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# Insecticidal activity of some medicinal plant essential oils combined with Proteus® against greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) under greenhouse conditions

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## Abstract

*Trialeurodes vaporariorum* (Westwood) is one of the most important pests of vegetable and ornamental crops in the world. Generally, control of *T. vaporariorum* is based on the application of insecticides, but they are resistant to many synthetic chemicals. There is a necessity to evaluate non-chemical and low-risk ways against the pest. Accordingly, medicinal plants are promising because they are safe to human, eco-friendly and biodegradable components. However, chemical insecticides are an inevitable component of agricultural products. Hence, in the present study, we examined the synergistic/antagonistic interactions between *Agastache foeniculum* (Pursh) Kuntze, *Cuminum cyminum* L., *Ferulago angulata* (Schlecht) Boiss, *Foeniculum vulgare* Mill., *Origanum vulgare* Mill., *Satureja hortensis* L., *Trachyspermum ammi* I. Sprague and *Ziziphora tenuior* L. essential oils with Proteus® against *T. vaporariorum* adults and eggs. Probit analysis of essential oils showed that *O. vulgare* and *T. ammi* were the most effective essential oils against *T. vaporariorum* adults that both exhibited $LC_{50}$ values equivalent to 0.44 µL/L air. *F. vulgare* essential oil had the highest mortality against the eggs of *T. vaporariorum* ($LC_{50}$= 30.60 µL/L air). All essential oils synergized efficacy of Proteus® against adults of *T. vaporariorum*. The inclusion of *Z. tenuior* essential oil with Proteus® led to antagonistic interaction against *T. vaporariorum* eggs.

**Key words:** *Trialeurodes vaporariorum*, Medicinal plant, Essential oil, Proteus®, Synergistic effect

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## Introduction

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) is an important and significant pest in greenhouses in the most parts of the world (Wang *et al*., 2003; Malais & Ravensberg, 2004). *T. vaporariorum* decreases the yield and quality of plants due to direct feeding and transmission of many plant viruses (Skaljac *et al*., 2010). The pest sucks cell sap of the leaves and produces honeydews that adversely impacts plant photosynthesis (Ali *et al*., 2005). *T. vaporariorum* has a very vast host range consist of more than 300 types of 82 plant families that the most important ones are Cucurbitaceae, Solanaceae, Fabaceae and among the greenhouse vegetables, the most important ones are tomatoes, cucumbers and some ornamentals like chrysanthemum, poinsettia etc. It is well documented that chemical control is an effective method to control the pests (Pavela, 2009) and chemical insecticides are still important management tools of the greenhouse whitefly (Wang *et al*., 2003). Proteus® (the new commercial mixture of thiacloprid and deltamethrin) is a powerful and wide-spectrum insecticide (Sekeroglu *et al*., 2011). It is a contact and systemic insecticide. In recent decades, many new insecticides with different mode of actions have been developed for the management of whiteflies. Pesticides can cause serious risks to human health and the environment, so natural components such as plant materials could be useful, since they are easy to extract, safe and do not persist in water and soil (Isman, 2000). We selected Proteus® because it is a powerful and wide-spectrum insecticide with short pre-harvest interval and low effect on natural enemies (Elbert *et al.,* 2001). This insect has also developed immunity to many conventional insecticides. Several essential oils have been found to be effective against all insect stages, including eggs, nymphs and adults of *T. vaporariorum* without adverse effect on beneficial insects. Therefore, we examined some medicinal plant essential oils (*Agastache foeniculum*, *Cuminum cyminum*, *Ferulago angulata*, *Foeniculum vulgare*, *Oreganum vulgare*, *Satureja hortensis*, *Trachysperum ammi* and *Ziziphora tenuior*) against the pest. We also examined the antagonistic/synergistic interactions between mentioned essential oils combined with Proteus® against *T. vaporariorum* eggs and adults.

## Materials and Methods

### Insect rearing

Adults of *T. vaporariorum* were collected by an aspirator from the greenhouse of Department of horticulture at Urmia University, Urmia, Iran. Trials were carried out in the years 2015 and 2016 in greenhouse of Department of Plant Protection, Faculty of Agriculture, Urmia University, Urmia, Iran. The colony was mass reared on tomato (an important host of greenhouse whitefly). The collected adults were transferred on the tomatoes with 4-5 leaves. Experiments were conducted after three generations of *T. vaporariorum* rearing. Trials were carried out under greenhouse conditions at 27 ± 2 °C, 65±5% RH and a photoperiod of 16 L: 8 D. In order to access a cohort, following Muniz and Nombela technique (2001), small cages with a few changes were used.

### Insecticide

The insecticide used was Proteus® (110 OD, 100 g/l thiacloprid and 10 g/l deltamethrin, Bayer CropScience, New Zealand) that is a broad spectrum insecticide.

### Essential oils

Essential oils were extracted from seeds of *T. ammi*, *C. cyminum* and *F. vulgare* and leaves of *S. hortensis*, *Z. tenuior*, *F. angulata* and *O. vulgare* and flowers of *A. foeniculum*. They were subjected to hydro distillation using a modified Clevenger-type apparatus.

## Essential oils

## Bioassays

Bioassay trials were carried out following Rahman and Schimdt technique (1999) and Negahban *et al*. (2007). The concentration ranges of essential oils were determined by preliminary dose setting experiments. In this respect, filter papers were placed in the cap of leaf cages and impregnated with the required concentration of essential oils, then they covered with parafilm. One tomato leaf was placed in each leaf cage, then 20 one to two-days-old adults of *T. vaporariorum* were transferred into the leaf cages by an aspirator. They were exposed to vapors of essential oils of *A. foeniculum*, *C. cyminum*, *F. angulata*, *F. vulgare*, *O. vulgare*, S. *hortensis*, *T. ammi* and *Z. tenuior* (before the cages were capped). These experiments were carried out under greenhouse conditions at 27 ± 2 °C, 65±5% RH and a photoperiod of 16 L: 8 D. Each concentration had three replications and each experiment was replicated three times. Mortality was counted after 24 h. To examine the sensitivity of egg hatch of the pest to the essential oils, one tomato leaf containing 20 one-day-old eggs of *T. vaporariorum* was placed in leaf cages. They were exposed to vapors of essential oils of *A. foeniculum*, *C. cyminum*, *F. angulata*, *F. vulgare*, *O. vulgare*, S. *hortensis*, *T. ammi* and *Z. tenuior*. These trials were carried out similar to adults. Mortality was recorded after 10 days. Each experiment was replicated three times.

## Insecticide

Trials were carried out following Prabhaker *et al*. (1988) method with a slight modification. Tomato leaves were dipped in determining concentrations of Proteus® that were determined by preliminary dose setting experiments. For controls, the leaves were dipped in distilled water. When the water evaporated and leaves were dried, 20 one to two-days-old adults of *T. vaporariorum* were transferred into leaf cages by an aspirator. These experiments were carried out under greenhouse conditions at 27 ± 2 °C, 65 ± 5% RH and a photoperiod of 16L : 8D. Mortality was counted after 24 h. Each experiment was replicated three times. In order to survey egg hatch of greenhouse whitefly, one tomato leaf containing 20 one-day-old eggs of *T. vaporariorum* was dipped in determining concentrations of Proteus® that were determined by preliminary dose setting experiments. After water evaporating and the leaves were dried, they were placed in leaf cages. These trials were carried out similar to adults. Mortality was recorded after 10 days. Each experiment was replicated three times.

## Plant essential oils combined with Proteus®

In order to assay whether there was an antagonistic or synergistic interaction between essential oils combined with Proteus®, sub-lethal concentrations ($LC_{25}$) of essential oils were combined with $LC_{25}$ of Proteus®. Therefore, one tomato leaf was dipped in sub-lethal concentration of Proteus®. When the water evaporated and the leaf was dried, the leaf was placed in leaf cage and 20 one-day-old adults were transferred into leaf cages. Then $LC_{25}$ of essential oils were immersed with filter paper. Finally, the leaf cage was covered with parafilm. This trial was carried out for all essential oils separately. Mortality was recorded after 24 h. Each experiment was replicated three times. To examine whether the mentioned essential oils synergize performance of Proteus® against the eggs of *T. vaporariorum*, one tomato leaf containing 20 one-day-old eggs was dipped in sub-lethal concentration of Proteus®. When the water evaporated and the leaf was dried, the leaf was placed in leaf cage. Then filter paper was immersed in $LC_{25}$ of essential oils. Finally, the leaf cage was covered with Parafilm. This trial was carried out for all essential oils separately. Mortality was recorded after 10 days. Each experiment was replicated three times.

## Data Analysis

In order to determine $LC_{50}$ and $LC_{25}$ values, the data were analyzed using the probit procedures with SPSS for Windows® release 16. The percentage data were transformed into $\arcsin\sqrt{x}$ before statistical analysis. To determine synergistic/antagonistic interactions, experiments were conducted following

Gisi (1991). The relationship between data was assayed by analysis of variance (ANOVA) and correlation analysis. The means were separated by using the Tukey test.

## Results

According to table 1, *T. ammi* and *O. vulgare* showed the highest efficacy against *T. vaporariorum* adults, which both exhibited $LC_{50}$ value equivalent to 0.44 µL/L air.

جدول ۱- تجزیه پروبیت سمیت برخی از اسانس‌های گیاهی روی حشرات کامل سفیدبالک گلخانه

**Table 1- Probit analysis of the toxicity of some essential oils to *T. vaporarium* adults**

| Compound | $\chi^2$ | Slope ± S.E | $LC_{25}$ (µL/L air) | $LC_{50}$ (µL/L air) | $LC_{90}$ (µL/L air) |
|---|---|---|---|---|---|
| *A. foeniculum* | 1.65 | 3.28 ± 0.85 | 1.76 | 2.12 | 2.96 |
| *C. cyminum* | 1.70 | 2.61 ± 0.70 | 2.80 | 3.52 | 5.08 |
| *F. angulata* | 1.93 | 2.26 ± 0.66 | 0.72 | 0.96 | 1.68 |
| *F. vulgare* | 1.77 | 2.00 ± 0.66 | 1.68 | 2.16 | 3.56 |
| *O. vulgare* | 2.68 | 1.78 ± 0.25 | 0.16 | 0.44 | 2.32 |
| *S. hortensis* | 2.78 | 2.97 ± 0.61 | 3.00 | 3.28 | 3.80 |
| *T. ammi* | 2.33 | 2.55 ± 0.51 | 0.28 | 0.44 | 1.04 |
| *Z. tenuior* | 3.02 | 3.22 ± 0.87 | 3.12 | 3.99 | 4.35 |

Probit analysis of Proteus® demonstrated that it was toxic for adults ($LC_{50}$= 1.11 ppm) (Table 2).

جدول ۲- تجزیه پروبیت سمیت پروتئوس روی حشرات کامل سفیدبالک گلخانه

**Table 2- Probit analysis of the toxicity of Proteus® to *T. vaporarium* adults**

| Compound | $\chi^2$ | Slope ± S.E | $LC_{25}$ (ppm) | $LC_{50}$ (ppm) | $LC_{90}$ (ppm) |
|---|---|---|---|---|---|
| Proteus® | 1.87 | 2.73 ± 0.51 | 0.73 | 1.11 | 2.44 |

Results presented in table 3 show that fennel had the highest toxicity on hatch eggs of greenhouse whitefly which exhibited $LC_{50}$ value equivalent to 30.60 µL/L air.

جدول ۳- تجزیه پروبیت سمیت برخی از اسانس‌های گیاهی روی تخم سفیدبالک گلخانه

**Table 3- Probit analysis of the toxicity of some essential oils to *T. vaporarium* eggs**

| Compound | $\chi^2$ | Slope ± S.E | $LC_{25}$ (µL/L air) | $LC_{50}$ (µL/L air) | $LC_{90}$ (µL/L air) |
|---|---|---|---|---|---|
| *A. foeniculum* | 1.76 | 5.21 ± 0.68 | 36.80 | 50.80 | 68.40 |
| *C. cyminum* | 2.57 | 2.08 ± 0.65 | 42.00 | 54.00 | 88 |
| *F. angulata* | 1.34 | 3.89 ± 0.71 | 39.90 | 51.04 | 93.24 |
| *F. vulgare* | 1.53 | 1.21 ± 0.20 | 2.40 | 3.60 | 6.44 |
| *O. vulgare* | 3.14 | 2.32 ± 0.31 | 34.40 | 50 | 100.48 |
| *S. hortensis* | 1.32 | 1.80 ± 0.09 | 70.40 | 75.6 | 86.36 |
| *T. ammi* | 0.97 | 2.17 ± 0.40 | 23.60 | 38.80 | 98 |
| *Z. tenuior* | 1.49 | 1.90 ± 0.11 | 67.60 | 74.40 | 89.76 |

Albeit, essential oil of ammi was toxic for eggs of the pest ($LC_{50}$ value = 38.80 µL/L air). Proteus® exhibited $LC_{50}$ value equivalent to 31.08 ppm against the eggs of *T. vaporariorum* (Table 4).

جدول ۴- تجزیه پروبیت سمیت پروتئوس روی تخم سفیدبالک گلخانه

**Table 4- Probit analysis of the toxicity of Proteus® to *T. vaporarium* eggs**

| Compound | $\chi^2$ | Slope ± S.E | $LC_{25}$ (ppm) | $LC_{50}$ (ppm) | $LC_{90}$ (ppm) |
|---|---|---|---|---|---|
| Proteus® | 3.80 | 2.82 ± 0.35 | 20.01 | 31.08 | 88.39 |

The inclusion of *A. foeniculum*, *C. cyminum*, *F. angulata*, *F. vulgare*, *O. vulgare*, S. *hortensis*, *T. ammi* and *Z. tenuior* essential oils with Proteus® led to synergistic interaction against *T. vaporariorum* adults (Table 5).

جدول ۵- اثر سینرژیستی برخی از اسانس‌های گیاهی و پروتئوس روی حشرات کامل سفیدبالک گلخانه

**Table 5- Synergistic interactions between some essential oils and Proteus® against *T. vaporarium* adults**

| P + essential oils | %mortality ± S. E. | | interaction |
|---|---|---|---|
| | Expected | Observed | |
| P + EO of *A. foeniculum* | 58.01 ± 1.80 | 73 ± 2.82 | synergism |
| P + EO of *C. cyminum* | 57.69 ± 2.12 | 81 ± 3.03 | synergism |
| P + EO of *F. angulata* | 76.62 ± 2.25 | 83 ± 2.98 | synergism |
| P + EO of *F. vulgare* | 76.73 ± 2.56 | 80 ± 3.03 | synergism |
| P+ EO of *S. hortensis* | 76.72 ± 2.00 | 80 ± 3.00 | synergism |
| P+ EO of *T. ammi* | 56.69 ± 2.05 | 70 ± 2.03 | synergism |
| P+ EO of *Z. tenuior* | 76.00 ± 2.45 | 80 ± 2.99 | synergism |

In the case of *T. vaporariorum* eggs exposed to a mixture of all essential oils (except for *Z. tenuior*) and Proteus®, the synergistic interaction was recorded (Table 6). The inclusion of *Z. tenuior* essential oil with Proteus® led to antagonistic interaction against *T. vaporariorum* eggs (Table 6).

جدول ۶- اثر سینرژیستی برخی از اسانس‌های گیاهی و پروتئوس روی تخم سفیدبالک گلخانه

**Table 6- Synergistic and antagonistic interactions between some essential oils and Proteus® against *T. vaporarium* eggs**

| **P + essential oils** | **%mortality ± S. E.** | | **interaction** |
|---|---|---|---|
| | **Expected** | **Observed** | |
| P + EO of *A. foeniculum* | 70.20 ± 2.01 | 75 ± 1.90 | synergism |
| P + EO of *C. cyminum* | 56.69 ± 2.05 | 80 ± 4.03 | synergism |
| P + EO of *F. angulata* | 56.69 ± 2.05 | 80 ± 4.03 | synergism |
| P + EO of *F. vulgare* | 70.00 ± 2.95 | 83 ± 3.13 | synergism |
| P+ EO of *S. hortensis* | 56.72 ± 1.35 | 70.69 ± 2.34 | synergism |
| P+ EO of *T. ammi* | 76.69 ± 2.35 | 86.69 ± 4.00 | synergism |
| P+ EO of *Z. tenuior* | 45.53 ± 1.25 | 40 ± 0.78 | antagonism |

## Discussion

*T. vaporariorum* adults were susceptible to essential oils combined with Proteus®. In the present study, *T. ammi* and *O. vulgare* essential oils exhibited high insecticidal activity against adults of *T. vaporariorum*. Proteus® had a high efficacy against *T. vaporariorum* adults. Essential oils are valuable secondary metabolites obtained through steam distillation of medicinal plants and herbs (Yatagai, 1997). Most of them have no persistence and are non-toxic to human and environment (Hjorther *et al*., 1997). Khorrami and Soleymanzade (2016) investigated efficacy of some medicinal plant essential oils, extracts and Powders against adults of *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) and they found that essential oils of *T. ammi* and *F. vulgare* were effective on the pest ($LC_{50}$= 0.64 and 0.72 µL/L air, respectively). Regnault–Roger and Hamraoui (1993) evaluated the influence of aromatic essential oils of *O. vulgare* (L.), *Thymus vulgaris* (L.) and *Eucalyptus globules* Labill. on *Acanthoscelides obtectus* (Say) and they reported that *O. vulgare* resulted in more mortality of pest.

Our results showed that egg hatching was significantly reduced by *F. vulgare* essential oil. We demonstrated that in the case of *T. vaporariorum* eggs exposed to a mixture of Proteus® and essential oils, the synergistic interaction was recorded (except for *Z. tenuior*). Sampson *et al*. (2005) tested insecticidal activity of 23 essential oils on *Lipaphis pseudobrassicae* (Davis) and they showed that the most effective essential oils were *Rosmarinus officinalis* (L.), *S. hortensis*, *Mentha piperita* (L.) and *F. vulgare* (Miller), which elicited high fumigant toxicity. Choi *et al*. (2003) were tested 53 plant essential oils against eggs, nymphs and adults of *T. vaporariorum* and they reported that Pennyroyal and peppermint oils gave 89 and 83% mortality. They showed that caraway seed, lemongrass, oregano, and spearmint oils were the most effective compounds against eggs of the pest. Górski (2004) assayed the effectiveness of natural essential oils in the monitoring of greenhouse whitefly (*T. vaporariorum*). He noted that greenhouse whitefly (*T. vaporariorum*) reacted most intensively to *Ocimum basilicum* oil. The addition of this oil on yellow sticky traps caused a significant increase in trapped insects in comparison with the control (with no aromatic substance). Dehghani and Ahmadi (2013) demonstrated that the highest anti-oviposition effect after 3 days caused by essential oil of *A. millefolium* (67.77%), while the lowest anti-oviposition index was for aqueous extract of *C. cyminum* (48.78%). Also the aqueous extract of *C. cyminum* showed the highest repellent effect on the greenhouse whitefly after 3 d (66.11%). Thielert and Hungenberg (2007) investigated the biological effect of O-D (oil dispersion) formulation insecticides such as Proteus®, Confidor® and biscayain and also EC, SC and SE formulations against important sucking-biting insects like peach and cotton aphid. Their results showed that all the insecticides with O-D formulations were more effective than the others and had a better knockdown effect and durability. Generally, based on our results, interaction of essential oils with Proteus® has insecticidal effectiveness against *T. vaporariorum* adults and eggs. The selection of Proteus® and medicinal plant essential oils was based on low side effects on natural enemies, bio-degrability, lower-risk to beneficial organisms, human and environment. Hence, *A. foeniculum*, *C. cyminum*, *F. angulata*, *F. vulgare*, *O. vulgare*, S. *hortensis*, *T. ammi* and *Z. tenuior* essential oils combined with Proteus® can be applied to control the pest infestations as a part of an integrated pest management strategy. Although, the combination of mentioned essential oils combined with Proteus® is very effective for controlling the pest, but additional research is needed.

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# اثر حشره‌کشی تعدادی از گیاهان دارویی در ترکیب با پروتئوس روی سفیدبالک گلخانه تحت شرایط گلخانه‌ای

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## چکیده

سفیدبالک گلخانه *Trialeurodes vaporariorum* (Westwood) یکی از مهم‌ترین آفات سبزیجات و گیاهان زینتی در جهان است. به‌طور کلی کنترل این آفت بر پایه استفاده از حشره‌کش‌ها است، اما آن‌ها به بسیاری از مواد شیمیایی مصنوعی مقاوم شده‌اند. ارزیابی روش‌های غیر شیمیایی و کم‌خطر برای سفیدبالک گلخانه ضروری به نظر می‌رسد. بر این اساس، گیاهان دارویی به دلیل بی خطر بودن برای انسان، سازگار بودن با محیط زیست و خاصیت تجزیه پذیری، ترکیباتی امیدوار کننده می‌باشند. با این حال، ترکیبات شیمیایی اجزای اجتناب‌ناپذیر در تولیدات کشاورزی هستند. از این رو در تحقیق حاضر ما اثر سینرژیستی/آنتاگونیستی اسانس گیاهان گل مکزیکی *Agastache foeniculum*، زیره سبز *Cuminum cyminum*، چویل *Ferulago angulata*، رازیانه *Foeniculum vulgare*، مرزنجوش *Origanum vulgare*، مرزه *Satureja hortensis*، زنیان *Trachyspermum ammi* و کاکوتی *Ziziphora tenuior* را با حشره‌کش پروتئوس روی حشره کامل و تخم سفیدبالک گلخانه مورد بررسی قرار دادیم. تجزیه پروبیت اسانس‌ها نشان داد که مرزنجوش و زنیان روی حشرات کامل سفیدبالک بسیار موثر هستند به‌طوری‌که مقدار $LC_{50}$ این دو اسانس برابر با ۰/۴۴ میکرولیتر بر لیتر هوا بود. اسانس رازیانه بیشترین میزان مرگ و میر را روی تخم سفیدبالک گلخانه داشت (مقدار $LC_{50}$، ۳۰/۶۰ میکرولیتر بر لیتر هوا بود). ترکیب تمامی اسانس‌ها با پروتئوس دارای اثر سینرژیستی روی مرحله حشره کامل سفیدبالک گلخانه بود. ترکیب اسانس کاکوتی با پروتئوس روی مرحله تخم اثر آنتاگونیستی نشان داد.

**واژه‌های کلیدی**: سفیدبالک گلخانه، گیاه دارویی، اسانس گیاهی، پروتئوس، اثر سینرژیستی

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