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The Impact of Several Plant Extracts and Essential Oils on Pistachio Psylla (*Agonoscena pistaciae*) and Its Natural Enemy, *Chrysoperla carnea*

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ABSTRACT

The common pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer (Hem.: Aphalaridae), is one of the most important pests of pistachio orchards in Iran. Green lacewing, *Chrysoperla carnea*, as a general predator found abundantly in Iranian pistachio gardens, loses its performance under the impression of pesticides spraying. Nowadays, considering environmental pollutions, human health and the importance of healthy and organic nutrition, extensive researches are carried out around the world in order to replace the synthetic products with plant materials. In this study, the mortality effect of two ethanol extracts of *Tribulus terrestris* and *Tagetes erecta* and two essential oils of *Mentha pulegium* and *Rosmarinus officinalis* were studied on *A. pistaciae* and its natural enemy *C. carnea*. The results showed that, although essential oils do not have appreciable effect on mortality of this psylla, but the extracts have high mortality potential on psylla nymphs, especially on N1 nymphs and the low influence on 1-2 days green lacewing larvae. The plant extracts and essential oils have not effect on 5-6 days green lacewing larvae. The results show that the plant derived chemicals and ethanol have not negative effect on hatching rate of the natural enemy. Among these treatments, *R. officinalis* essential oil has negative effect on eggs hatching rate, so its use is not recommended. Therefore with no impact of plant material on natural enemies, we can use green lacewing and herbal extracts in integrated pest control.

Introduction

Pistacia vera L. (Anacardiaceae: Sapindalis), is one of the most important horticultural products in Iran that has been cultivated in various parts of the country for a long time and has great economic value (Hassani *et al.*, 2009). *Agonoscena pistaciae* Burckhardt and Lauterer, 1989 (Hemiptera : Psyllidae), is one of the most important pests of pistachio which are distributed in many pistachio growing areas in the world such as Iran, Turkey, Iraq, Syria, Armenia, Turkmenistan and Greece (Burckardt and Lauterer, 1989; Lauterer *et al.*, 1998; Anagnou–Veroniki *et al.*, 2008). It is named "common pistachio psylla" (Mehrnezahd, 2002).

Nymphs and adults of this pest suck sap from leaves and produce large amounts of honeydew. Direct feeding causes reduced plant vigor, defoliation, stunting, poor yield and bud drop. Gardeners named it "dry sap" (Samih *et al.*, 2005). Spreading and infesting the pest, compels us to change our policies on chemical control to reduce the usage amount of pesticides, and find a way to diagnose and use non-chemical methods and protect natural enemies.

Chrysoperla carnea Stephens (Neuroptera: Chrysopidae), is used as one of the best natural enemies in pest control program. The efficiency of *C. carnea* as a biological controlling factor in crops,

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horticulture and greenhouse cultivation has been confirmed (Hagley and Mikes, 1987). This predator has been highlighted by researchers due to its broad predatory host range (Hydron and Whitcomb, 1979), wide distribution (New, 1975), an insatiable polyphagous larvae, resistance to some pesticides and relative coverage in mass production (Hassan *et al.*, 1985; Medina *et al.*, 2001; Schuster and Stansly, 2000; Pappas *et al.*, 2011). Benefitting from this biological agent, has led to reduction in pesticides usage and their impact on non-target species, groundwater pollution and cost of control while increasing the health of food stuff.

In recent years, efforts have been made to replace chemical pesticides with compounds of low risk and stability in nature. In this regard, some secondary plant metabolites that play an important role in natural defense and pest control are taken into consideration (Georges *et al.*, 2007). The purpose of the present study is evaluating the mortality rate of two ethanol extracts of *Tagetes erecta* and *Tribulus terrestris* and two essential oils of *Mentha pulegium* and *Rosmarinus officinalis* on *A. pistaciae* N_1 and N_5 nymphs and *C. carnea* larvae as a natural enemy, in order to utilize these plant materials to control the common pistachio psylla. *T. erecta* is a plant that is compatible with the warm and dry environment and cultivated in green areas in most parts of Iran, so extraction of plant material from this plant is very economical. *T. terrestris* plant is known as weed pistachio gardens, so extraction of effective substances on pistachio pests is economical. Two plants of *R. officinalis* and *M. Puligium* are highly economical for the production of herbal derivatives, and their impact on pests is mentioned in many sources.

T. terrestris, is a plant that Weaver *et al.* (1994), examined the insecticidal effects of its extracts against Mexican bean beetle *Zabrotes subfasciatus* and achieved favorable results. Effects of *T. erecta* on the fourth instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinque-fasciatu* were tested by Dharmagadda *et al.* (2005). In a research, *T. erecta* was evaluated on neonate larvae of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) a major maize pest in the world (Salinas-Sánchez *et al*., 2012). The acetone leaf extract (500 ppm) of *T. erecta* induced an antifeedant effect, causing a 50% reduction of larval weight in comparison with the control. Larval weights were drastically reduced at 7 d, but even more so at 14 d, when *T. erecta* extracts also caused substantial mortality. Three leaf extracts of *T. erecta* caused high larval mortality, with hexane (48%), acetone (60%) and ethanol (72%). Further *T. erecta* leaf extracts caused pupal mortalities of 40-80% (Salinas-Sánchez *et al.*, 2012).

El-Sheikh *et al.* (2016) examined the larvicidal and repellent effect of the crude ethanol, acetone and petroleum ether extract leaves of *T. terrestris*, against 3rd instar larvae and adults of mosquito, *Aedes aegypti* the vector of dengue fever was evaluated. They concluded that, these extracts can be used as an effective alternative to the existing synthetic pesticides for the control of *A. aegypti*. Zekri *et al.* (2013) studied the phytochemical and insecticidal activity of *M. pulegium* oils from Morocco against *Sitophilus Oryzae*. The aim of this study was to validate the therapeutic properties of *M. pulegium* by conducting a phytochemical study, to determine the chemical composition of its essential oils and evaluate its insecticidal activity against stored cereals pests. This effect is influenced by the tested doses and exposure periods. The potential of this plant to be used to control stored product insects was discussed.

Pavlidou *et al.* (2004) examined the effect of essential oils of *M. pulegium* on *Drosophila melanogaster* and *Bactrocera oleae* with good results. Also, Pavela (2005) studied the effect of 34 plant oils such as *M. pulegium* on *Spodoptera littoralis* larvae. *R. officinalis*, is a plant that Prajapati *et al.* (2005) applied its essential oil on *A. aegypti*, *A. stephensi* and *C. quinque-fasciatu* and at last introduced it as the pernicious and repellent agent. In another research, investigated the chemical composition of essential oils from *R. officinalis* samples collected in spring in Moyen-Atlas (Morocco) and to test their insecticide activity on survival of the pest locust *Dociostaurus*

maroccanus (El Ghadraoui *et al*., 2015). The extract was active against locusts, as estimated by the determination of the LT50 (lethal time 50). LT50 obtained was around 2.25 days for females and 1.9 days in males. This effect can be attributed to one or several compounds of the extract. Given the high humidity of produced faeces, disturbance of water intake by rectum seems to be the most probable cause of mortality (El Ghadraoui *et al.*, 2015).

Materials and Methods

Host plant (pistachio)

The experiment was conducted in a pistachio orchard at Kerman, Iran. To have pistachio seedlings for the tests, used Fandoghi variety seeds which were dried in the sun. Seeds were removed from their bony shell and put with water and a few drops of liquid detergent in tissue culture glasses for 30 min and placed on a shaker until washed thoroughly. Then the mixture of water and liquid detergent was poured out and the seeds were washed with distilled water. Then every 10 disinfected seeds were placed between two circular filter papers. Papers containing the seeds were placed in appropriate petri dishes and the dishes were put in a nylon bag and kept them at 4°C (in the refrigerator) for 48 hours. After that, a few drops of distilled water were added to filter papers in petri dishes to provide moisture for germination and petri dishes were transferred into the growth chamber, keeping the temperature at 25±1°C, the relative humidity at 60±5% and photoperiod at 14:10 (light: dark). Petri dishes were visited on a daily basis and if seeds had high germination, they were transferred into seedling bags (10 cm diameter, 30 cm height). After about two months, seedlings had desired growth (30 cm in height and having at least 12 leaves) to be used in the experiment.

Chrysoperla carnea rearing

In order to create a colony of *C. carnea* insects, adults were transferred into PVC cylindrical tubes with 20 cm diameter and 23 cm height and both ends were covered with blue nets. Adults were fed on artificial diet consisting water, honey and yeast (1: 1: 1) every day. The mixture produced from mentioned substances were placed on paper strips and exposed to the insects. Required water was provided by wetting nets daily. After starting egg laying, the cylinders were replaced every day and the eggs were transferred to petri dishes. After hatching the eggs, *C. carnea* larvae were fed with egg of *Sitotroga cerealella* Olivier (Lpidoptera: Gelechiidae) and the moths' body. The larvae were periodically fed on *Aphis fabae* Scopoli (Hemiptera : Aphididae) reared in the laboratory, to maintain the predators' hunting abilities.

Preparation of plant extracts and essential oils

The dried flowers of *T. erecta* and *T. terrestris* were powdered mechanically using commercial electrical stainless steel blender for five minutes and then 100 gr powders were put in the Erlenmeyer flask (500 ml) with ethanol (purity 96%). After being stirred for one hour under the hood, the flask was well sealed by Parafilm M® and covered with aluminum foil to prevent direct light exposure. Then the Erlenmeyer flask containing plant extract and pulp was put in the refrigerator at 4°C for 48 hours. Then extracts were separated from the pulp by Whatman® cellulose filter paper (9 cm diameter) and then, needed concentration was prepared.

Essential oils content of *M. pulegium* and *R. officinalis* plants was obtained with hydro-distillation of grinded samples using Clevenger apparatus and after that, these compounds were poured into sterilized dish and stored in freezer.

Evaluate the effect of the extracts and the essential oils on mortality of N_1 and N_5 instars of Agonoscena pistaciae nymphs

For the purpose of the extracts effects, pistachio seedlings were transferred to a garden near Kerman. Fifteen pistachio seedlings were taken for each extract, 15 seedlings for ethanol as an extract solvent and 15 seedlings as the control (without any

substance). When seedlings got infected with *A. pistaciae* N_1 and N_5 nymphs separately, first recording was made and the number of insects on three leaves (top, middle and down) of each plant was counted. The counted leaves were marked by color ribbons and the plants sprayed by the extracts of 20 $mg.mL^{-1}$ concentration or ethanol. After 48 hours, the mortality percent in different treatments were measured by counting the number of dead nymphs per leaf. The nymphs who showed no reaction to the stimulation by a brush were considered as dead.

In order to evaluate the effect of essential oils on mortality of *A. pistaciae* N_1 and N_5 nymphs, exactly at the same time and place where seedlings got infected by psylla, fifteen pistachio seedlings were considered for each essential and fifteen plants for the control (without the use of any material). At first, the nymphs on marked leaves were counted and then, the sheets of white paper with dimensions of 3 cm by 3 cm impregnated with 100 µl of essential oil were prepared. Three papers were hung from each seedling by a thread in a way that upper, middle and lower parts of seedlings were covered by them. After 48 hours, the second recording was done by counting the number of dead nymphs in each leaf and the percentage mortality of the pest was calculated.

Effect of the extracts and the essential oils on mortality of Chrysoperla carnea larvae

For investigating the plant ethanol extracts effects on mortality of *C. carnea*, 10 petri dishes containing ten 1-2 and 5-6 days old larvae were used separately and the bio- tests were sprayed by the extracts (20 $mg.mL^{-1}$). The same numbers of larvae were treated with ethanol 96% and also similar numbers without use of any material, as a control. The larvae were fed with enough food on a daily basis. Due to cannibalistic characteristic of lacewing larvae, some straw was placed in dishes that could be used as shelters when needed. After 72 hours, the number of dead larvae was counted and mortality rate was calculated.

To perform this experiment by essential oils, the fumigation method was used. So that, for each essential oil, 15 replications and for each replication, a small plastic container (A container with a 5.5 cm diameter, 3 cm height and 70 ml volume) containing larvae five 1-2 and 5-6 days old green lacewing larvae was considered. *S. cerealella* eggs were provided as the food for the larvae. The lid of each container had a hole covered with mesh to allow ventilation. The small plastic container was transferred into a larger cylindrical container (A container with a 6.5 cm diameter, 8 cm height and 250 ml volume). The large container walls were covered with tissue paper which was impregnated by 100 µl of each essential oil. Control container was treated without any substance. Then the lids of the large containers were quickly put down and the dishes were sealed completely by Parafilm M®. After 72 hours, the number of dead larvae was counted and mortality rate was calculated.

The effect of ethanolic extracts and essential oil on eggs hatching rate of green lacewing

For the purpose of the extracts effects on eggs hatching rate of *C. carnea*, 10 petri dishes containing ten 1 day old eggs were selected and the bio- tests were sprayed by the extracts (20 $mg.mL^{-1}$). The same numbers of eggs were treated with ethanol 96% and also similar numbers without use of any material, as a control. After the extraction, the dishes were monitored daily and the hatching eggs were counted and the larvae were removed from the dishes.

To investigate the effect of essential oils on the green lacewing 1 day old eggs, the eggs were transferred to the essential oil test vessels mentioned earlier in the experiments and the experiment was performed using fumigation. So that, for each essential oil, 10 container and 10 eggs in each container were selected. The experiment was carried out like the previous test. In this method, the lacewing eggs that had been laid on paper were placed at the bottom of the cylindrical containers with a volume of one liter. The large container walls were covered with tissue

paper which were impregnated by 100 µl of each essential oil. Control container was treated without any substance. Then the lids of the large containers were quickly put down and the dishes were sealed completely by Parafilm M®. The essential oil on the paper evaporates and affects the eggs. After 24 hours, the number of hatching larvae was counted daily and removed from the dishes.

Statistical analysis

In order to affirm the basic assumptions of the data to be analyzed, they were first tested for the normal distribution and the homogeneity of variance using the Bartlett test (Köhler *et al*., 2002). The data that had not conformed to the assumptions of normal distribution were transformed to conform to the assumptions, using the Box-Cox formula:

$Y = X2 - 1/\lambda$ if $\lambda \neq 0$,

$Y = \ln X$ if $\lambda = 0$,

Where: Y – the transformed value, X – the untransformed value, and $0 < \lambda < 1$ (Anonymous, 1996).

All statistical analyses were carried out using StatPlus. The first phase was calculating means and then finding the variations among them. During the next phase, the significant difference among the means was determined in 5 percent probability. All obtained data were inferred from a one-way analysis of variance (ANOVA) using Fisher's LSD test.

Results

According to the results achieved from studying of mortality rate of plant extracts and essential oil on *A. pistaciae* N_1 and N_5 nymphs (Figs. 1 and 2), the mortality rate of *T. terrestris* is 95.6 ± 0.9% (Fig. 1) and 48.63 ± 7.23%, respectively (Fig. 2). In two figures, the extract of *T. terrestris* has the highest percentage of mortality among nymphs. There is no significant difference between *T. terrestris* and *T. erecta*. But *T. terrestris* and *T. erecta* showed significant difference with ethanol, essential oils of *M. pulegium* and *R. officinalis* as well as control. Also, ethanol has significant difference with essential oils and control. There is no meaningful difference between essential oils and control ($P \leq 0.05$). Generally the mortality percentage of N_1 nymphs is more than N_5 nymphs.

The experiments of the plant extracts and essential oils effects on mortality of C. carnea 1-2 days old larvae showed that, T. erecta extract has the highest percentage of mortality on larvae of this natural enemy. The mortality rate of T. erecta is 31.04± 4.29% and T. terrestris is 29.30±3.57%. There is no meaningful difference between mortality rates of the larvae treated by these two extracts. Essential oils of M. pulegium showed significant difference with control ($P \leq 0.05$), but it had not meaningful difference with essential oil of R. officinalis ethanol and control (Fig. 3).

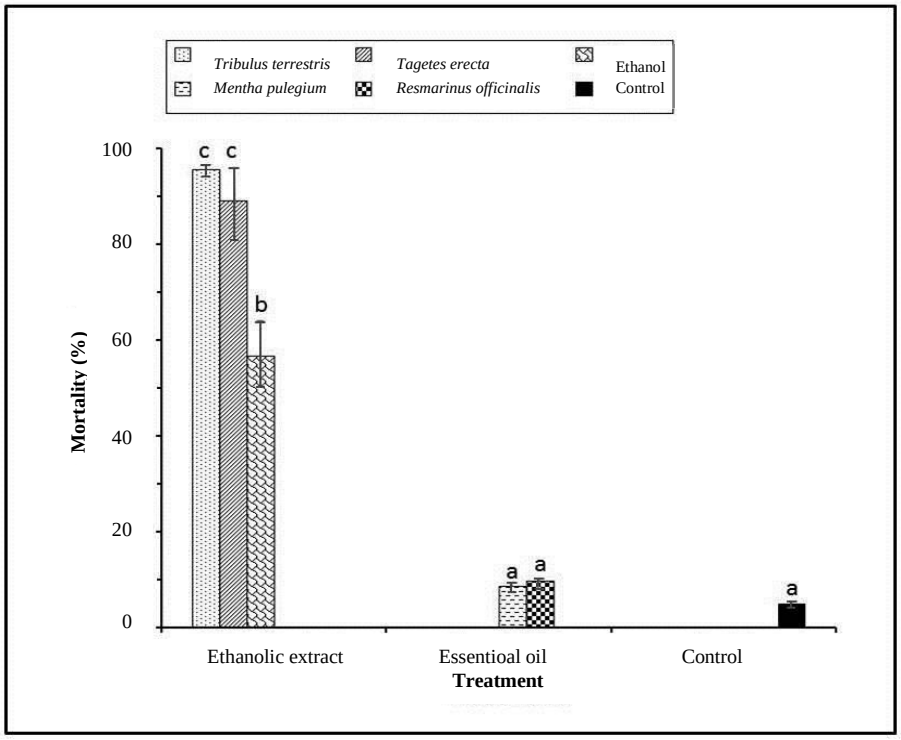

Fig. 1. Mortality rate of ethanol extract of *Tribulus terrestris* and *Tagetes erecta* and essential oils of *Mentha pulegium* and *Rosmarinus officinalis* on *Agonoscena pistaciae* N_1 nymphs

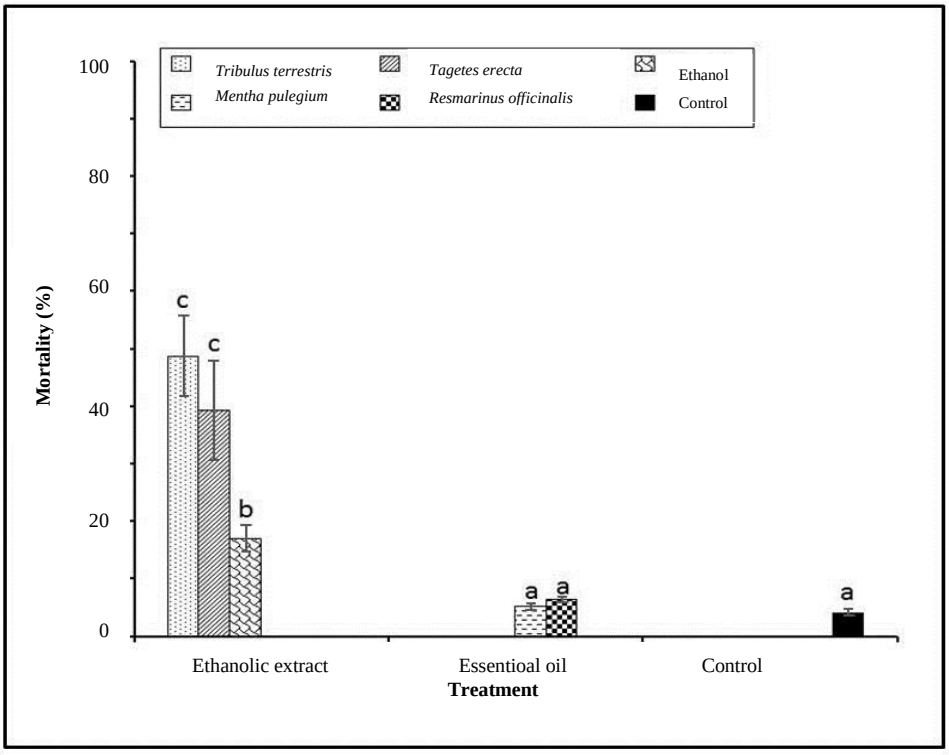

Fig. 2. Mortality rate of ethanol extract of *Tribulus terrestris* and *Tagetes erecta* and essential oils of *Mentha pulegium* and *Rosmarinus officinalis* on *Agonoscena pistaciae* N_5 nymphs

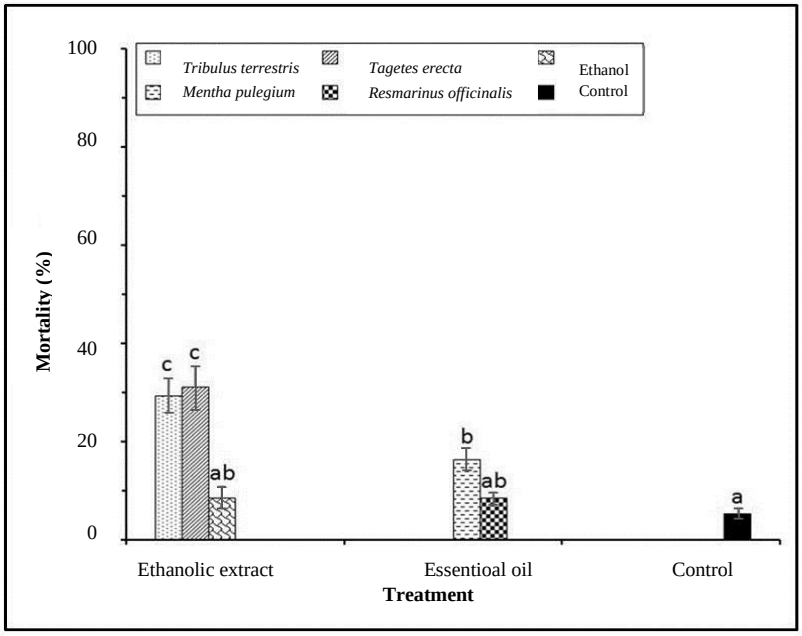

Fig. 3. Mortality rate of ethanol extract of *Tribulus terrestris* and *Tagetes erecta* and essential oils of *Mentha pulegium* and *Rosmarinus officinalis* on 1-2 day old larvae of *Chrysoperla carnea*

In the test of the plant extracts and essential oils effects on mortality of *C. carnea* 5-6 days old larvae showed that, fortunately, the effect of plant extracts on larval mortality of this natural enemy is very minimal. There is no meaningful difference between mortality rates of the larvae treated with different treatments (Fig. 4).

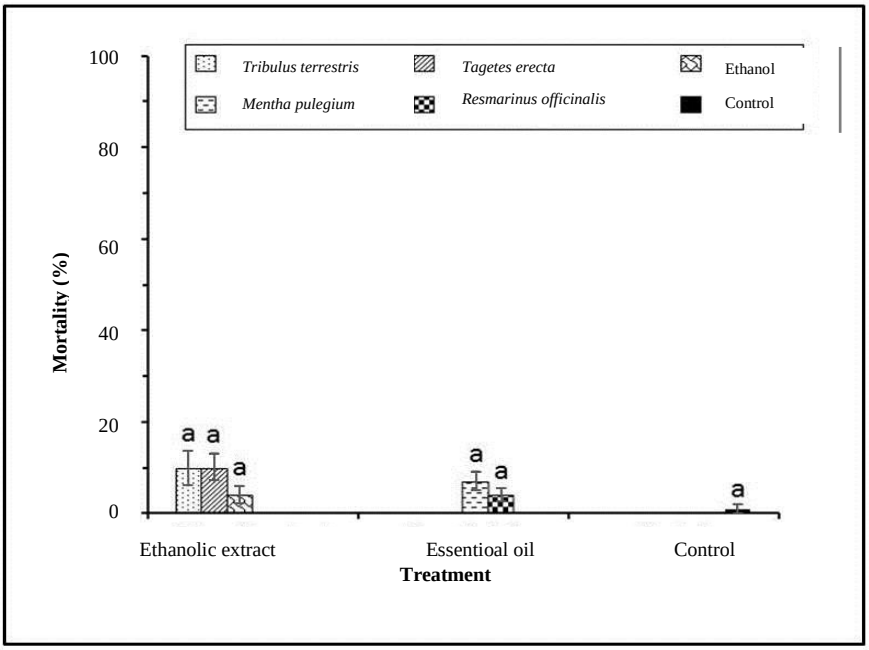

Fig. 4. Mortality rate of ethanol extract of *Tribulus terrestris* and *Tagetes erecta* and essential oils of *Mentha pulegium* and *Rosmarinus officinalis* on 5-6 day old larvae of *Chrysoperla carnea*

In the research, the effect of ethanolic extracts and essential oil on eggs hatching rate of green lacewing was tested. There is no significant difference between the plant extracts, essential oil of *M. pulegium* and control. But essential oils of *R. officinalis* has significant difference with the others ($P \leq 0.05$) (Fig.

5). Ethanol had the highest of hatching rate (85.0± 4.28).

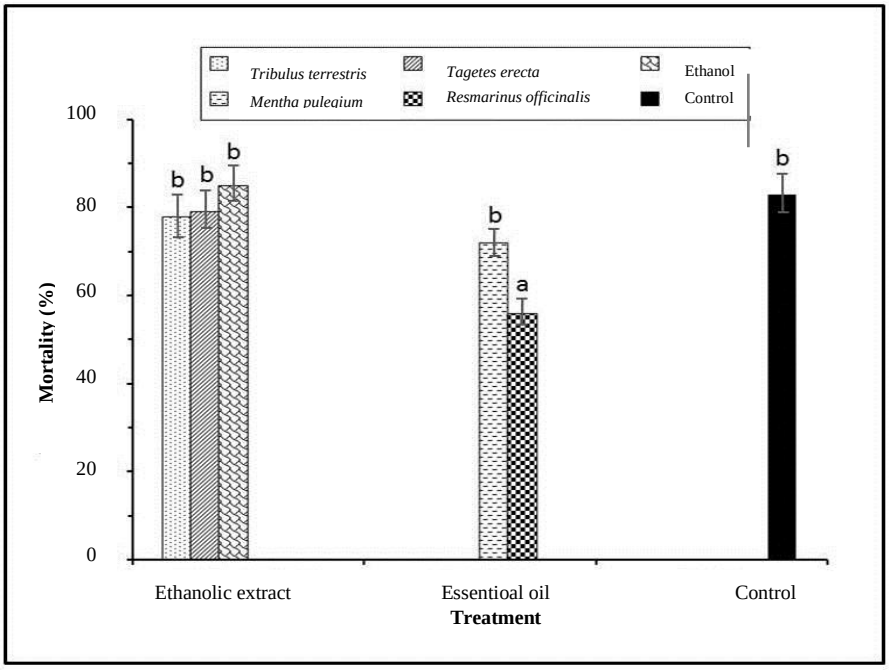

Fig. 5. The effect of ethanol extract of *Tribulus terrestris* and *Tagetes erecta* and essential oils of *Mentha pulegium* and *Rosmarinus officinalis* on eggs hatching rate of green lacewing.

Discussion

About the impact of used material on N_1 and N_5 nymphs mortality, it can be said that the ethanol extract of *T. terrestris* has The highest effect on pistachio psylla nymphs primarily. But it showed more effective on N_1 nymphs compared to N5 nymphs. Because, N_1 nymphs are more sensitive. After that, ethanol extract of *T. erecta* stands on the second place and ethanol has the least effect on nymphs. The results show that both extracts have not significant difference together. But they have acceptable effect in controlling the nymphs of this pest. Of course, this extraordinary effect is justified according to the previous experiments such as: the results of Dharmagdda *et al.* (2005) who examined the effect of *T. erecta* essential oil on fourth instar larvae of the *A. aegypti*, *A. stephensi* and *C. quinque-fasciatu*; nematicide effect of *T. erecta* expressed by Siddiqui Mansoor and Alam (1988); pesticide effect of ethanol extract of *T. terrestris* on *Z. subfasciatus* (Weaver *et al.,* 1994) and considering the results of the experiment done by Islam and Talukder (2005) who analyzed the effect of seed and leaves extracts of *T. terrestris* against *T. castaneum*. According to the results, essential oils had no effect on mortality of nymphs. This is due to the properties of volatile plant essential oils.

The results of experiments related to mortality effect of substances on larvae of the green lacewing, indicate that the mortality rate of *T. terrestris*, *T. erecta* extracts on green lacewing 1-2 days old larvae is more than other treatments. Among essential oils, *M. pulegium* had the highest effect that it showed not significant difference with ethanol and *R. officinalis.* But the plant extracts and essential oils had not mortality on green lacewing 5-6 days' old larvae. This is due to the sensitivity and being small of 1-2 days old larvae than 5-6 days old larvae. Although this effect is very minimal in comparison with its impact on some pests such as three species of stored product pests that Ayvaz *et al.* (2010) demonstrated that *M. pulegium* causes 100% mortality on them. Therefore, it can be said that these essential oils are safe for *C. carnea* five-day old larvae. The analysis and comparison of effect rate of *T. terrestris* and *T. erecta*

ethanol extracts and essential oils and ethanol on eggs hatching rate of green lacewing showed that among these treatments only *R. officinalis* essential oil has negative effect on hatching rate of eggs. So its use is not recommended because this essential oil has a negative effect on the natural enemy. Ethanol extracts had no effect on hatching rate of the natural enemy. So with proper timing can be used in pest control.

This study was carried out to replace chemical pesticides with plant compounds and finally, according to the least effect of used materials on mortality of the green lacewing as natural enemies, it is hoped that, after conducting more researches, we will be able to use them in further pest management programs, especially controlling *A. pistaciae*.

Considering that the ethanolic extract of the two plants has a good effect on pistachio psylla control and minimal side effects on natural enemy, green lacewing, in addition to preparation ethanol extract of these plants are negligible cost, the formulation of recent plant extracts is possible as a plant insecticide.

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