

Fumigant Toxic Action and Repellent Effects of Plant Essential Oils against Two Spotted Spider Mite *Tetranychus urticae* koch (Acari: Tetranychidae)

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ABSTRACT

Three plant essential oils extracted by hydro-distillation from three medicinal plants namely, clove, *Eugenia caryophyllata* (Myrtaceae), basil, *Ocimum basilicum* and peppermint, *Mentha piperita* (Lamiaceae) were tested for their fumigant toxic action and repellency effects against adults of the two spotted spider mite, *Tetranychus urticae*. A filter paper diffusion bioassay without allowing direct contact and dipping method respectively was used. Responses varied according to oil type and dose. In a petri dish 90x10mm as a test chamber at the high dose 200 µl /L air, clove oil gave 100% mortality after 24h of exposure against adult *T. urticae* whereas 87.5 and 85.1% mortality were obtained with basil and peppermint oils respectively. The LC₅₀ values of essential oils of clove, basil, and peppermint against adult *T. urticae* were 32.2, 66.7 and 62.5 µl/L air, respectively. This indicates that the vapors of essential oil of clove exhibited the highest toxic effect on mites whereas basil and peppermint showed relatively the least toxic action. Essential oils of clove, basil, and peppermint proven to have repellent effects with ED₅₀s of 1.63, 3.43 and 4.88% respectively. The present results show that essential oils of the tested plants have the potential to be used in integrated pest management programs as safer alternative to conventional acaricides to control *T. urticae* on economic plants in commercial agriculture.

keywords: Medicinal plants, Essential oils, Natural acaricides, *Tetranychus urticae*.

INTRODUCTION

Plant essential oils are aromatic oily liquids isolated from different plant parts and widely used as food additives. They have received much attention as resources of new types of pest control agents with novel modes of action (Isman, 2000). The components of these essential oils are mainly monoterpenes and sesquiterpenes. These compounds called secondary plant metabolites that play a vital role in plant defense against insects and pathogens via toxicity and repellency effects (Karban & Chen, 2007; Eldoksch & El-Sebae, 2009). Many essential oils and their active constituents have been reported to exhibit various antifungal, antibacterial, insecticidal and insect antifeeding properties (Saleh et al., 1984; El-Sebae, 1987; Isman, 2000; Choi et al., 2004; Eldoksch & El-Sebae, 2005; Eldoksch et al., 2009).

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most serious pests of vegetables, fruits and ornamental plants in Egypt and worldwide (Eldoksch et al., 1991; Hosny et al., 2003). It is well documented that about 1,200 plant species of which more than 150 are economically important have been reported as the mite's host (Zhang et al., 2003). Control of *T. urticae* is primarily dependent on repeated application of conventional synthetic acaricides. Although effective, their repeated use led to side effects on the environment including disruption of natural biological control systems, development of mite resistance, persistent residues in soil and water and human health hazards.

Under greenhouse conditions mite reproduction is high and life cycle is short and repeated use of synthetic pesticides or acaricides resulted in rapid resistance to acaricides in the mite population (Sertkaya et al., 2010). It has been reported that spider mites have developed resistance to more than 80 synthetic acaricides and recorded as more than 400-times (Wu et al., 1990; Dagli and Tunc, 2001). As a result the efficacy of the pesticides or acaricides being reduced and the cost of chemical control continue to be increased.

These problems have highlighted the need for the development of selective mite control alternatives, particularly with fumigant action (Lee et al., 2004) for application in greenhouses where ventilation can be controlled. Plant essential oils may be an alternative source of new compounds for mite control in greenhouses because they are volatile chemicals mostly non toxic to mammals, birds and fish, and are commonly used as fragrances and flavoring agents for food and beverages. Furthermore, they act against insects and mites via neuro-toxic effects by which they affect octopamine pathways and GABA-gated chloride ion channels (Grundy & Still, 1985; Enan, 2001). The lack of octopamine receptors in vertebrates likely accounts for the profound mammalian selectivity of essential oils as insecticides (Kostyukovsky et al., 2002). So, many research efforts have been focused on plant essential oils as potential sources of commercial pest control agents (Isman & Machial, 2006).

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The present work was conducted to investigate the fumigant toxicity and repellency effects of essential oils extracted from three plant species namely, clove, *Eugenia caryophyllata* (Myrtaceae), basil, *Ocimum basilicum*, and peppermint, *Mentha piperita* (Lamiaceae) against the two spotted spider mite *T. urticae* Koch. Acaricidal mode of action of the tested plant essential oils is also discussed.

MATERIALS AND METHODS

Plant material and essential oils extraction method

Three aromatic plants namely clove, *Engeniacaryophyllata*, basil, *Ocimum basilicum*, and peppermint, *Mentha piperita* were used for essential oil extraction and acaricidal activity evaluation. Fresh leaves of basil, peppermint and ground clove buds (500 g each) were subjected to hydrodistillation for 4h and the oils were extracted by a Clevenger-type apparatus. The oils were separated from water by dichloromethane extraction, dried with anhydrous sodium sulphate and then stored in sealed vials at low temperature prior to bioassays. The oil yield was calculated via the relation of the obtained volatile oil volume to the mass of plant material used in the extraction (Table 1.)

Tested organism

The two-spotted spider mite *T. urticae* used for the bioassay was reared and maintained on lima bean plants (Fabaceae: *Phaseolus lunatus* L.). Seedlings 3-4 weeks after germination at a temperature of 27 ± 1 C, relative humidity of 70 ± 5 % and 12 h photophase were used. Adult stage of mites was used for different bioassay experiments.

Fumigant bioassay

The toxicity of the test essential oils to *T. urticae* was evaluated by a filter paper diffusion bioassay without allowing direct contact (Pascual-Vullalobos & Robledo, 1998). Lima bean leaves 3 weeks old were collected, and disks of 3cm in diameter were punched from each leaf. The leaf disks with adult mites (10 adults per leaf disk) were placed on four layers of filter paper in a Petri dish plate (90x10mm) which was used as a test chamber to evaluate the fumigant toxicity on adult mites. To prevent a direct contact between the mites and the tested oils, the various amounts of each test essential oil were applied to filter paper (5x2 cm) fixed on the inner surface of the petri dish. Each filter paper received the concentrations 1,2,4,8 or 10 microlitre (μ l) of essential oils using micropipette, which correspond to 20, 40, 80, 160 and 200 μ l L⁻¹ air (μ l /L air) respectively. Each plate was then covered with parafilm to prevent any loss of volatile essential oils. Each concentration was replicated five times with each replicate (a Petri dish) consisting of one disc with 10 adult mites. The control

consisted of a similar setup but without essential oils. Mortality was recorded after 24, 48 and 72h after exposure. Mites were counted as dead if no irritability was observed after stroking them with a fine hair brush using a stereoscopic microscope.

The LC₅₀ and LC₉₀ values were calculated at 24 h after treatment by probit analysis (Finney, 1971). Data were corrected for control mortality using Abbott's (1925) formula. Data also were analyzed using analysis of variance (ANOVA) and treatment means were compared using Duncan's multiple range test.

Repellent activity test

The repellency tests were made according to the method described by Kogan & Goeden (1970). Leaf disks of lima beans of 4.0 cm diameter were used to evaluate the repellent activity of the essential oils. Half of the disk was immersed for 5 seconds in an ethanol solution of the essential oil in four concentrations, 0.5, 1.0, 2.0 and 4% . After drying, the other half of the disk was immersed in pure ethanol, which served as control. Each half circle was immersed in such a way that an area of 0.3 cm between the two halves, where the mites were collected, remained intact. The leaf was collected on four layers of filter paper wetted by water in a petri dish 9 cm diameter. 10 female adults of mites were put on each disk, each treatment was repeated 5 times. The repellent activity evaluation was made after 24 h, where mites present on each half of the leaf disk were counted. Mites found in the neutral area were considered as repellent or attracted, based on their proximity to the blank or to the treatment. The Repellent Index (RI) of the oils was calculated according to the equation: $RI = (C - T / C + T) \times 100$ proposed by Pascual - Villalobos & Robledo (1998), Where C = number of mites on control diet, T = number of mites on treated diet. RI varying from -100 (Total attractancy) to +100 total repellency), with 0 meaning no effect.

RESULTS AND DISCUSSION

Fumigant toxic action of essential oils on adult mites

The fumigant toxicity of three plant essential oils extracted by water distillation from three aromatic plants (Table 1.) was evaluated against adult females of the two spotted spider mite *T. urticae* and the results presented in Table (2) in terms of LC_{50s}, LC_{90s}, their confidence limits and slope values. The data in Table (2) indicate that the clove oil vapors exhibited the most toxic action against the spider mite after 24 h of exposure with LC50 value 32.2 μ l /L air, followed by peppermint oil (62.5 μ l /L air) and then basil oil with LC₅₀ value of 66.7 μ l /L air.

The data also indicated that responses varied according to oil type and dose. The vapors of clove oil caused 100 % mortality to *T. urticae* at the high

concentration (200 μl /L air) 24 h post-treatment, whereas basil and peppermint oils produced 87.5 and 85.1% mortality, respectively.

Clove oil exhibited also 67.1 and 86.3% mortality at 80 μl /L air and 160 μl /L air, respectively whereas basil and peppermint oils at the concentrations 80 and 160 μl /L air caused 54.7 and 69.3% mite mortality respectively for basil oil ;and 58.6 and 78.5% mite mortality respectively for peppermint oil. The slope values for clove, basil and peppermint are 3.25, 1.63 and 1.88 respectively. Thus, it is shown that clove essential oil became more effective with increase in the concentrations. Basil and peppermint oils are of the lowest slope values showing that their efficacy on the mite are not as pronounced as clove oil.

Table (3) indicates the fumigant toxicities of tested essential oils after 48 and 72h of treatment. The data indicated that there is no significant differences between mean of percent mortality at 48 h (Fig.1) and that of 72 h post-treatment when applying the same oil concentrations except for peppermint oil at 200 μl /L air which exhibited 85.1 and 95.3% mortality after 48 and 72 respectively . Mortality in the solvent treated control was < 4% after 24 h and 4% after 48 and 72 of exposure. The promising fumigant toxicities against adult *T.urticae* in the present work may be due to such active compounds present in the tested essential oils (Bouwmeester et al., 1998). The main components of clove, basil and peppermint oils are the

monoterpenes eugenol, linalool and menthol respectively (Trease and Evans, 1985). Plant essential oils are potential products for *T.urticae* control because they have little or no harmful effects on nontarget organisms and the environment (Miresmaili et al.2006). The biological activity of clove, *E.caryophyllata* (Myrtaceae) which exhibited high fumigant toxicity against mites in the present work has been investigated (Chaieb et al., 2007) against many biological organisms, it has antifungal, antibacterial, antiviral, and insect repellent activity. Furthermore clove essential oil and its main constituent eugenol showed contact acaricidal activity against adult *T.urticae*(Eldoksch et al., 2009) and also against the poultry red mite, *Dermanyssus gallinae* De Geer, the most important haematophagous ectoparasite of domestic poultry (Soon- Il Kim et al.,2004).

The mode of action of plant essential oils and their bioactive components was suggested by Enan (2001) who indicated that the toxicity of constituents of essential oils against insect pests may be related to the octopaminergic nervous system of insects. There is another suggestion that some monoterpenes may inhibit cytochrome P450- dependent monooxygenases (De-Oliveira et al., 1997). Also a relationship between monoterpene toxicity or inhibition of acetylcholinesterase (AChE) and insecticidal or acaricidal activity was previously reported by Ryan & Byrne (1988). These demonstrate that the mode of action and target sites of essential oil monoterpenes are various.

Table 1. Plants used

| Latin Name | Family name | Common name | Part used | Oil yield (%) |
|------------------------------|-------------|-------------|-----------|---------------|
| <i>Eugenia caryophyllata</i> | Myrtaceae | Clove | Buds | 12.2 |
| <i>Mentha piperita</i> | Lamiaceae | Peppermint | Leaves | 1.5 |
| <i>Ocimum basilicum</i> | Lamiaceae | Basil | Leaves | 0.8 |

Table 2. Efficacy of different concentrations of essential oils on adults of two – spotted spider mite *T.urticae*

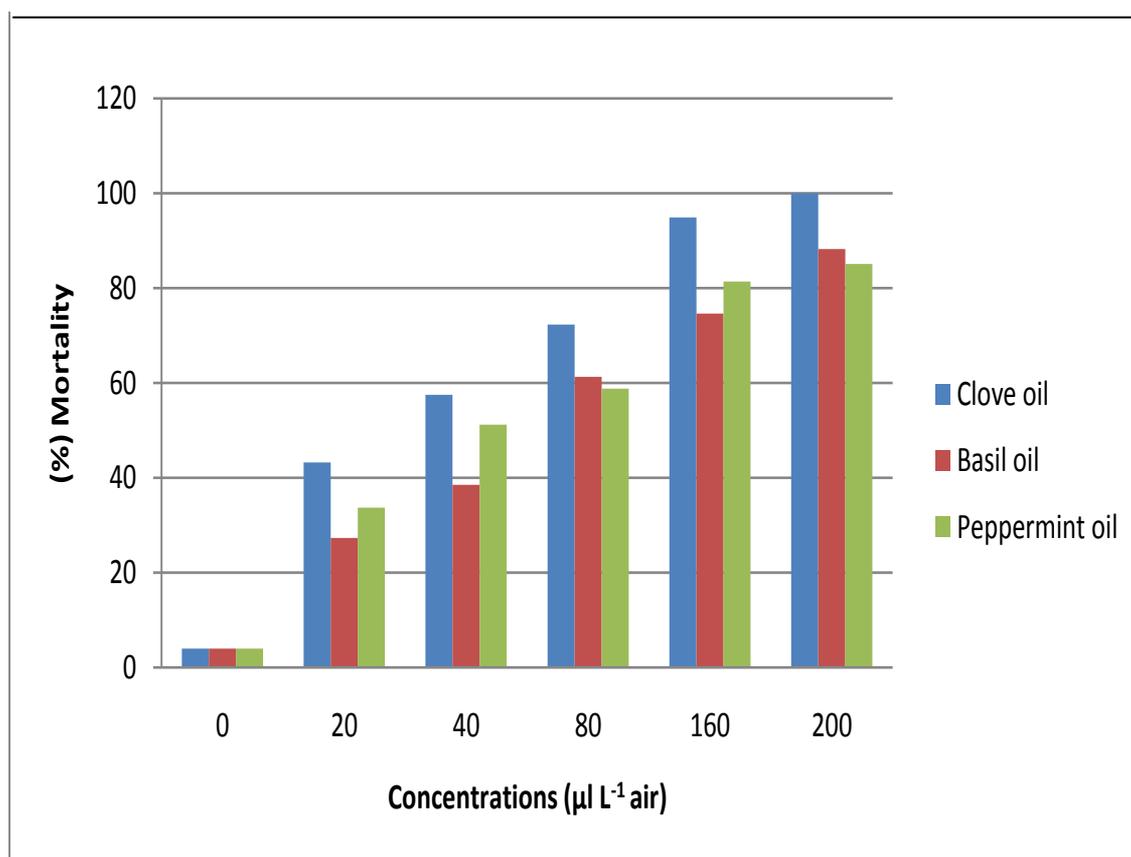
| Concentration (μL^{-1} air) | Mean % Mortality, LC ₅₀ , LC ₉₀ , confidence limits and slop values 24 h post-treatment | | |
|--|---|----------------|----------------|
| | Clove oil | Basil oil | Peppermint oil |
| 0.0 | 3.4f | 3.4f | 3.4e |
| 20.0 | 38.2 e | 22.5e | 24.2d |
| 40.0 | 55.6 d | 36.2d | 41.2c |
| 80.0 | 67.1c | 54.7c | 58.6b |
| 160.0 | 86.3b | 69.3b | 78.5a |
| 200.0 | 100a | 87.5a | 85.1a |
| LC ₅₀ | 32.2 | 66.7 | 62.5 |
| (confidence limits) | (26.8-38.4) | (55.5-80.0) | (52.0-75.0) |
| LC ₉₀ | 174.75 | 245.2 | 301.2 |
| (confidence limits) | (145.6-209.7) | (204.3-294.2) | (251.0-361.4) |
| Slope value \pm SE | 3.25 \pm 0.4 | 1.63 \pm 0.2 | 1.88 \pm 0.2 |

Mean values followed by different letters in the same column differ significantly at 0.05 level according to Duncan's multiple range test.

Table 3. Toxicity of different concentrations of volatile oils on adults of two-spotted spider mite *T. urticae*

| Concentration μL^{-1} air | Mean % Mortality 48 and 72h posttreatment | | | | | |
|---|---|-------|-----------|-------|----------------|-------|
| | Clove oil | | Basil oil | | Peppermint oil | |
| | 48h | 72h | 48h | 72h | 48h | 72h |
| 0.0 | 4.0e | 4.0e | 4.0f | 4.0e | 4.0d | 4.0e |
| 20.0 | 43.2d | 45.7d | 27.3e | 31.5d | 33.7c | 35.3d |
| 40.0 | 57.5c | 57.5c | 38.5d | 38.5d | 51.2b | 54.6c |
| 80.0 | 72.3b | 78.7b | 61.3c | 63.0c | 58.8b | 62.3c |
| 160.0 | 94.9a | 100a | 74.6b | 77.1b | 81.4a | 83.5b |
| 200.0 | 100a | 100a | 88.2a | 88.2a | 85.1a | 95.3a |

Means followed by different letters in the same column differ significantly at 0.05 level according to Duncan's multiple range test.

**Fig. 1. Toxicity of Different Concentrations of Essentials Oils on Two- Spotted Spider Mite 48h after treatment****Repellency effects against adult *T. urticae***

As shown in table (4) repellency index (RI %) and ED_{50} for the essential oils of the tested plants against *T. urticae* adult was calculated.

The data indicate that repellency index was increased with increase in concentrations of the essential oils. Concerning clove oil treatment at concentrations 0.5, 1.0, 2.0 and 4.0 % repellency index (RI%) were 20, 38, 71 and 88%, respectively. The same trends were

exhibited for the other two essential oils of basil and peppermint that showed increase in level of repellency index (RI%) when concentrations were increased (Fig.2).

The data also showed that ED_{50} values of clove, basil and peppermint essential oils were 1.63, 3.43 and 4.88%, respectively (Table 4). This results revealed that clove oil, from *E. caryophyllata* buds exerts the greatest repellency effect on the tested mite followed in a descending order with basil oil and then peppermint oil.

The current results are in agreement with that reported by (Mansour et al., 1986) who indicated that different essential oils from 14 species of Lamiaceae caused mortality and showed repellency effects in adult mite, *T.cinnabarinus*(Boisd.).

Also, (Choi et al., 2004) indicated that rosemary oil from *Rosmarinusofficinalis* has contact toxicity while the oils of caraway seed and lemon eucalyptus have fumigant activity and repellency effects against *T.urticae*. It is of interest to mention that the commercial formulation of rosemary oil based pesticides (Hexicide, Ecol, Sporam) gave high toxicity against *T.urticae*but it does not have acute toxicity on the predatory mite *Phytoseiuluspersimilis* showing that commercial

formulation based on plant essential oils can be effectively used in an integrated pest management program (Isman&Machial, 2006).

In conclusion and based on the results obtained from the current research it can be stated that essential oils from clove buds, basil and peppermint leaves proved to have fumigant toxicity and repellency effects against the two spotted spider mite, *T.urticae* and could be used for mite control as environmentally safe natural acaricides.

These results are in accordance with those obtained by El-Sayed (1989) who recorded that the hatchability were statistically insignificant in all conducted treatments of larval starvation throughout the rearing period of the Eri - silkworm *Philosamia ricini* Boisd.

Table 4. Repellency Index, ED₅₀, ED₉₀ and slop value of essential oils on adult mite *T.urticae*

| Essential oil | Concentration (%) | Repellency Index (%) | ED ₅₀ (%) | ED ₉₀ (%) | Slop value |
|----------------|-------------------|----------------------|----------------------|----------------------|------------|
| Clove oil | 0.5 | 20 | 1.63 (1.33-1.95) | 4.35 (3.62-5.22) | 3.35 |
| | 1.0 | 38 | | | |
| | 2.0 | 71 | | | |
| | 4.0 | 88 | | | |
| | | | | | |
| Basil oil | 0.5 | 6 | 3.43 (2.85-4.11) | 6.52 (5.43-7.82) | 2.81 |
| | 1.0 | 14 | | | |
| | 2.0 | 38 | | | |
| | 4.0 | 60 | | | |
| | | | | | |
| Peppermint oil | 0.5 | 8 | 4.88 (4.06-5.85) | 7.68 (6.40-9.21) | 2.27 |
| | 1.0 | 6 | | | |
| | 2.0 | 26 | | | |
| | 4.0 | 38 | | | |
| | | | | | |

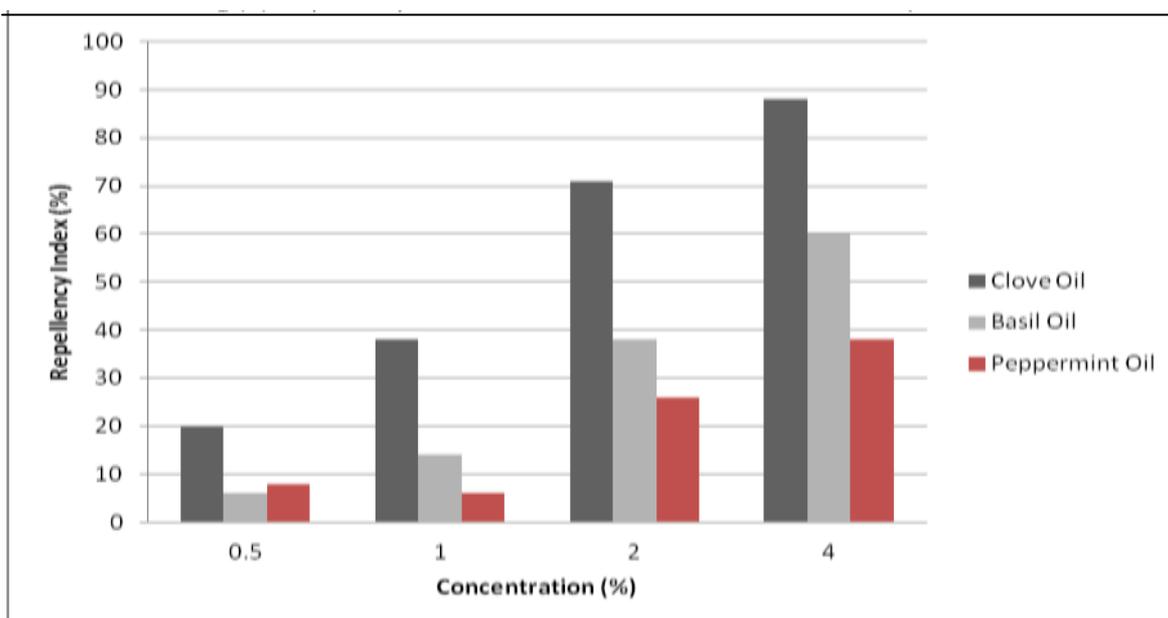


Fig. 2. Repellency Index of Essential Oils on Adult Mite *Tetranychus urticae*

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الملخص العربي

دراسة الفعل السام البخارى والتأثير الطارد لثلاثة من الزيوت النباتية ضد الحلم العنكبوتى ذو

البقعتين تترانيكس يورتيكا

حمدي علي الدكش، يوسف محمد دوير، عنتر قناوى

نسبة قتل 87.5، 85.1% على الترتيب وكان التركيز الذى يقتل 50% من الأفراد المعاملة هو 32.2، 62.5، 66.7 ميكروليتر/لتر هواء لكل من القرنفل، النعناع الفلفلى والريحان على الترتيب وتوضح النتائج أن بخر زيت القرنفل اظهرت التأثير السام البخارى الأعلى بينما زيوت الريحان والنعناع الفلفلى فقد أظهروا التأثير السام البخارى الأقل نسبياً. أوضحت النتائج أيضاً أن الزيوت الأساسية للقرنفل والريحان والنعناع الفلفلى تملك تأثير طارد ضد الأفراد الكاملة للحلم العنكبوتى وكان التركيز الذى يطرد 50% من الأفراد المعاملة يعادل 1.63، 3.43، 4.88% على الترتيب. وهذا يوضح أن الزيوت النباتية المختبرة لها تأثير سام بخارى وأيضاً فعل طارد ضد الحلم العنكبوتى ومن المحتمل استخدامها في برامج لمكافحة المتكاملة للآفات كبداية للمبيدات الأكاروسية التقليدية لمكافحة الحلم العنكبوتى على النباتات الاقتصادية في الزراعات التجارية.

تم استخلاص ثلاثة من الزيوت النباتية الأساسية المتطايرة بواسطة التقطير المائى من ثلاثة نباتات طبية وعطرية وهى القرنفل (براعم)، الريحان (أوراق) والنعناع الفلفلى (أوراق) وتم دراسة الفعل السام البخارى والتأثير الطارد لهذه الزيوت ضد الأفراد الكاملة للحلم العنكبوتى ذو البقعتين تيترانكسيورتيكا باستخدام طريقة الانتشار بورق الترشيح بدون السماح بالتلامس المباشر وطريقة الغمر على الترتيب. درجة الاستجابة للحلم العنكبوتى اختلفت تبعاً لنوع الزيت المستخدم والتركيز المطبق. تم استخدام أطباق بتري سعة 10×90 مم كغرفة اختبار.

أوضحت النتائج أنه عند التركيز العالى 200 ميكروليتر/لتر هواء فان بخر زيت مستخلص براعم القرنفل أعطى 100% نسبة قتل للأفراد الكاملة للحلم العنكبوتى (الأكاروس) بعد 24 ساعة من التعرض بينما زيت مستخلص الريحان والنعناع الفلفلى فقد أعطى