatchability of *Callosobruchus maculatus* eggs after the treatment with rosemary EO. The difference in the amount of phenolic metabolites these oils contained may account for the variability in their impact on the eggs' capacity to hatch.

In this study, fatty acids and their esters were the main components of rosemary EO based on GC/MS analysis. Several studies have been conducted to correlate insecticidal activity with the chemical structure of fatty acids and their esters. According to Chen and Dai (2015), 9-octadecenoic acid, ethyl ester, a structurally-similar compound, shows the potential to be devel-

**Table 1**
GC–MS analysis report for essential oils.

| **Anise** | | | **Rosemary** | | | | **Eucalyptus** | |
|---|---|---|---|---|---|---|---|---|
| **R.T.** | Name of compound | % | R.T. | Name of compound | **%** | **RT** | Name of compound | **%** |
| 6.829 | α-Pinene | 0.011 | 6.836 | α-Pinene | 1.041 | 9.145 | D-Limonene | 0.002 |
| 7.866 | β-Pinene | 0.028 | 7.192 | Camphene | 2. 324 | 10.659 | L-Fenchone | 2.008 |
| 9.043 | p-Cymene | 0.042 | 7.872 | β-Pinene | 0.137 | 10.952 | Terpin diacetate | 2.010 |
| 12.224 | Citronellal | 0.519 | 9.049 | p-Cymene | 1.103 | 11.079 | camphene | 0.040 |
| 12.536 | l-Menthone | 0.122 | 9.150 | D-Limonene | 1.103 | 12.122 | Camphor | 14.262 |
| 12.345 | Isopulegol | 0.390 | 9.219 | Camphor | 14.595 | 12.281 | l-Menthone | 0.050 |
| 13.352 | Estragole | 0.408 | 12.05 | Eucalyptol | 12.497 | 12.377 | Isoborneol | 0.008 |
| 15.036 | 4-methoxy- Benzaldehyde, | 7.413 | 12.60 | propan-2-ol | 0.260 | 17.034 | Eugenol | 0.019 |
| 15.666 | Anethole | 26.175 | 13.18 | α-Terpineol | 1.397 | 24.916 | 2-propyl tetradecyl ester | 0.050 |
| 17.740 | 2-Propanone | 2.939 | 15.40 | Bornyl acetate | 1.063 | 31.774 | Ethanol | 4.761 |
| 19.686 | 1,5-dimethyl-4-hexenyl | 1.086 | 17.40 | Eugenol | 2.210 | 32.538 | Tetrapentacontane | 2.278 |
| 20.208 | β -Bisabolene | 0.339 | 21.78 | Caryophyllene oxide | 0.456 | 33.028 | Eicosane | 7.140 |
| 20.539 | *trans*-calamenene | 0.202 | 31.40 | 9,12-Octadecadienoic acid(Z, Z) | 30.03034wq | 33.218 | 1,8- Cineole | 3.701 |
| 21.805 | 2(1H)-Pyridinone | 7.201 | 31.90 | 9-Eicosyne | 7.205 | 33.333 | Octadecane | 1.256 |
| 23.122 | alpha.-ethyl-4-methoxy | 2.310 | 36.241 | 1-palmitate | 2.005 | 33.568 | Docosane | 5.076 |
| 23.593 | 2-Butanone | 1.269 | 38.411 | 9,17-Octadecadienal | 1.231 | 33.766 | Menthol | 1.525 |
| 24.229 | 4-Methoxyphenyl | 2.491 | 38.567 | 3-hydroxypropyl ester | 21.099 | 34.332 | Octatriacontyl pentafluoropropiona | 4.261 |
| 32.798 | 4-Hexanoylresorcinol | 1.337 | 38.826 | 2,3-hydroxypropyl ester | 6.273 | 34.701 | 1-Chloroeicosane | 1. 542 |
| 33.327 | 2-Heptanone | 1.239 | 39.063 | 2-Chloroethyl linoleate | 8.467 | 34.917 | Tetracosane | 2.311 |
| 35.509 | Benzamide | 5.353 | 41.266 | 2-Butanone | 0.368 | 35.280 | Heneicosane | 9.813 |
| 35.763 | Acetamide | 2.275 | | | | 35.598 | 1-Eicosene | 3. 811 |
| 35.865 | 4,4′-Dimethoxychalcone | 5.079 | | | | 39.231 | 3-oxo-1-butenyl | 0.241 |
| 36.590 | 3-Methyl-5-nitrosotropone | 4.015 | | | | | | |
| 36.940 | Ethanone | 9.658 | | | | | | |
| 37.920 | 1-Propanone | 3. 750 | | | | | | |
| | **Garlic** | | | | | | **Clove** | |
| 9.056 | o-Cymene | 0.006 | | | | 16.073 | 2,4-Decadienal | 3.127 |
| 9.151 | D-Limonene | 0.005 | | | | 17.091 | Eugenol | 32.823 |
| 12.071 | Camphor | 0.010 | | | | 34.268 | Pentadecane | 1.017 |
| 12.555 | cis- l-Menthone | 0.005 | | | | 34.357 | Octadecane | 1.078 |
| 12.866 | Terpinen-4-ol | 0.002 | | | | 34.618 | Tetracosane | 1.439 |
| 17.046 | Eugenol | 0.013 | | | | 34.694 | Eicosane | 6.028 |
| 20.577 | p-Menth-8(10)-en-9-ol | 0.031 | | | | 34.840 | Heneicosane | 11.329 |
| 23.415 | 1-Hydroxypyrene | 0.242 | | | | 35.203 | Tricosane | 3.619 |
| 24.585 | 2-propyl tridecyl ester | 1.007 | | | | 36.148 | Pentacosane | 2.671 |
| 25.768 | Hexadecane | 1.871 | | | | 36.291 | Tetracosane | 1.017 |
| 27.359 | 1-Decanol | 2.134 | | | | 36.478 | Hexacosane | 1.450 |
| 30.381 | 1-Eicosene | 2.431 | | | | 40.108 | Squalene | 0.366 |
| 31.876 | Ethanol | 2.961 | | | | 40.673 | Acetophenone | 11.160 |
| 32.519 | Octadecane | 1.988 | | | | 40.997 | 3-Methyl-5-nitrosotropone | 1.967 |
| 32.805 | Tetrapentacontane | 0.616 | | | | 41.456 | Benzothiophene-3-carbohydrazide | 9.917 |
| 33.536 | Docosane | 1.658 | | | | 41.839 | 6H-Purin-6-one | 1.011 |
| 34.122 | Octatriacontyl pentafluoropropiona | 11.522 | | | | 42.055 | Silane | 0.289 |
| 34.901 | Eicosane | 9.751 | | | | | | |
| 35.261 | Heneicosane | 8.575 | | | | | | |
| 39.523 | Triacontyl acetate | 0.621 | | | | | | |

oped as new natural acaricide for controlling carmine spider mites (*Tetranychus cinnabarinus*). Also, Jiang et al. (2018) identified 9, 12-octadecadienoic acid (20.9 %), as a major constituent in the extract of *Robinia pseudacacia* and it exhibited strong insecticidal activities against cotton aphid and cabbage aphid. In addition, 9, 12-octadecadienoic acid was the major fatty acid in the *Jatropha curcas*

**Table 2**
Mortality percent (mean ± SEM) of the essential oil emulsion against adult females of *Eutetranychus orientalis* after 72 h.

| **Conc** | **Anise** | **rosemary** | **Garlic** | **Eucalyptus** | **Cloves** |
|---|---|---|---|---|---|
| **Mortality (mean ± SEM)** | | | | | |
| **4 %** | 77.7 ± 1.2$^{a}$ | 68.88 ± 2.31$^{a}$ | 66.65 ± 1.15$^{a}$ | 66.66 ± 1.73$^{a}$ | 64.44 ± 2.56$^{a}$ |
| **3 %** | 73.33 ± 1.73$^{b}$ | 60.00 ± 1.2$^{b}$ | 53.33 ± 2.5$^{b}$ | 57.77 ± 2.31$^{b}$ | 55.55 ± 2.89$^{b}$ |
| **2 %** | 68.8 ± 1.2$^{c}$ | 44.44 ± 2.31$^{c}$ | 51.11 ± 0.64$^{b}$ | 55.55 ± 1.15$^{b}$ | 42.22 ± 1.28$^{c}$ |
| **1 %** | 40.00 ± 1.15$^{d}$ | 35.55 ± 0.58$^{d}$ | 33.33 ± 1.34$^{c}$ | 33.33 ± 1.2$^{c}$ | 33.33 ± 1.35$^{d}$ |
| **Control(0.05 % of Tween-80)** | 9.33 ± 0.67$^{e}$ | 9.33 ± 0.67$^{e}$ | 9.33 ± 0.67$^{d}$ | 9.33 ± 0.67$^{d}$ | 9.33 ± 0.67e |
| **F value** | 562.68 | 290.45 | 181.52 | 234.91 | 121.28 |
| **P value** | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

Data are means ± standard error means (SEM). Superscript of the same letter in cells of the same column is non-significant. Superscript of different letters in cells of the same column is significant ($P \leq 0.05$).

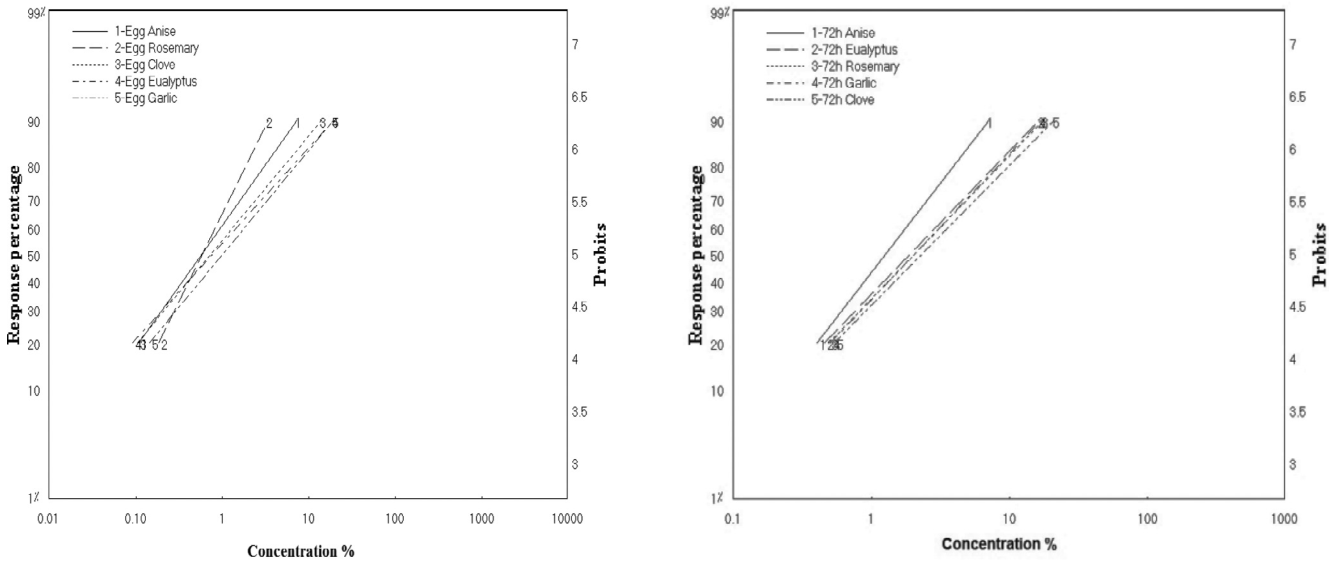


**Fig. 1.** Ldp-lines of toxicity effect of anise, rosemary, eucalyptus, clove and garlic essential oil on eggs adult females and of *Eutetranychus orientalis*.

**Table 3**
Ovicidal effect of the essential oil emulsion against eggs of *Eutetranychus orientalis* (Klein) after 7 days.

| Conc | Anise | rosemary | Garlic | Eucalyptus | Cloves |
|---|---|---|---|---|---|
| **Un-hatched egg (Mean ± SEM)** | | | | | |
| 4 % | 84.00 ± 2.31[a] | 93.33 ± 1.73[a] | 73.33 ± 1.73[a] | 73.33 ± 2.89[a] | 77.33 ± 2.08[a] |
| 3 % | 77.33 ± 1.73[a,b] | 88.00 ± 2.89[a] | 65.33 ± 2.08[b] | 70.66 ± 2.08[a] | 70.66 ± 2.89[a] |
| 2 % | 74.66 ± 2.89[b] | 77.33 ± 4.04[b] | 62.66 ± 2.88[b] | 65.33 ± 2.88[a] | 68.00 ± 4.04[a] |
| 1 % | 60.00 ± 2.89[c] | 66.66 ± 1.73[c] | 49.33 ± 1.15[c] | 53.33 ± 1.73[b] | 54.66 ± 2.31[b] |
| Control (0.05 % of tween 80) | 25.00 ± 2.88[d] | 25.00 ± 2.88[d] | 25.00 ± 2.88[d] | 25.00 ± 2.88[c] | 25.00 ± 2.88[c] |
| F value | 83.5 | 77.04 | 68.04 | 62.85 | 36.2 |
| P value | <0.001 | <0.001 | <0.001 | <0.001 | **<0.001** |

Data are means ± standard error means (SEM). Superscript of the same letter in cells of the same column is non-significant. Superscript of different letters in cells of the same column is significant ($P \leq 0.05$).

**Table 4**
$LC_{50}$, and $L_{C90}$ values with their confidence limits for essential oils on adults of *Eutetranychus orientalis* after 72 h.

| Essential oil | $LC_{50}$ | Lower limit% | Upper limit% | Toxicity index | Slope | $LC_{90}$ |
|---|---|---|---|---|---|---|
| **Anise** | 1.27 | 0.72 | 1.67 | 100 | 1.70 | 7.19 |
| **Rosemary** | 1.91 | 1.36 | 2.88 | 99.33 | 1.36 | 8.03 |
| **Cloves** | 2.33 | 1.62 | 3.50 | 79.49 | 1.32 | 21.55 |
| **Eucalyptus** | 2.06 | 1.20 | 2.63 | 75.83 | 1.36 | 16.79 |
| **Garlic** | 2.06 | 1.38 | 2.86 | 57.62 | 1.38 | 17.38 |

$LC_{50}$: Lethal concentration of essential oil required to kill 50% of *Eutetranychus orientalis*.
$LC_{90}$: Lethal concentration of essential oil required to kill 90% of *Eutetranychus orientalis*.
Index compared with anise.

**Table 5**
$LC_{50}$, and $LC_{90}$ values with their confidence limits for essential oils on eggs of *Eutetranychus orientalis* (Klein) after 7 days.

| Essential oils | $LC_{50}$ | Lower limit% | Upper limit% | toxicity index | slope | $LC_{90}$ |
|---|---|---|---|---|---|---|
| **Anise** | 0.593 | 0.156 | 0.954 | 100 | 1.16 | 7.537 |
| **Rosemary** | 0.597 | 0.276 | 0.864 | 99.33 | 1.67 | 3. 476 |
| **Coves** | 0.746 | 0.173 | 1.174 | 79. 49 | 0.99 | 14.563 |
| **Eucalyptus** | 0.782 | 0.143 | 1.247 | 75.83 | 0.91 | 20.049 |
| **Garlic** | 1.029 | 0.362 | 1.478 | 57.62 | 0.99 | 20.131 |

Index compared with Egg anise.
$LC_{50}$: Lethal concentration of essential oil required to kill 50% of *Eutetranychus orientalis*.
$LC_{90}$: Lethal concentration of essential oil required to kill 90% of *Eutetranychus orientalis*.

oil that exhibited anti-oviposition and ovicidal effects on cowpea bruchid, *Callosobrochus maculatus* (Adebowale and Adedire, 2006). Furthermore, Satyan et al. (2009) found that the treatment with fatty acid mixtures can inhibit the growth of *Helicoverpa armigera* larvae. The acaricidal effects of these fatty acids were attributed to their interfering with the nematode cuticle or hypodermis

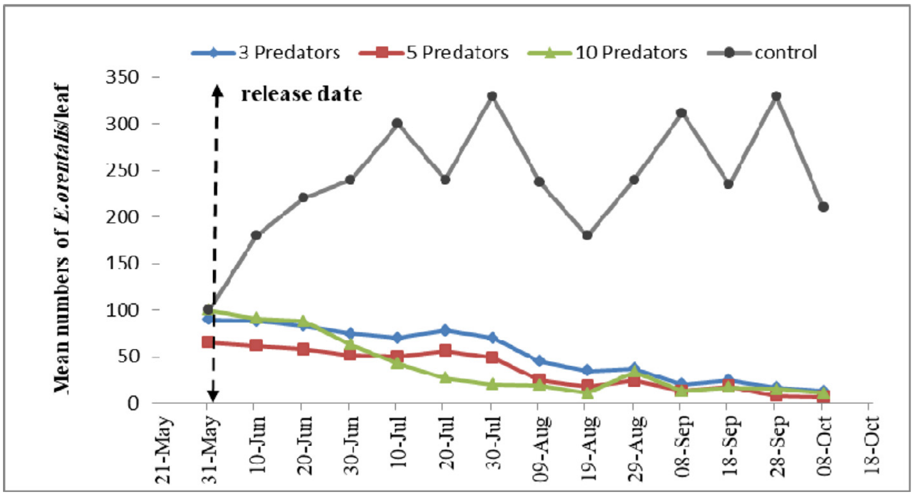


**Fig. 2.** Mean number of *Eutetranychus orientalis* after 3, 5 and 10 release of *Euseius scutalis* on seedling of mandarin in season 2021.

**Table 6**
Reduction percent of Eutetranychus *orientalis* after 3, 5 and 10 release of *Euseius scutalis* on seedling of mandarin in season 2021.

| Sampling date | Reduction % of *E. orientalis*/ 20 leaves after release of *E. scutalis* | | |
|---|---|---|---|
| | 3 predators/ **mandarin** seeding | 5 predators/ **mandarin** seeding | 10 predators/ **mandarin** seeding |
| **10-Jun** | 45.67 | 47 | 49.46 |
| **20-Jun** | 58.03 | 59.44 | 60 |
| **30-Jun** | 65.27 | 66.66 | 73.75 |
| **10-Jul** | 74.07 | 74.35 | 85.66 |
| **20-Jul** | 63.88 | 64.1 | 88.75 |
| **30-Jul** | 76.43 | 77.15 | 93.93 |
| **09-Aug** | 78.9 | 83.77 | 91.98 |
| **19-Aug** | 75.69 | 82.69 | 93.12 |
| **29-Aug** | 82.87 | 84.61 | 85.83 |
| **08-Sep** | 92.87 | 93.58 | 95.83 |
| **18-Sep** | 88.17 | 88.21 | 92.76 |
| **28-Sep** | 94.27 | 96.27 | 98.48 |
| **08-Oct** | 93.12 | 94.06 | 94.76 |
| **Mean** | 76.10[a] | 77.84[a] | 84.95[b] |
| **F-value** | 15.38 | | |
| **P-value** | 0.0001 | | |
| **L.S.D at 0.05** | 3.48 | | |

Superscript of the same letter is non-significant. Superscript of different letters is significant ($P \leq 0.05$).

via a detergent/solubilization effect or through their direct interaction with the lipophilic regions of target plasma membranes (Davis et al., 1997).

Our results proved the impact of the predatory mite, *E. scutalis* in the management of the citrus brown mite, *E. orientalis* using three rates of release during 5 months as its release induced a significant reduction in the number of *E. orientalis* during the full growing season. Similarly, Metwally et al. (2010) found that the release of *E. scutalis* reduced the percent of *T. urticae* on apple seedling. Also, Heikal et al. 2019 found that the numbers of *E. orientalis* on the navel orange trees were affected by releasing of *E. scutalis* as a biological control agent.

Statistically, there were significant differences between the three releasing rates. The rate of 10 predatory mites /seedling was the most appropriate one with an average reduction of 84.95 %. There was no significant difference between the rates of 3 and 5 predatory mites/seedling. This study did not record any kind of damage to the seedling, but concerned the impact of different release rates of *Euseius scutalis* on populations of *E. orientalis* infesting mandarin seedlings only. Similarly to EL-Halawany et al. (1993), the release of *Euseius scutalis* caused a high percent reduction (89.5 %) in *Brevipalpus californicus* on the Baladi orange tree. Also, El-Ghobashy (2012) evaluated the efficacy of the predatory mite, *Euseius scutalis* against citrus brown mite, *Eutetranychus*

**Table 7**
Number of *Euseius scutalis* per 20 leaves for each sampling date on seedling of mandarin in season 2021.

| Sampling date | Number of *E. scutalis* / 20 leaves | | | | |
|---|---|---|---|---|---|
| | 3 predators/ **mandarin** seeding | 5 predators/ **mandarin** seeding | 10 predators/ **mandarin** seeding | Mean Temp. | Mean RH. |
| **10-Jun** | 3 | 6 | 5 | 33.51 | 30.67 |
| **20-Jun** | 4 | 10 | 10 | 31.43 | 34.34 |
| **30-Jun** | 7 | 11 | 13 | 34.18 | 45.47 |
| **10-Jul** | 7 | 12 | 15 | 32.89 | 51.37 |
| **20-Jul** | 5 | 9 | 11 | 36.01 | 26.99 |
| **30-Jul** | 6 | 11 | 12 | 32.62 | 44.57 |
| **09-Aug** | 7 | 15 | 17 | 33.35 | 49.69 |
| **19-Aug** | 4 | 9 | 14 | 32.39 | 49.16 |
| **29-Aug** | 11 | 7 | 10 | 33.74 | 47.15 |
| **08-Sep** | 15 | 15 | 22 | 34.29 | 40.97 |
| **18-Sep** | 10 | 12 | 17 | 27.61 | 49.86 |
| **28-Sep** | 12 | 17 | 25 | 27.35 | 55.13 |
| **08-Oct** | 9 | 10 | 12 | 27.33 | 51.58 |
| **Mean** | 7.69[a] | 11.07[b] | 14.07[c] | | |
| **F-value** | 26.97 | | | | |
| **P-value** | 0.0001 | | | | |
| **L.S.D at 0.05** | 1.97 | | | | |

Superscript of the same letter is non-significant. Superscript of different letters is significant ($P \leq 0.05$).

*orientalis* on the citrus trees and they found an 82.88 % reduction in prey population density.

At all release rates, there was a reduction in the density of *E. orientalis* after release. The lowest reduction rate of the *E. orientalis* was at the density of 3 predatory mites /seedling. Thus, the higher release rates (higher densities of predators) resulted in greater reductions of the *E. orientalis*.

In conclusion, the results obtained in the present study indicated that *Euseius scutalis* is an important factor for the population reduction of *E. orientalis* on mandarin seedlings under field conditions. This predatory mite could therefore be suitable as a biological control agent for this pest with the release rate of 10 predators per seedling as it introduced the highest percent of reduction in the mite pest.

Further studies in the combination of predatory mites and essential oil active ingredient will represent a more efficient biological control strategy for *E. orientalis*.

## CRediT authorship contribution statement

**Mariam Mohamed Ibrahim Ata:** Methodology, Investigation. **Gamal Zidan El-Shahawy:** Supervision, Methodology. **Magdy Hussein Fawzy:** Supervision. **Abdel-Azeem S. Abdel-Baki:** Supervision, Methodology. **Saleh Al-Quraishyd:** Project administration, Funding acquisition. **Ahmed O. Hassan:** Supervision, Methodology. **Heba Abdel-Tawab:** Methodology, Investigation.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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