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EFFICACY OF TWO ESSENTIAL OIL EXTRACTS AND PREDATORY MITE *Phytoseiulus persimilis* IN SUPPRESSING THE POPULATION OF THE TWO-SPOTTED SPIDER MITE, *Tetranychus urticae* KOCH

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ABSTRACT

The potential of two plant extracts (peppermint *Mentha piperita* L. and clove *Syzygium aromaticum* L.) and a predatory mite species (*Phytoseiulus persimilis* Athias-Henriot) as well as their combinations were evaluated for their effect in reducing the population densities of the two spotted spider mite, *Tetranychus urticae* Koch, on detached mulberry leaves (*Morus nigra* L.).

The results of control pest experiment on the eight treatments showed that the total of spider mite populations was significantly different among treatments ($F_{7,\ 136}$ = 32. 74; $P < 0.001$). Likewise, the reduction percentage of the spider mite populations was significantly different among on the seven treatments ($F_{6,\ 105}$ = 3.01; $P < 0.01$). The highest total average number of spider mite population reduction was exhibited in the combination of *P. persimilis* with peppermint and clove together from all treatments

The total average number of spider mite population in the combination of *P. persimilis* with peppermint and clove together was significantly highest from all treatments (9.07 individual; with 97.93% redaction; LSD; $P < 0.05$), except the combination of *P. persimilis* with peppermint extract (9.67 individual; with 97.52% redaction), the combination of *P. persimilis* with clove extract (11.19 individual; with 96.55% redaction) or *P. persimilis* alone (11.28 individual; with 96.36% redaction). The lowest total average number of spider mite population was recorded for the clove extract alone, which was significantly different from all treatments (36.37 individual; with 83.17% redaction; LSD; $P < 0.05$) except peppermint extract alone (24.66 individual with 89.12% redaction).

These experiments suggested that releasing *P. persimilis* alone or its combinations are more favourable strategy for suppression of spider mite *T. urticae*.

Keywords: Plant extracts; peppermint *Mentha piperita*; clove *Syzygium aromaticum*; predatory mite *Phytosiulus persimilis*; spider mite *Tetranychus urticae*.

INTRODUCTION

Problems associated with the use of synthetic insecticides led researchers to look for natural plant protection compounds such as botanical insecticides. Botanical products are useful tools in many pest management programs because they are effective and specifically target plants natural enemies (Isman, 2006).

The two spotted spider mite, *Tetranychus urticae* Koch, is polyphagous and can devastate many crops (Walter and Proctor, 1999). It is widely distributed throughout many agricultural systems causing great economical loss to many crop species (e.g. Easterbrook *et al.,* 2001). *Tetranychus urticae* control in Egypt has been almost exclusively on pesticides. Unfortunately, spider mite has developed resistance to most available pesticides and the loss of acaricidal efficacy as a result of resistance mite populations is the major problem encountered (Ay, 2005). There is no doubt that widespread indiscriminate pesticide application causing pollution to the health hindered the successful application of such control. So, the intensive use of acaricides in the last few years not acceptable in the modern criteria of integrated pest management (IPM) programs, leading to an increasing interest for alternative pesticides which derived from natural plants (Nauen *et al*., 2001). Many predaceous phytoseiid mites are now used as biological control agents in various agricultural ecosystems, and are important predators of phytophagous mite populations in IPM programs on outdoor and greenhouse crops. *Phytoseiulus persimilis* Athias-Henriot is one of the most important specialist indigenous predators of tetranychid mites (McMurtry and Croft, 1997). It is widely found on various crops and it is considered one of the main predatory mites used in IPM in Egypt. Recently, the extracts of plants have provoked interest as sources of natural products (El-Sharabasy, 2010). Since the discovery of pyrethroids, Phytochemicals biological activities have demonstrated a great utility as pharmaceuticals and pest-management agents since decades ago (Choi *et al*., 2004). Essential oils with potential and or their constituents extracted from herbs have been used as flavouring and fragrances in food, beverage and cosmetic industries. Also they have been recognized to repel insects for at least as long, and in recent years have been demonstrated to have both contact and fumigant toxicity to insect pests (Choi *et al*., 2004; Jang *et al*., 2005). There is also growing evidence that certain essential oils are effective antibacterial and antifungal agents (Opender and Dhaliwal, 2001).

The interaction between a pest species and its single biocontrol agent or a combination of multiple agents is a controversy that has been addressed by both biological control practitioners (Ehler, 1990) and theoreticians (May and Hassell, 1981; Kakehashi *et al.,* 1984; Hassell and May, 1986).

A laboratory experiment was conducted using the two plant extracts (peppermint *Mentha piperita* and clove *Syzygium aromaticum*) and a predatory mite species (*Phytoseiulus persimilis*) to assess whether a single or a combination of two or three control agents would provide the maximum control of the two-spotted spider mite, *T. urticae* on detached mulberry (*Morus nigra*).

MATERIALS AND METHODS

For evaluating the effect of the two plant extracts (namely peppermint *Mentha piperita* and clove *Syzygium aromaticum*) and the predatory mite, *P. persimilis* as well as their combination against the two-spotted spider mite, *T. urticae* Koch.

Rearing Technique of Mites:

A pure culture of the phytophagous mite, *T. urticae* was reared at the Acarological greenhouse in Agric. Zoology and Nematology Dept., Faculty of Agriculture, Al-Azhar University.

Phytophagous mites, *T. urticae*, were cultured on the lima bean plants in the greenhouse. Three plants were put in a large tray (50 x 35 x 20 cm) and infested with 50 gravid spider mites. Each plant pot was placed on an empty inverted pot to avoid water logging the plant roots, as the tray contained water to prevent the mites from escaping. Two infested plants were replaced with fresh uninfested plants once a week to ensure the continuation of the culture.

Predatory mite species, *Phytoseiulus persimilis* was obtained from at Agricultural Research Center, Giza, Egypt. It was reared on two-spotted spider mite. *P. persimilis* were reared using methods modified from (McMurtry and Scriven, 1965), large plastic boxes 26 x 15 x 10 cm. were used, cotton pad were placed in the middle of each box, leaving a space provided with water as a barrier to prevent predatory mites from escaping. Excised bean leaves highly infested with *T. urticae* were provided every day as food source for mites. Water was added to the plastic pan whenever required, to prevent the mites from escaping. The culture units for all species were kept at room temperature (25 ± 3 °C).

Extraction Procedures:

Extraction by water: Plant parts (Leaves of the peppermint and cloves of the clove) were collected and dried at Pharmacological Department, Faculty of Pharmacy, British University at Alshrouk City, Cairo, using 200 gm powder of each were extracted for 24/h in methanol (MeoH) (1 gm: 7ml methanol: 3ml Water), and then blended for 15 minutes using conical Flask and maceration the mixtures 3 days with shaking each day and then filtrate the mixtures. The mixtures were transferred to shaking bottles in a rotary evaporator adjusted to 60°C until dry. Evaporate the mixtures till draymen total extract. The crude extracts were weighed and kept in refrigerator. Series of dilutions of the plant extracts were prepared using distilled water to make the concentrations.

Experimental design

An experimental Petri dish (10 cm diameter) consisted of a mulberry leaf disc (6 cm diameter) kept upside down on a filter paper (9 cm), which was placed on the same-sized saturated cotton wool (1 cm thick). Water was replaced, as required, to prevent the mites from escaping and to keep the leaf disc turgid.

A total of 40 experimental Petri dish was divided into seven treatments and a control, with five replicates in each treatment. The experimental Petri dishes were maintained at 25 ± 3°C, 16L: 8D photoperiod and 60 ± 15 % RH.

Experimental procedure

The experiment started with 100 eggs and 10 healthy gravid spider mite females placed on each mulberry leaf disc. To obtain eggs, leaf discs were infested with 14-18 healthy, gravid spider mite females for 24 hours. The number of eggs was adjusted to 100 by removing or adding eggs with a fine camel hairbrush, also the healthy gravid spider mite females was adjusted to 10 individuals.

The leaf discs with the laid eggs were dipped in the tested concentration for 10 seconds, and the excess solution was dried off by filter paper (Tawadrous, 2005).

Into each replicate firstly for a single agent: (six predatory mite individuals or a plant extract with concentration 12%), next each two agents: (three predatory mite individuals with a plant extract with concentration 6%) or both plant extracts concentration 6%). Finally all of them: two predatory mite individuals with both of the plant extracts with concentration 4%) as shown in table 1. The control had 10 spider mite females and 100 spider mite eggs without any predatory mite species or any plant extracts.

Table 1: Number of predatory phytoseiid mite species and/or different percentage of the plant extract concentrations in each treatment

Treatments	No. of predatory females	Extract concentrations
Phytoseiulus persimilis (P)	6	-
Peppermint extract (M)	-	12%
Clove extract(C)	-	12%
P. persimilis and peppermint (PM)	3 +	6%
P. persimilis and clove (PC)	3 +	6%
Peppermint and clove (MC)	-	6% + 6%
P. persimilis, peppermint and clove (PMC)	2 +	3% + 3%
Control (spider mites only)	-	-

The reduction percentages of the average population number of phytophagous species were calculated according to the equation of Henderson and Titton, 1955).

$$\text{Reduction} = 1 - \frac{\text{Treatment after x control before}}{\text{Teaiment before x control after}} \times 100$$

Statistical analysis: One-way analysis of variance (ANOVA) and mean comparison using Fisher's least significant difference (LSD) were conducted for the number of spider mite, using the software packages SPSS 16.0.0 (USA) for windows. Values observed in mixed treatments and the values expected on the basis of single treatments were compared using Student's t-test. Significance was tested at $P \leq 0.05$.

RESULTS AND DISCUSSION

The two plant extracts (peppermint *Mentha piperita* L. and clove *Syzygium aromaticum* L.) and a predatory mite species (*Phytoseiulus persimilis* Athias-Henriot) as well as their combinations were used to evaluate their effect in reducing the population densities of the two spotted spider mite, *Tetranychus urticae* Koch, on detached mulberry leaves in the laboratory.

Figure 1 shows that the relation between time (day) and the mean average numbers of the spider mite (individual) for the different life stages of the control treatment. Also, Figure 2 shows that the same relation between time (day) and the mean average numbers of the spider mite (individual) but for the previously mentioned seven treatments and the control.

Overall daily changes in spider mite populations and their extinction

Control group: For the life stages (Figure 1), the number of spider mite eggs increased until day four and then the population decreased due to hatching of the initial eggs to movable immature stages (larvae, after that they moulting to nymphal stages). The number of the movable immature stages increased from day four to eight, and then stabilised until day 10. After that, the immature stages decreased as some of them moulting to adult stage and the rest were passed on to reached to zero on day 17. After day 11, the adults started to increase until day 17, hence the number of adults decreased due to food limitation (the leaf resources were exhaust) and extinction on day 18. As a whole, the total spider mite population started to increase rapidly until day five, then stabilised until day 7 and reached to the top on day eight (285 individuals; table 2).

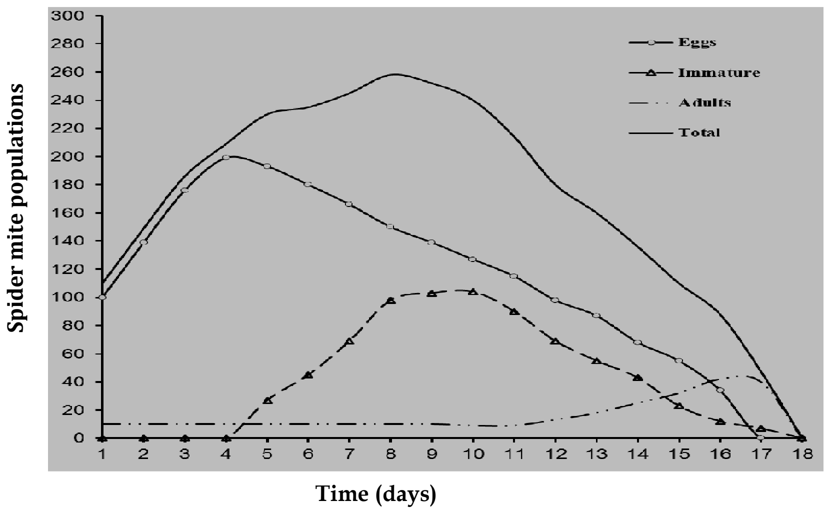

Figure 1: Population size of different spider mite stages (Control treatment) and the total through the experiment

After day 10, the population decreased sharply due to death of the majority of different stages due to the exhaustion of plant leaf juice and, the population slowed to extinction on day 18. The daily total average number of spider mite in this treatment (the control) was 169.39 individual).

Treatment groups: After spraying the plant extracts and releasing the predatory species, the total average number of spider mite population had significant difference among treatments ($F_{7, 136}$ = 32. 74; P < 0.001).

Table 2 shows that the average number of spider mite population and the reduction percentage for all treatments, on detached mulberry leaves.

The combination of *P. persimilis* with both plant extracts (the three agent together) exhibited the lowest total average number of spider mite which was significantly different from all treatments (9.07 individual; LSD; $P < 0.05$; Table 2), except the combination of *P. persimilis* with peppermint extract (9.67 individual), the combination of *P. persimilis* with clove extract (11.19 individual) or *P. persimilis* alone (11.28 individual). The highest total average number of spider mite population was recorded for the clove extract alone, which was significantly different from all treatments (36.37 individual; LSD; $P < 0.05$) except peppermint extract alone (24.66 individual; Table 2). The observed value of prey peak density for any combination was significantly lower than the expected value ($P < 0.05$; Table 2) calculated from the single treatment data.

Table 2: The population average numbers of spider mite, *T. urticae* their expected values and their corresponding reduction percentage (%) by the predatory mite species, peppermint, clove and their combinations as well as the control treatment

Treatments	Observed Average ± SE	Expected#	Max.	Reduction %
Phytoseiulus persimilis (P 6)	11.28 ± 6.89ab	-	110.00	96.36%$_{A}$
Peppermint extract (M 12%)	24.66 ± 8.36$_{b}$c	-	110.00	89.12%$_{AB}$
Clove extract (C 12%)	36.37 ± 9.94c	-	110.00	83.17 % $_{B}$
(P 3 + M 6%)	9.67 ± 6.38a	17.97	110.00	97.52% $_{A}$
(P 3 +C 6%)	11.19 ± 6.61ab	23.82	110.00	96.55% $_{A}$
(M 6% + C 6%)	12.03 ± 6.38ab	30.51	110.00	96.27% $_{A}$
(P2+ M4% +C4%)	9.07 ± 6.28a	24.10	110.00	97.93% $_{A}$
Control (spider mites only)	169.39 ± 17.74d	-	285.00	-

In all cases the average of spider mite populations (Eggs, adults and total of spider mite; Figure 1) declined immediately after spraying the plant extracts and releasing the predatory mite species (showing type-I dynamics).

It was driven to extinction within the shortest time by the combination of *P. persimilis* with both plant extracts together*,* the combination of *P. persimilis* with peppermint extract and the *P. persimilis* alone on day 5. After that, there was the combination of *P. persimilis* with clove extract on day 6, then the combination of peppermint with clove extracts on day 8, next was the peppermint extract alone on day 9. Lately, it was the clove extract alone on day 12, compared with control treatment (Figure 3), which slowed to extinction on day 18 (Figure 2). However, the total population peak density showed no significant difference among the seven treatments. Which had the same peak density of spider mite populations (110 individuals), which was the original starting number.

Figure 2: Effect of single or combined biocontrol agents (the two plant extracts (peppermint and clove) and a predatory mite on extinction time of spider mite populations

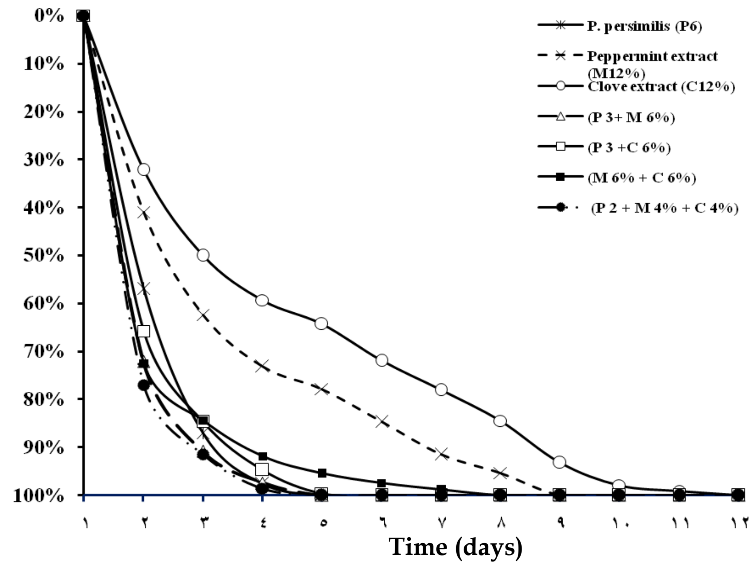

Figure 3: Shows the reduction percentages of spider mite species, *T. urticae* under different treatments

Similarly, there was a significant difference in the redaction percentages of the spider mite populations among the different treatments ($F_{6, 105}$ = 3.01; P < 0.001; Table 2), with the same pattern. The investigation was taken daily, until the reduction percentages of phytophagous mite were reached to 100% as shown in Figure 3.

The combination of *P. persimilis* with both plant extracts together exhibited the highest reduction percentage of spider mite which was significantly different from all treatments (97.93% redaction; LSD; P < 0.05; Table 2), except the combination of *P. persimilis* with peppermint extract (97.52% redaction), the combination of *P. persimilis* with clove extract (96.55% redaction) or *P. persimilis* alone (96.36% redaction). The lowest reduction percentage of spider mite population was recorded for the clove extract alone, which was significantly different from all treatments (83.17% redaction; LSD; P < 0.05) except peppermint extract alone (89.12% redaction; Table 2). Several studies have been conducted in different countries to assess the effect and potential of plant extraction for controlling the pest without the use of pesticides without economic damage to the crop (e.g. Hoda *et al*., 1989; Kotb, 2003; ElMougy and Alhabeb, 2009; Hussein *et al*., 2013. Successful biocontrol can be obtained in many cases (e.g. Brødsgaard and Enkegaard, 1997; Messelink *et al.,* 2005& 2006). Our result is closely and agree with Gorski and Piatekm *(*2008) and El-Zemity *et al*. (2009) who recorded that the peppermint oil was the best extract to control of *T. urticae* with 92.70% redaction.

The predatory mite *P. persimilis* alone or its combination was good at driving spider mite populations to extinction rapidly. These results agree with (Gould, 1971; French *et al.,* 1976; Mori and Saito, 1979), *Phytoseiulus persimilis* type I as a specialist predator of all species of genus *Tetranychus* (McMurtry and Croft, 1997); and could provide the best control of this pest, *T. urticae.*

In conclusion, the experiment suggested that releasing *P. persimilis* alone or its combinations are more favourable strategy for suppression of spider mite *T. urticae* than spraying by the two tested plant extracts each of them alone, which maybe require more spray times through the season while the *P. persimilis* maybe require one release.

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كفاءة اثنـان مـن المسخلصـات النباتيـة والمفتـرس الاكاروسـى *Phytosiulus persimilis* في وقف تعداد الأكاروس النباتي ذو البقعتين الـ *Tetranychus urticae*

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تم استخدام نوعين من المسخلصات النباتية (مستخلص أوراق النعناع الـ Peppermint *Mentha piperita* ومستخلص قرون الـقرنفل الـ Clove *Syzygium aromaticum*) والمفترس الاكاروسـى الـ *Phytosiulus persimilis* كلاً على حدا (منفردا) أو اشتراك كل اثنين معا أو اشتراك الثلاثـة فى آن واحد لدراسة وتقييم تأثيرهم فى خفض تعداد الأكـاروس العكنبـوتى الـ Spider mite, *Tetranychus urticae* فى المعمل على اوراق التوت المنفصلة.

وقد اوضحت نتائج تجربة مكافحـة الآفـه علـى وجـود اختـلاف معنـوى فـى تعداد الأكـاروس العنكبـوتى بين المعـاملات المختلفـة ($F_{7,\ 136}$ = 32. 74; P < 0.001). وايضـاً يوجد اختلافَـاً معنويـاً فـى نسبة خفض تعداد الأكاروس العنكبوتِى الـ *T. urticae* بين المعاملات ($F_{6,\ 105}$ = 3.01; P < 0.01).

وقد أظهرت معاملة اشتراك أو وضع الثلاث عناصر معا (رش كلا من مستخلص أوراق النعنـاع الـ Peppermint وقرون الـقرنفل الـ Clove مع اطـلاق المفترس الأكاروسـى الـ *P. persimilis*) الأقل تعدادا للأكاروس العنكبوتى الـ *T. urticae* عن بـاقى المعـاملات حيث كانـت (9,07 فرد اكـاروس عنكبـوتى بمعدل نسبة خفض 97,93% LSD; P < 0.05) باستثناء كلا من معاملـة المفترس الاكاروسـى الـ *P. persimilis* مـع مسـتخلص النعنـاع الـ Peppermint (9,67 فـرد أكـاروس عنكبـوتى بنسـبة خفـض 97,52%) أو اشـتراك المفتـرس الاكاروسـى الـ *P. persimilis* مـع مسـتخلص الـقرنفل الـ Clove (11,19 فرد أكـاروس عنكبـوتى بنسبة خفض 96,55%) او المفترس الاكاروسـى الـ *P. persimilis* منفردا (11,28 فرد أكاروس عنكبوتى بمعدل نسبة خفض 96,36%).

وقد سـجل الرش بمستخلص القرنفل الـ Clove منفردا أعلـى تعداد للأكاروسـى العنكبـوتى الـ *T. urticae* حيث سجل (36,37 فرد أكاروس عنكبوتى بنسبة خفض 83,17% LSD; P < 0.05) باستثناء الرش بمستخلص النعناع الـ Peppermint منفردا حيث سجل (24,66 فرد أكاروس عنكبـوتى بنسبة خفض 89,12%).

وتوصـى التجربة أن اطلاق المفترس الاكاروسى الـ *P. persimilis* منفردا او مشتركاً مـع عناصـر اخرى يعطى استراتيجية المكافحة المفضلة لآفة الاكاروس العنكبوتى الـ Spider mite, *T. urticae*.