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The effects of plant extracts of lemon-scented tea tree [*Leptospermum petersonii* (*Myrtaceae*)] on *Tetranychus urticae* Koch (*Acarina: Tetranychidae*)

Abstract

The two-spotted spider mite [*Tetranychus urticae* Koch (*Acarina: Tetranychidae*)] is a very serious pest worldwide, causing considerable damage to vegetables, flowers and fruit crops. Control is difficult mainly due to mite resistance to conventional acaricides. The application of plant extracts to control insects can be an effective alternative. In this study, the efficacy of extract obtained from *Leptospermum petersonii* Bailey (*Myrtaceae*) was tested as an alternative to conventional acaricides. Using two different methods, bioassays were used to determine the effects of varying concentrations (0.1, 0.3, 0.4 and 0.5%) of the extracts. Experiments were carried out using 3cm diameter leaf disks of *Phaseolus vulgaris* L. In addition, the effects of the extracts on mite reproduction and oviposition were investigated. In the leaf dipping method, the extract at 0.5% concentration caused the highest mortality in the nymph (100%) and adult (100%) stages. In the lowest concentration bioassays, the adult mites laid lower numbers of eggs compared to the untreated control. The highest effect occurred at a concentration of 0.5% while the lowest effect was at 0.1%. No ovicidal effects of the plant extracts were observed.

Keywords: Two-spotted spider mite, *Lepospermum petersonii*, acaricidal effect

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Introduction

Pesticides have been used since War II World to control pests and improve product quality. Especially in developed countries, the use of pesticides is much more. However, careless use of pesticides without adhering to the safety norms and recommended practice has created health risks to humans, natural enemies, and environment, to commodities containing pesticide residues.[1,2] Therefore, in recent decades, there is an increasing demand for food safety and quality such as strict regulations on the import of products the amount of residues in commodities. In addition, public noticed that the side effects of pesticides on environmental pollution and the safety foods. In this regard, scientists have focused on the search for alternatives to widely used chemical pesticides, including biopesticides.

Biopesticides are naturel materials derived from animals, plants and bacteria that are used for pest control.[3] The major source of biopesticides substances is the plant kingdom. Some plants contain some bioactive compounds that are biological actions against insects, including repellent, antifeedant, anti-ovipositional and growth regulatory activities.[4,5] The use of plant derivatives as an alternative to chemical insecticides has been studied throughout the world. It is determined that over 20,009 plant species have been reported to possess pest control properties.[6] The most famous examples are the neem tree, Azadirachta indica A.Juss. And Melia azaderach L (*Meliaceae*) These plants have been used for several thousands of years against insect pests. There are some commercial products from A.[7]

Lemon-scented tea tree oil (LSO), obtained from the native Australian plant *Leptospermum petersonii* (FM. Bailey), and has a range of chemical components. Information on the use of LSO is very limited. LSO have antifungal and insecticidal properties. There are very few studies on this subject. For example, Hood et al.[8] revelaed that essential oil volaties *L. petersonii* caused both directly and indirectly on both *Candida albicans* and *Aspergillus fumigatus* to produce growth inhibition. It was found that the extracts obtained from *L. petersonii* showed strong mortality effect against *L. decemlineata* larvae, and caused decrease in the number of eggs laid.[9] In addittion, this oil showed good antifungal activity against *Phytophthora cactorum* (Lebert & Cohn) Schroet, *Microsporum canis* (Bodin), *Trichophyton mentagrophytes* (Charles-Philippe Robin Sabour), and *Microsporum gypseum* (Guiart & Griggorakis).[10] Morover Erdogan and Sever,[11] revealed that LSO showed the highest insecticidal effect on *Phthorimae opeculella* Zeller (*Lep Gelechiidae*).

The two spider spotted mite *Tetranychus urticae* Koch (*Acarina: Tetranychidae*) is one of the most important pests which are responsible for yielding losses to many horticultural ornamental and agronomic crops. The mite has got about 1,200species of plants, of which more than 150 are economically significant.[12] According to Tsagkarakkou et al.[13] *T. urticae* causes remarkable economic loss by reaching high density, especially the conditions of greenhouse are appropriate to grow this mite. Therefore, the rate of damage, which causes this mite, is high throughout the year. *T. urticae* is produced by feeding a characteristic yellow speckling on the T. uritace produce silk webbing that is easily visible when the high population of this pest.[14] The purpose of the study was to determine the acaricidal activity of *L. petersonii* extracts on T urticae.

Materials and methods

Mite rearing

T. urticae was reared in the laboratory at 25±1°C and under long daylight (18light: 6dark) and 65%-70% relative humidity on potted bean. The bean plants (*Phaseolus vulgaris* L.) used in the experiment all was grown in greenhouse.

Effect of Lemon-scented tea tree oil on *T. urticae*

Ovicidal effect

Bean leaf discs were placed into petri dishes on moisturized filter paper and females of the same age were put on leaf discs. The eggs

were counted after two days. Ten eggs were placed in every petri dish and the other eggs removed. Then eggs were sprayed with different concentrations of extract (17-20µl/cm_2) using a small hand held sprayer. The numbers of hatched larvae were counted.

Acaricidal Effect on Larvae and Adult

Leaf-Dipping Method; from untreated bean leaves 3cm in diameter disc was punched out. These discs were then dipped into the test solutions (for extract 1%, 3%, 6%, 12% and Neem Azal T/S 500mL/100-liter water) for one minute. The control disc was dipped in 0.01% Triton X-100 solution. Then left to dry for 30minutes The treated leaf discs were placed into petri dishes, which lined with moistened filter paper. Then 10adults and larva of *T. urticae* were introduced onto the treated discs in separate petri dishes. Same procedure was used for control.[15]

Spraying Method, bean leaf discs were placed into Petri dishes on moisturized filter paper. Then 10adult *T. urticae* transferred onto the disc and using a hand held sprayer leaf discs were sprayed (17-20µl/cm2) with different concentration (1%, 3%, 6%, 12%), control (untreated) discs were sprayed with (0.01% Triton X-100). After the spraying left to dry for 15minutes when adults dried, the treated *T. urticae* was transferred to untreated leaf discs.[15]

Effect on egg laying capacity

Green bean leaf discs dipped for 3-5seconds in prepared concentrations then dried for 30minutes were placed in petri dishes with ten adults. After 48hours of feeding on treated green bean leaves, mites were given untreated green bean leaves. The experiment was repeated 10times. Daily monitoring was done for fourteen days and the total number of eggs was recorded.[16]

For all experiments, first instar larvae and 3-days old adults were used. In every experiment, 10immature stages and 10adult mites were used. Four concentrations and untreated control were used for all bioassays. Experiments were carried out using (3cm diameter) leaf discs of green bean leaves. Data collection started after 1, 3, and 6days by counting the number of living larvae and adults. The experiments were conducted in a climate chamber at 25±1°C and under long daylight (18:6 light: dark).

Statistical analysis

The effect was calculated according to Abbott.[17] The obtained results were submitted to a variance analysis and the mean values were compared by Duncan's test (P=0.05) calculated by the program SPSS 20.6.

Results and discussions

Ovicidal Effect

All of the eggs treated were hatched. It is determined that *L. petersonii* extract did not have an ovicidal effect. The hatched larvae continued to develop as it was control.

Acaricidal Effect on Larvae and Adult

Leaf-dipping method; as shown Table 1 & Figure 1, the mortality of larvae placed on leaf discs treated with concentrations of 0.1, 0.3, 0.4 and 0.5was 69, 80, 90 and 100 % respectively. The highest effect occurred at a concentration of 0.5% while the smallest effect was at 0.1%. Statistical analysis showed significant differences among the treatments, but there was no difference between concentrations 0.3, 0.4 and 0.5% (F= 26.84; P < 0.05).

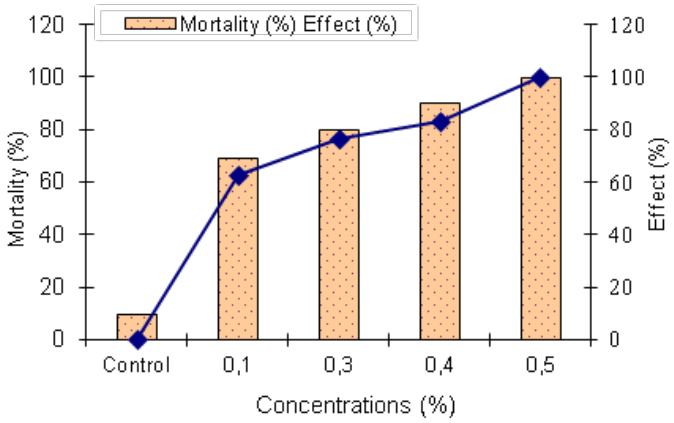

Figure 1 Mortality and effect of extract of *L. petersonii* on larva of *T. urticae*. (Leaf-dipping method)

Table 1 Mortality and effect of extract of *L. petersonii* on *T. urticae* (Mean ±SE)*

Concentrations (%)	Mortality (%)					
	Leaf-dipping method				Spraying method	
	Larva		Adult		Adult	
	Mortality (%)	Effect (%)	Mortality (%)	Effect (%)	Mortality (%)	Effect (%)
0.1	69	62.45±3.^{0b}	72	63.45±4.0^{b}	68	62.50±3.30^{b}
0.3	80	76.34±3.2^{a}	88	79.46±3.2^{a}	80	70.60±3.89^{ab}
0.4	90	83.23±2.4^{a}	96	96.34±2.1^{a}	88	78.89±3.47^{a}
0.5	100	100.00±0^{a}	100	100.00±0^{a}	90	87.67±2.52^{a}
Control	10	0	10	0	9	0

*Within columns, means ± SE followed by the same letter are not significantly different (Duncan's multiple range test)

The mortality of adult placed on leaf discs treated with concentrations of 0.1, 0.3, 0.4 and 0.5% was 69, 80, 90 and 100 % respectively. The highest effect occurred at a concentration of 0.5% while the smallest effect was at 0.1%. According to statistical analysis, the concentration of 0.1was different from other concentrations, but there was no difference between 0.3, 0.4, and 0.5concentrations (27.65; P < 0.05) (Table 1 & Figure 2).

Spraying method; according to the data in Table 1 and Figure 3, the mortality of spraying treated with concentrations of 0.1, 0.3, 0.4 and 0.5%was 68, 80, 88 and 90 % respectively. The highest effect

occurred at a concentration of 0.5% while the smallest effect was at 0.1%. Statistical analysis showed significant differences among the treatments, but there was no difference between concentrations 0.4 and 0.5% (F= 24.72; P < 0.05)

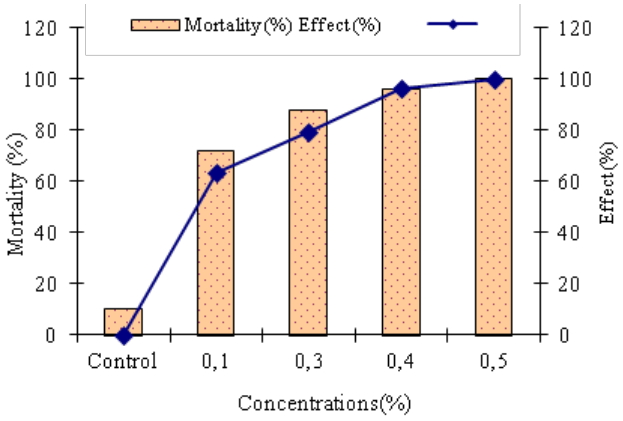

Figure 2 Mortality and effect of extract of *L. petersonii* on adult of *T. urticae* (Leaf-dipping method)

Effect on egg production

For all concentrations with the maximum number of eggs obtained from the control. The least number of eggs was found at 0.5%concentration with number of eggs laid reduced significantly by increasing concentration. Statistically analysis showed that control was different group from the other concentrations. There was no difference between concentrations 0.4 and 0.5 % (F=28.43; P< 0.05) (Table 2 & Figure 4).

Table 2 Effect number of eggs of extracts of *L. petersonii*

Concentrations (%)	Number of eggs
0.1	76.80 ± 5.45[b]
0.3	41.20± 4.15[ab]
0.4	17.25 ± 2.29[a]
0.5	12.80 ± 2.11[a]
Control	151.90 ± 6.31[c]

On *T. urticae* (Mean ± SE)*

In recent years, because of the adverse effect of pesticide use, alternative control methods have been researched for *T. urticae*. Some of the alternative control methods including acaricidal effects of the plant essential oils, plant preparations and secondary metabolites on two-spotted spider mites have been researched.[18,19] There were no references in the literature about the extracts *L. petersonii* on *T. urticae*. However, there are references of using extract of *L. petersonii* against other insects. For example, the efficacy of LSO was evaluated against the diamondback moth (*Plutella xylostella* L, *Lep Plutellidae*). According to this study feeding activity and development of larval stages were significantly reduced on broccoli leaves that had been dipped in LSO. Oviposition deterrence was also found when an adult stage was exposed to treated leaves. Fecundity dropped by 50% at concentrations of 0.5%. The LC50 value for third instar larvae was estimated to be 2.93% 7days after treatment. In addition, the oil was also tested at concentrations from 0.5 to 6% for oviposition deterrence of an egg parasitoid of the diamondback moth, *Trichogramma* pretiosum (Riley). LSO deterred parasitisation in choice tests but not in no-choice tests. LSO did not cause mortality of T. pretiosum during 24hours in a contact toxicity test. It was concluded that LSO had no significant effects on the parasitoid, and therefore LSO is compatible with this biocontrol agent for integrated management of the diamondback moth.[20] It was found that the extracts obtained from *L. petersonii* showed strong mortality effect against *L. decemlineata* larvae, and caused decrease in the number of eggs laid 9. Erdogan and Sever11 revealed that fourth instar larva of about were used in tuber dipping and larvae dipping methods. In tuber dipping method, the highest mortality (100%) occurred at concentration of 0.4% while the smallest mortality was at 0.05% the extracts of *L. petersonii*.

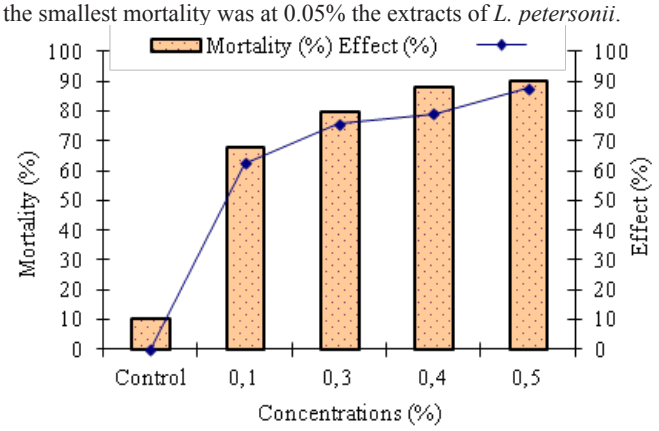

Figure 3 Mortality and effect of extract of *L. petersonii* on adult of *T. urticae* (Spraying method).

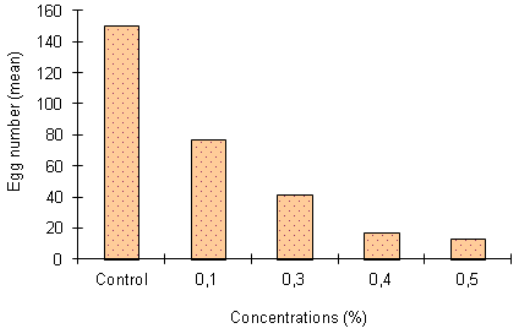

Figure 4 Effect number of eggs of extracts of *L. petersonii* on *T. urticae*.

LSO contains mainly monoterpenes, sesquiterpenes, with β-caryophyllene or globulol/viridiorol/ spathulenol as major components. The existence of a third chemotype, Penfold's 'variety B' contains geraniol acetate (21–38%) and geraniol (21–29%), as well as citral (neral and geranial) as principal components of the oil20. It is thought that these mainly substances are caused by a high rate of mortality. There are many of studies about the extracts Melaleuca alternifolia (Maiden&Betche) (*Myrtaceae*). *L. petersonii* is the same family to *M. alternifolia*. There are two commercial preparations obtained from the active ingredient *M. alternifolia*. These are called Gamma-T-ol (tea tree oil, 75% Terpene) and Fungatol (tea tree terpinene-4-ol). There are some references of studies done using these commercial preparations against mites and insects. For example, it was revealed that the highest mortalities were observed at 3.50% concentration of Fungatol and 3.60% concentration of Gamma-T-ol. The mortalities caused by 3.50 and 3.60% concentrations of Fungatol and Gamma-T-ol, respectively.[21] Also Kasap et al.[22] revealed that after 1, 24 and 48h, the highest concentration of Fungatol (3.50%) and Gamma-T-ol (3.605) obtained from *M. alternifolia* had caused 0, 18, 42% and 0, 20, 48.9% mortality on cotton aphid (*Aphis gossypii*) Glover, (*Hemiptera:Aphididae*) respectively. It was determined

that formulations (Fungatol, Gamma-T-ol, Fungatol plus neem and Gamma-T-ol plus neem) had LC50 values that indicated formulations were highly efficacious against cotton aphid.[23] It was revealed that the Fungatol-Neem spray (50.0-001) was found to be the most effective extract while Fungatol was moderately effective on the tomato leaf miner Tuta absoluta (*Lepidoptera*: *Gelechiidae*) under laboratory conditions.[24] In another study, Bayindir et al.[25] revealed that the commercial production named Fungatol, Gamma-T-ol, Fungatol plus neem and Gamma-T-ol plus neem showed negative effective on reproductively and development of M. persicae.

Conclusion

It was found out that the extract of *L. petersonii* had a high rate mortality two spotted spider mite. In both methods, similar results were obtained and there was not a significant difference on the mortality when leaf dipping method was compared with direct spraying on the plant. Increased concentration lead to increased larval mortality. The contact and feeding on treated leaves lead to high mortality of adults. The extract of *L. petersonii* can be useful to control *T. urticae* populations on vegetable plants grown through Integrated Pest Management (IPM) and organic systems of agriculture.

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Conflicts of interest

Conflict of interest Author declares there is no conflict of interest.

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