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Original article

Toxicity of *Rosmarinus Officinalis* Essential Oil to the Pest *Tetranychus Urticae* Koch (Acari: Tetranychidae) and its Predator *Phytoseiulus Persimilis* Athias-Henroit (Acari: *Phytoseiidae*)

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Abstract

This study aims to determine the chemical composition of *Rosmarinus officinalis* (Lamiales: Lamiaceae) and its acaricidal effect on the pest mite *Tetranychus urticae* Koch (Acari: Tetranychidae). and its predator *Phytoseiulus persimilis* Athias-Henroit (Acari: Phytoseiidae). The analysis of the chemical composition of the essential oil extracted was carried out on gas chromatography coupled with mass spectrometry. Three major compounds found in the essential of *R. officinalis* were α-pinene (32.64%), β-Humulene (8.71%), and Camphene (5.95%). The toxicity of *R. officinalis* oil was performed using inhalation bioassay on both *T. urticae* and its predator *P. persimilis*. The results obtained showed high toxicity on the mite than to its predator with a mortality rate of 80% and 16.11% respectively at the dose of 4%. The fertility of females, the hatching of eggs, and the emergence of adults in *T. urticae* were also studied using contact bioassay where a total reduction of all three biological parameters was recorded at the dose of 4%. Besides, the application of this oil will also allow the conservation of *P. persimilis*. Altogether, the combination of toxicity on *T. urticae* and preservation of its predator gives *R. officinalis* essential oil a propitious potential for the control of the pest mite *T. urticae* and maintenance of environmental balance.

Keywords: Essential oil, Toxicity, *Rosmarinus officinalis, Tetranychus urticae, Phytoseiulus persimilis*.

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INTRODUCTION

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae), is responsible for significant yield losses in many horticultural, ornamental and agricultural crops worldwide (Hoy, 2011; Vacante, 2016). This mite damages more than 1500 plants of which more than 150 are economically important (Migeon & Dorkeld, 2016). *T.urticae* causes necrotic stain formation as the result of leaf damage, and in high populations it causes drying and leaf fall (Brandenburg & Kennedy, 1987). In Morocco, TSSM is one of the most redoubtable pests for many crops. Strawberries, the dominant crop in the Loukkos perimeter (northern Morocco), are considerably affected by this pest. The control of spider mites, as well as other strawberry pests, is based on the systematic and widespread use of many plant protection products. On the other hand, this excessive use of synthetic pesticides has led to an increase in the populations of the pest mite *T. urticae* due to the development of resistance to these products (Tirello et *al*., 2012 ; Rahayu & Mairawita, 2018). Moreover, it resulted in the elimination of many natural enemies of these mites by reducing the production of eggs and their reproductive capacity which resulted in a reduction in the predation pressure on the mite thus affecting biodiversity (Prischmann et *al*., 2005; Kumral et *al*, 2009). Among these natural enemies, the mite *Phytoseiulus persimilis* Athias-Henroit (Acari: Phytoseiidae), a specific predator for TSSM among other Phytoseiidae which are rather generalists, has been well affected by the unwise use of synthetic pesticides.

Therefore, public demand for research solutions based on biological control has become the main driving force for the use of biopesticides while maintaining natural balances. In this regard, several studies have reported the toxic potential of essential oils (EOs) on different species of the Tetranychidae family (Pavela et *al*., 2016). These are effective products that are comparable or even better than synthetic products since they are pest specific and therefore harmless to non-target organisms needs a reference. These biopesticides work by contact and inhalation and have different modes of action. Essential oils seem to have no specific cellular targets because of the diversity of their constituents (Houël, 2011). This characteristic would make it possible in particular to limit the rate of development of resistance. Indeed, some authors have described an absence of resistance or sometimes a low resistance to these products (Bakkali et *al*., 2008 ; Attia et *al*., 2015). For this reason, EOs are considered to be the green pesticides of the future. However, the use of these oils in the field to fight against pests must guarantee the conservation of predators, which will maximize integrated pest management (Shi & Feng, 2004 ; Roobakkumar et *al*., 2010) and that will be more appropriate than the use of synthetic pesticides (Erdogan et *al*., 2012). In this sense, previous studies have mentioned that pests are generally more sensitive to EOs than their predators (Hany & El-Zahi, 2011; Elhalawany & Dewidar, 2017). Furthermore, an effective pest control program must target the toxicity of the products used on the target and also its various biological parameters. In this context, essential oils and plants extract effect on fertility, egg hatching and adult emergence have been well documented in the literature (Arshad et *al*., 2014; Kheirkhah et *al*., 2015; Adel et *al*., 2015). In this sense, Momen et al. (2014) reported a reduction in egg laying in females of TSSM treated with the essential oil of *Melissa officinalis L.* (Lamiales: Lamiaceae). Another study by Mead, (2012) reported a reduction in the hatching percentage of *T. urticae* eggs treated with EO of *Cymopogon citratus* (Cyperales: Poaceae) to a significant extent compared to the control group. For their part, Kawka & Tomczyk (2002) mentioned significant mortality of the preimaginal stages of *T. urticae* on leaves previously treated with an extract based on dry leaves of *Salvia officinalis* (Lamiales: Lamiaceae) causing the obstruction emergence of adults.

The Lamiaceae family is highly recognized for its antimicrobial activity and its activities against many arthropods (Biljana et *al*., 2007; Tong & Coats, 2010). Among these aromatic plants, Rosemary, *Rosmarinus officinalis* (Lamiales: Lamiaceae), a shrub found in the wild around the Mediterranean, occupies an important place due to its antibacterial activity and its insecticidal/acaricidal effects (Martinez-Velazquez et *al*., 2011; Laborda et *al*., 2013; Ben Slimane et *al*., 2015).

In the same perspective, this present work aims to test the acaricidal potential of *R. officinalis* essential oil on the pest mite *T. urticae* and its predator *P. persimilis and* evaluate its effect on three biological parameters such as fertility, egg hatching and the emergence of adults of TSSM to assure an efficient control of this pest.

MATERIALS and METHODS

Plant Material

Rosmarinus officinalis plant was harvested in May (2017) in the town of Dar Ben Karrich (Latitude: 35.50, Longitude: -5.42), located in the Tetouan region of northwestern Morocco. Botanical identification was carried out at the regional center for agricultural research in Tangier, Morocco.

Essential Oil Extraction

The extraction of the essential oil was carried out by hydrodistillation on a Clevenger type apparatus (Pyrex quickfit-United kingdom). 100g of the aerial part of the plant was introduced and the whole was boiled for 2 to 3 hours. At the end of the hydrodistillation process, the essential oil is separated from the water phase and stored at 4°C in the dark for further using.

Chemical Composition Analysis

The analysis of the chemical composition of the extracted essential oil was carried out on gas chromatography coupled with mass spectrometry, equipped with a TR-5MS column (30 m x 0.25 mm x 0.25 µm), the temperature of the column is programmed from 50 to 240 degrees Celsius at a reason of 5°C/min. The identification of essential oil constituents is done by comparing the mass spectrum of each peak separated by CG with those available on a library of standards, as well as by comparing the Kovats index calculated for each compound with those reported by the literature (Adams, 2001). The GC/MS is connected to a computer system that manages a mass spectrum library.

Plant Animal

Collection and Mass Breeding of Mites

Both *T. urticae* and *P. persimilis* are collected from infested plants collected from strawberry plants in Laouamra (Latitude: 31.79 ; Longitude: -8.71), located in the Larache region of northern Moroccoduring the month of April in 2017 . Dust mites are kept farmed on green bean plants (*Phaseolus vulgaris* L.) under laboratory conditions at 25±1°C, 70±5% relative humidity and a 16L: 8D photoperiod and without any exposure to pesticides. The seedlings of the host plants were made in separate bins and pots. Peat was disinfected by two successive autoclaving and was used as a planting substrate to prevent the introduction of soil pathogens.

To carry out mass breeding, individuals representing the stumps are transferred using brushes (one to two brushes per strain) to bean plants. At the same time, two massive farms are maintained in the laboratory: the breeding of the strain of *T. urticae* and that of the strain of *P. persimilis*. The strain of *T. urticae* is kept reared on healthy green bean plants, while the *P. persimilis* strain is reared on green bean plants infested with spider mites. These must be continuously brought to the breeding of *P. persimilis*. Spider mites are used as food for Phytoseiidae.

Toxicological Bioassays

***Inhalation toxicity*:** This test aims to study the inhalation effect of *R. officinalis* EO on the adult population of the pest *T. urticae* and its predator *P. persimilis*. Doses of 0.5, 1, 2, 4, and 8% of this oil were prepared. The choice of these doses was based on previous studies (Amer et *al*., 2001). In 50 ml-volume jars, cotton was sprayed with a 9.6 ±0.83 $\mu l/cm^2$ of each dose using a manual sprayer (Butt-Goettel, 2000). This quantity is proportional to the number of mites used. The cotton was suspended with a wire on the inside of the lid. Five pairs of *T. urticae* were added into each jar and sealed tightly. Dead individuals in each jar were counted after 24, 48, and 72h of the start of the test. Control was conducted soaking cotton in distilled water and a few drops of 0.1% Triton-X100. Five repetitions were performed for each dose. The same procedure was performed on adults of the predatory mite *P. persimilis*. Ten adults of *T. urticae* were placed with the five adults of *P. persimilis* tested.

***Toxicity Test on the biological parameters of Tetranychus urticae*:**

Female fecundity:

The method was conducted using fresh bean leaves applied to moistened filter paper. In the Petri dish (10 cm), five females of *T. urticae* were placed on the underside of each leaf of disk bean and then sprayed with different doses of EO. Five repetitions were performed for each dose tested (5 females per replicate). Females were kept at 25 °C and 16 L: 8D photoperiod for five days. Eggs laid by females were counted daily. The control dishes received only distilled water.The fertility of females was evaluated by counting the eggs laid by the female for 5 consecutive days where: Fecundity = Number of eggs laid / female

- Fecundity Reduction Percentage

This percentage makes it possible to assess the effect of the EO tested on the fertility of females of *T. urticae*. This percentage is calculated according to the following formula:

%Fecundity Reduction=1- (Rt / Rc)

Where: Rt: Fertility with Treatment

Rc: Fertility in the Case of the Witness

- Hatch rate:

After counting the eggs laid, the hatch rate is calculated as follows:

Egg hatch rate = (number of eggs hatched / number of eggs laid) X 100

- Toxicity coefficient (E)

The toxicity coefficient is determined by the percentage of egg mortality and their percentage of hatching. This coefficient of toxicity is calculated according to the following formula:

E = 100% - (100% - M) X R

Where: M: Percentage of egg mortality corrected by Abbott formula (1925).

R: Number of hatched eggs produced by treated females / Number of hatched eggs produced by untreated females.

- Adults emergence rate:

The emergence rate is the ratio, expressed as a percentage of the number of adult mites emerged to the number of eggs laid per female calculated as follows:

Emergence rate = (Number of adults emerged / Number of eggs laid) X 100

Statistical Analysis

The mortality percentages obtained are corrected by the Abbott formula (1925) to verify that no excess mortality was observed and to correct the observed mortality. All data in each experiment were evaluated using analysis of variance (ANOVA) with one or two factors (dose, duration of treatment). Each ANOVA is followed by a Tukey test to compare the averages two to two (Dagnelie, 1975).

Significant differences among means were detected by Tukey test P < 0.05. All statistical analyses were made using the Statistical Package for Social Scientists (SPSS) version 13.0.

RESULTS and DISCUSSION

Chemical analysis of *R. officinalis* EO identified seventeen compounds that account for about 75.6% of all compounds. The main constituents are: α-pinene (32.64%), β-humulene (8.71%), camphene (5.95%), 1.8-cineole (5.07%), β–pinene (3.56%), α-humulene (3.51%), camphor (3.34 %), P-cymene (2.78%), borneol (2.15%), ɤ-terpinen (2.07%), cadinene (1.52%), P-mentha-3.8 diene (1.21%) and linalool (1.08%) **(Table 1).**

Table 1: The main constituents of *R. officinalis* EO and their percentages, identified by CPG/SM.

Compound	**Retention time (min)**	**Calculated Kovats index**	**Kovats index, literature (DB5-ADAMS 2001)**	**AIR%**
α-pinene	7.35	937.30	939	32.64
Camphene	7.71	951.15	953	5.95
β-pinene	8.44	979.23	980	3.56
1.8-Cineole	9.95	1034.64	1033	5.07
P-cymene	9.72	1026.42	1026	2.78
Linalool	11.81	1101.06	1098	1.08
Camphor	13.23	1151.41	1143	3.34
Borneol	13.82	1172.34	1165	2.15
α-terpineol	14.11	1182.62	1189	0.53
ɤ-terpineol	14.48	1195.74	1183	0.59
Iso aromadendreneepoxide	24.69	1559.29	1579	0.70
Caryophyllene oxyde	25.29	1625,11	1581	0.19
α- humulene	21.54	1465.58	1455	3.51
ɤ-terpinene	10.69	1061.07	1062	2.07
P-mentha-3.8 diene	11.53	1091.07	1072	1.21
β-humulene	20.71	1431.98	1440	8.71
α-cadinene	23.17	1533.33	1538	1.52
Total				75.6

Many studies of the chemical composition of *R. officinalis* essential oil, from different regions, have been listed (Bekkara et *al*., 2007; Martinez-Velazquez et *al*., 2011; Megzari et *al*., 2015). The variability of the composition and the EO yield of this plant is due to intrinsic factors (genetics, subspecies, and age of the plantation) or extrinsic factors such as climate and growing conditions (geographic origin) or the extraction method. Chemical analysis of the EO in our sample showed the presence of terpenes: monoterpenes (α-pinene, β-pinene, camphene, 1.8-cineole, p-cymene, borneol, camphor, ɤ-terpinene) and sesquiterpene (β- humulene, α-humulene, α-cadinene). The EO of *R.*

officinalis is a mixture of terpene products which α-pinene is the majority component at a content of 32.64%. The other compounds are present in lower concentrations. Several research studies have proven the dominance of α-pinene in the chemical composition of *R. officinalis* essential oil (Martinez-Velazquez et *al*., 2011; Megzari et *al*., 2015; Zibaee & Khorram, 2015).

Biological control through the use of biopesticides of botanical origin presents a viable alternative to chemical control. Plant extracts contain large amounts of bioactive acaricidal and insecticidal compounds (or substances) that can be used in pest control (Lee, 2018 ; Tabet et *al*., 2018). In this regard, *R. officinalis* is a Lamiaceae that has drawn the attention of several researchers for its insecticidal/acaricidal properties (Labordaa et *al*., 2013; Jayakumar et *al*., 2017).

The variance analysis reveals that the inhalation effect of *R. officinalis* essential oil on *T. urticae* varies depending on the dose used (F=80.645 ; df=5 ; P<0.001). On average, 53.33 ; 55 ; 68.33 ; 80 and 96.66% of mortality was recorded at the respective doses of 0.5 ; 1 ; 2; 4 and 8%. Regarding the duration factor, the variance analysis shows that the mortality rate varies significantly during the 3 days of treatment (F=18.852 ; df=2 ; P<0.001) where 48.33% ; 57.5% and 70% of mortality was recorded in 1^{st},2^{nd} and 3^{rd} day respectively.

Regarding *P. persimilis*, the highest mortality rate observed was 16.11 and 35.43% for the highest two doses of 4% and 8% respectively after the three post-treatment days. Analysis of variance shows a significant difference between the five doses (F=50.190 ; df=5; P <0.001). Statistical data indicated that the mortality rate in *P. persimilis* did not change significantly after the three days of administration (F = 3.78 ; df = 2 ; P> 0.05). The mortality rate of 7.93% ; 10.15% and 13.35% was recorded day in the 1^{st}, 2^{nd} and 3^{rd} day respectively **(Figure 1 and 2).**

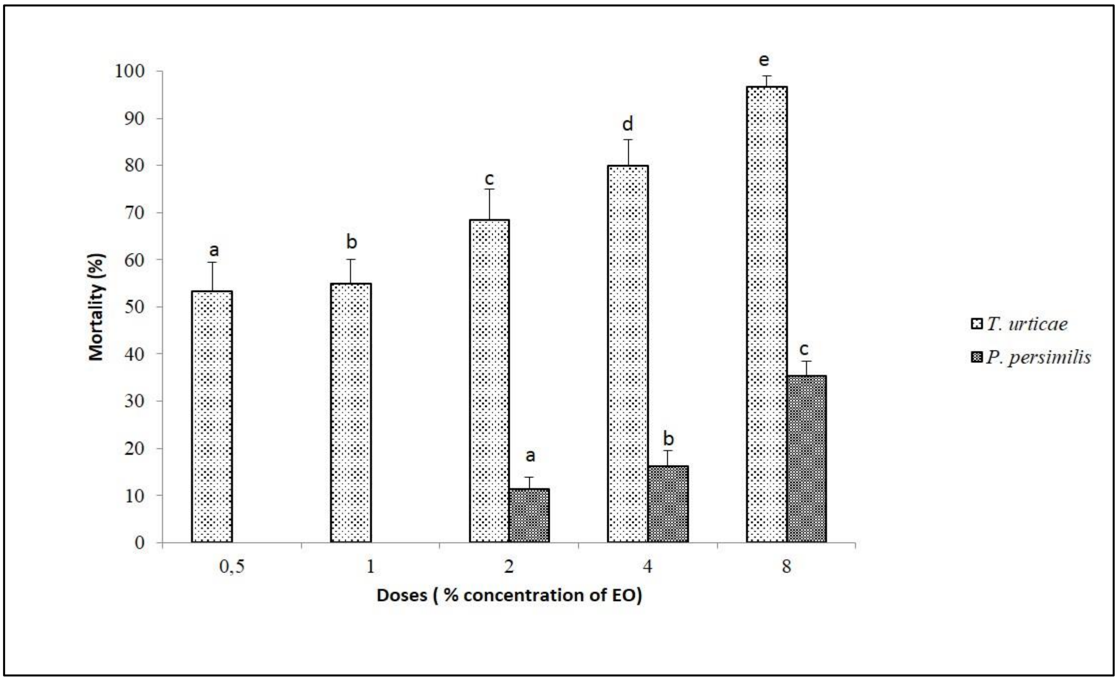

Fig. 1: Mean adults mortality percentage of *T. urticae* and *P. persimilis* treated by inhalation with 5 different doses of *R. officinalis* essential oil. Means (±SE) with the different letters are significantly different from one another at α = 0.05 level of significance (Tukey test).

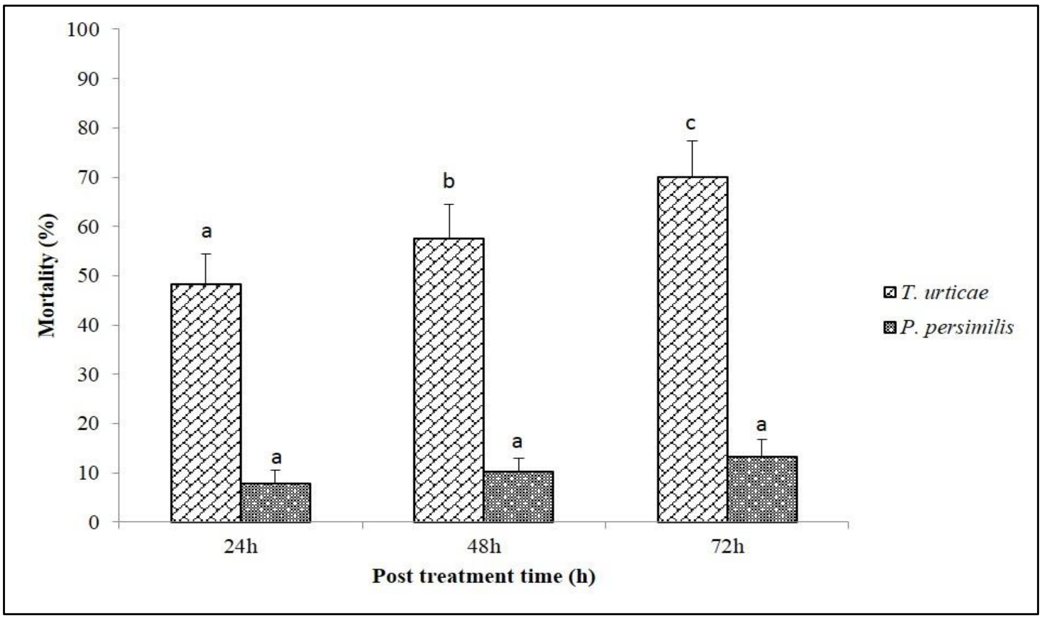

Fig. 2: Mean adults mortality percentage of *T. urticae* and *P. persimilis* recorded after 24, 48 and 72 h of application of *R. officinalis* essential oil. Means (±SE) with the different letters are significantly different/Means (±SE) with the same letter are not significantly different from one another at α = 0.05 level of significance (Tukey test).

The toxicity of EOs, including Rosemary essential oil, is due to the various terpene molecules that have demonstrated their physical and physiological effects (Roh et *al*., 2012; Amri et *al*., 2014;

Jayakumar et *al*., 2017). These compounds may contribute, jointly or independently, to adulticidal, larvicidal and ovicidal activity, reduction of the fecundity of females, decrease or inhibition of hatching of eggs, inhibition of emergence of adults and repulsion of culture enemies (Pavela, 2008 ; Lim et *al*., 2011). The acaricidal effect of several monoterpenes against *T. urticae* has been reported in several studies. The toxicity of α-pinene against *T. urticae*, for example, the predominant compound in our sample, has been described by Miresmailli et *al*. (2006) and Attia et *al*. (2011). In this vision, and under laboratory conditions, toxicological tests were carried out to demonstrate a significant efficacy by inhalation of *R. officinalis* essential oil against the adult population of *T. urticae* with an increase in this toxicity with time. EO vapors from *R. officinalis* eradicated almost all adults of *T. urticae* at the highest dose of 8%. On the other hand, and at the same dose, relatively low toxicity was recorded on the predator *P. persimilis*, the mortality rate of which was 35.43%.

The acaricidal activity of *R. officinalis* oil, observed in this study, has also been described by several authors. Miresmailli et *al*. (2006) observed total mortality in *T. urticae* mites treated, by contact, with a dose of 13.9 ml/l. The study by Labordaa et *al*. (2013) reported 95-100% mortality of *T. urticae* treated by contact at a dose of 0.2% . Esmaeily et *al*. (2017) reported high efficacy by inhalation. Indeed, they recorded a significant reduction in the longevity of males and females of *T. urticae* treated with Rosemary oil. However, Choi et *al*. (2004) reported moderate toxicity of this oil. These authors evaluated the inhalation toxicity of 53 essential oils, including rosemary, against *T. urticae* eggs and adults. Rosemary oil was not very toxic (mortality < 60%) compared to the seeds of caraway, citronella, lemon, eucalyptus, pennyroyal, and peppermint which were found to be very toxic (Mortality > 90%) on the mites tested. The results obtained by Amer et *al*. (2001) confirmed a mortality of 53.85% of *T. urticae* and a total mortality of *Eutetranychus orientalis* (Acari: Tetranychidae) treated with a dose of 2% of Rosemary EO after 10 days post-treatment. In the same sense, Isman & Machial (2006) have shown that a commercial formulation based on rosemary oil (Hexicide, EcoTrol, Sporam) offers high toxicity against *T. urticae* but it did not have any acute toxicity on the predatory mite *P. Persimilis* which is consistent with our remarks. The same finding was reported by Miresmailli & Isman (2006) who reported that *P. persimilis* was less sensitive to *R. officinalis* oil and EcoTrol (a Rosemary oil-based pesticide) than *T. urticae* in the laboratory and a greenhouse. A greenhouse trial showed that a single application of EcoTrol at the rate recommended on the label could reduce two-point spider mites by 52%. At this dose, EcoTrol did not cause any mortality in *P. persimilis*, nor did it affect their eggs. For their part, Hany & El-Zahi (2011) studied the acaricidal effect of Rosemary EO on *T. urticae* and its predator *P. persimilis*. They showed an acaricidal effect of this oil on female adults of both species with better toxicity to *T. urticae*. Besides, they indicated that the toxicity of this oil increases during the 72h after treatment. The same findings were reported by Elhalawany & Dewidar (2017) who confirmed an acaricidal effect of *R. officinalis* on *T. urticae*, of which LC_{90}=7.16% after 72 hours of treatment. This oil, according to these authors, was less toxic on the two predators *P. persimilis* and *Neoseiulus* *californicus* (Acari: Phytoseiidae) with LC_{90}=143.15% and LC_{90}=72.13% after 48h post-treatment respectively. These reports indicate lower toxicity of R. *officinalis* essential oil on predators than on the pest *T. urticae*.

In this study, *R. officinalis* EO significantly exhibited the biological parameters of *T. urticae*. At a dose of 4%, this oil completely reduced the fecundity of females, no egg was hatched was recorded and completely inhibited the emergence of adults.

Direct application of increasing doses of *R. officinalis* essential oil significantly reduces the fecundity of females of *T. urticae* (F=473.288 ; df=5; P<0.001). Females lay 9.5 ; 5.3 and 2.1 eggs when sprayed with low and intermediate doses of 0.5 ; 1 and 2% of Rosemary oil respectively for 5 consecutive days. Whereas, females laid no eggs when at higher doses (4 and 8%).

- Estimated percentage reduction in fertility in *T. urticae* after contact treatment of *R. officinalis* essential oil :

The percentages of fecundity reduction recorded are 43.45 ; 68.45 ; 87.5 ; 100 and 100% after contact treatment with respective doses of: 0.5 ; 1 ; 2 ; 4 and 8% of R. officinalis essential oil **(Figure 3).**

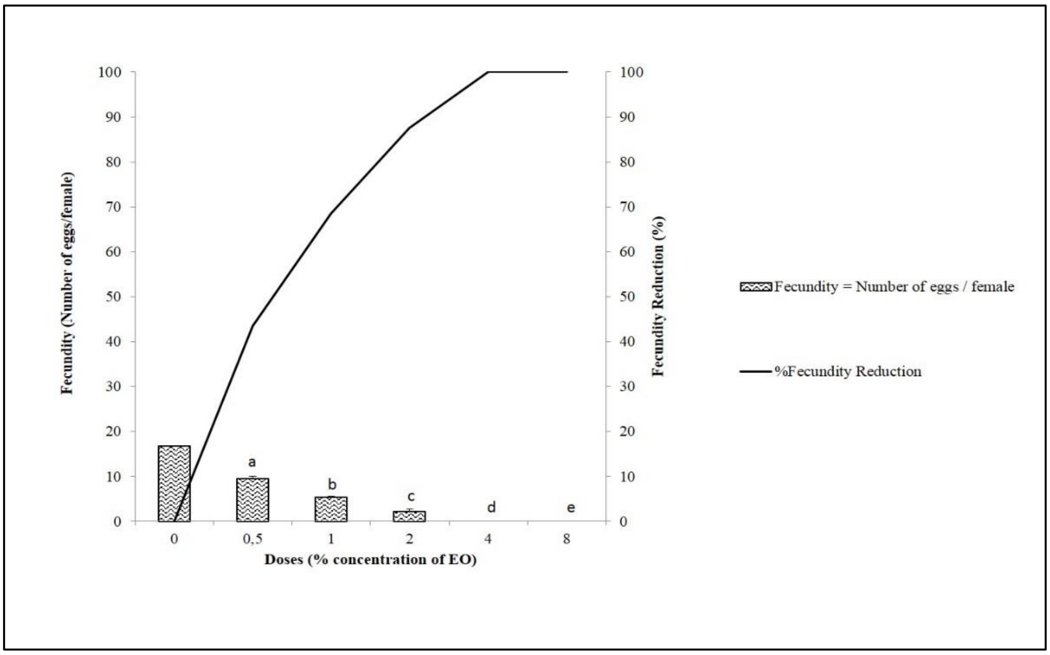

Fig. 3: Fecundity and percentage of fecundity reduction in females of *T. urticae* treated with the 5 doses of *R. officinalis* essential oil. Means (±SE) with the different letters are significantly different from one another at α = 0.05 level of significance (Tukey test).

The variance analysis shows that the use of increasing doses of Rosemary oil results in a significant reduction in the percentage of hatching eggs (F=455.606 ; df=5; P<0.001). The average hatching percentages retained from 47.32 to 19.73 and 0.72% after the application of 0.5 ; 1 and 2% of

Rosemary oil respectively. At the higher doses (4 and 8%) no egg hatching was recorded where 95% hatching rate was observed for control **(Figure 4).**

- Toxicity coefficient after contact treatment:

On average, the toxicity coefficients recorded 72.23 ; 79.66 ; 96.43 ; 100% and 100% after contact treatment with respective doses of: 0.5 ; 1 ; 2 ; 4 and 8% of *R. officinalis* essential oil. The variance analysis reveals that the increasing doses of Rosemary oil have different effects on the toxicity coefficient (F=2331.764 ; df=5 ; p<0.001).

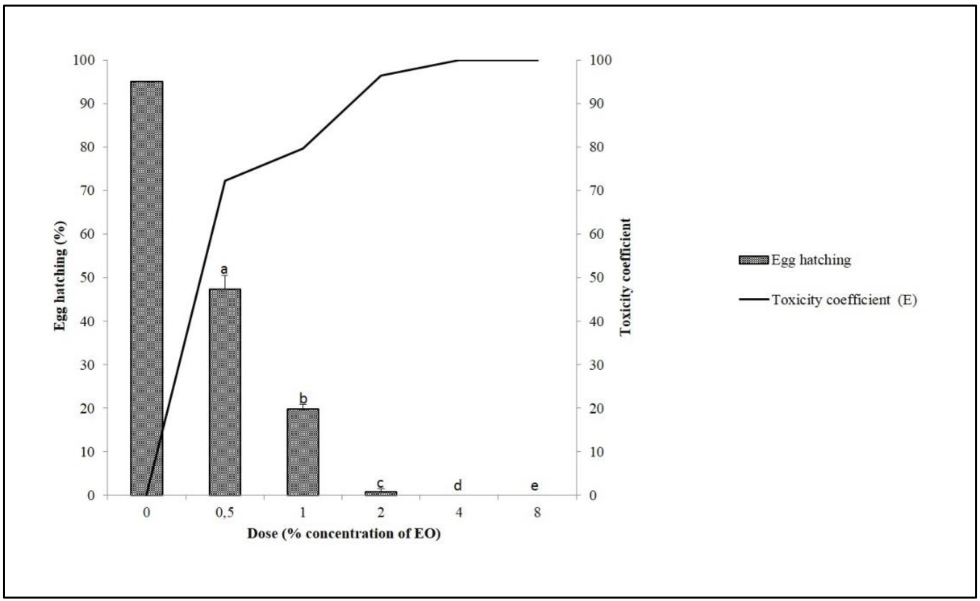

Fig.4: Mean egg hatching rate of *T. urticae* and the toxicity coefficient of increasing doses of *R. officinalis* essential oil. Means (±SE) with the different letters are significantly different from one another at $\alpha = 0.05$ level of significance (Tukey test).

Variance analysis reveals that increasing doses of *R. officinalis* oil have different effects on the emergence of adult larvae (F=88.409 ; df=5; p<0.001). The emergence of larvae was recorded at low and intermediate doses (0.5; 1 and 2%) with the following percentages 52.43, 35.37 and 11.12%. Contrariwise, applying higher doses (4 and 8%) completely prevents the emergence of larvae to adults **(Figure 5).**

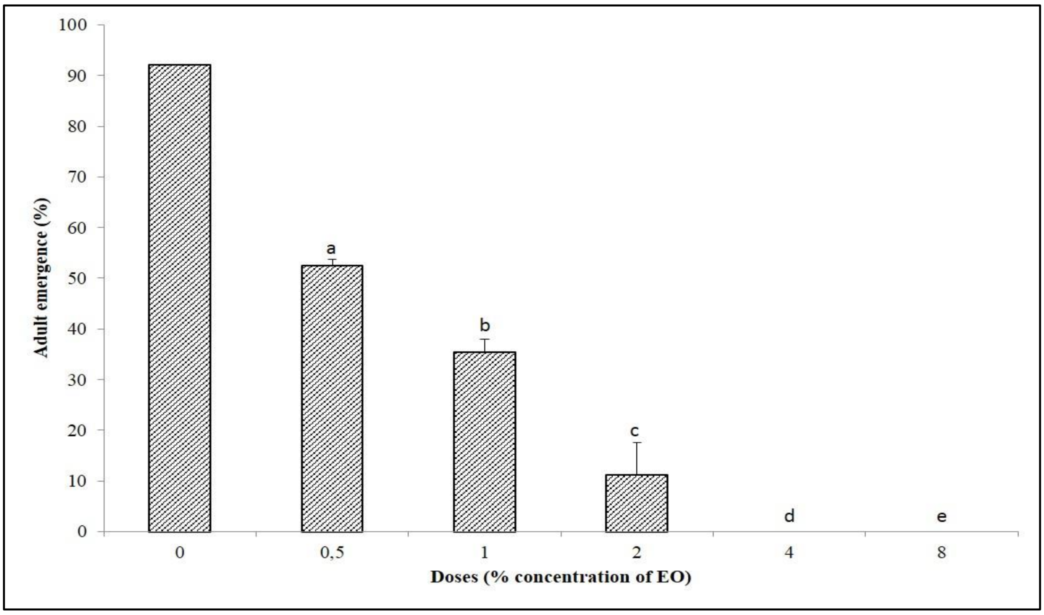

Fig. 5: Mean Adult emergence rate of *T. urticae* treated with 5 doses of *R. officinalis* essential oil. Means (±SE) with the different letters are significantly different from one another at α = 0.05 level of significance (Tukey test).

Previous studies have confirmed the effect of R. officinalis on the fecundity of T. urticae. According to Amer et al. (2001), the average number of eggs laid by females of T. urticae after 72h of treatment with Rosemary EO varied according to the concentrations used. The deterrent index laying recorded at the 2% concentration was 83.33 which is in agreement with our results. This oil also prevented females from E. orientalis to lay eggs. Laborda et al. (2013) reported a decrease in the total number of eggs laid by females of T. urticae at the increasing doses of oil and the reduction in the rate was significant when 0.25% of Rosemary extracts were sprayed on leaf discs. According to Esmaeily et al. (2017), R. officinalis EO has been shown to significantly reduce the fecundity of females of T. urticae. This was also mentioned in the study of Elhalawany & Dewidar (2017). In fact, in the control group, the number of eggs laid per female was 41.33 for 7 days. This number decreased in the treated batches to 27.38 ; 24.42 ; 18.33 ; 16.07 and 7.53 at concentrations 0.5 ; 1 ; 2; 3 and 4%. Also, *R. officinalis* was shown to have an ovicidal effect on *T. urticae* eggs which significantly reduced egg hatching (Yorulmaz et *al*., 2015). Rosemary EO also affects the emergence of larvae to adults. The study by Yorulmaz et *al*. (2015) reported high toxicity of this oil on *T. urticae* nymphs and therefore a reduction in their emergence into adults. The development of larvae into nymphs and nymphs into adults of *T. cinnabarinus* has also been studied by Najafabadi (2014). This oil reduced the number of larvae developed into nymphs to 127.7 compared to the control whose number was 145.7. The number of nymphs transformed into adults also decreased to 43.3 compared to the control (104.3).

Conclusion

The results of this work indicate a notable EO toxicity of *R. officinalis* against *T. urticae*. This bioproduct is a good candidate for controlling this pest of crops in the Loukkos region. Rosemary EO was also less toxic to *P. persimilis*, which will help protect predators. The combination of the toxicity of *R. officinalis* EO against the pest *T. urticae* with the use of predators will be more appropriate in integrated pest management. This study is based on laboratory data, so it remains important to direct an applied approach to carry out it in the field.

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