Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2024, 14(1), 8-16; doi: 10.15421/2024_543

ORIGINAL ARTICLE

Efficacy of different essential oils against the two spotted spider mites *Tetranychus utricae* (Acari: Tetraychidae) under laboratory conditions

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Received: *15 January, 2024; Manuscript No: UJE-24-125089;* ***Editor assigned:*** *17 January, 2024, PreQC No: P-125089;* ***Reviewed:*** *29 January, 2024, QC No: Q-125089;* ***Revised:*** *03 February, 2024, Manuscript No: R-125089;* ***Published:*** *12 February, 2024*

The risks posed by the application of synthetic insecticides against insects have led to significant environmental issues. Pest control strategies are being sought by the world to find alternative solutions. Various technologies, including microbial and botanicals, are included in the portfolio of bio-pesticide alternatives. Essential Oils (EOs) are significant category of botanicals that have gained significance due to their environmental safety. Insect pest and plant pathogenic fungi can be treated with essential oils, which possess a wide range of abilities such as insecticidal/antique-neutral, anti-fencing (anti-inflammatory), repellent/repellent, growth regulatory and anti–vector activities. Aromatic plants have given rise to their descendants through the development of diverse chemical defenses against insects during evolution. One of the most notorious and destructive arthropod pests, *Tetranychus urticae* Koch (Acari: Tetraychidae), is responsible for infesting more than 1000 plant species, including many economically important crops and ornamental plants. An investigation was carried out to determine the effectiveness of various essential oils against *Tetranychus Utricae*. The results indicated that the essential oils of lavender (*Lavendula officianlis*) had a 95% maximum toxicity against *Tetranychus urticae*. Citronella Java (*Cymbopogan winterianus*) essential oil showed a 90% toxicity level after the exposure. Two spotted spider mites (*Tetranychus urticae*) were found to be more susceptible to clove leaf (*Syzygium Aromaticum*) than Cardamom (Elettaria cardamum) essential oils. The conclusion was reached that essential oils had varying levels of toxicity to *Tetranychus urticae*.

Keywords: Pest control, Two Spotted spider mite (*Tetranychus utricae*), Citronella Java (*Cymbopogan winterianus*), Lavender (*Lavendula officianlis*), Clover leaf (*Syzygium Aromaticum*), Cardamom (*Elettaria cardamomum*), Toxicity.

Introduction

Two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is ubiquitous species, found on a variety of plants throughout the world. There are more than 1,200 plant species in this genus, including 150 economically important species (Zhang Z., 2003). Crops susceptible to mites are often protected with synthetic acaricide during hot and dry seasons, when severe spider mite outbreaks may occur (Riahi, E., et al., 2013). The Two spotted spider mite, *T. utricae* Koch (Acari: Tetranychidae), is one of the most common and destructive arthropods, parasitizing more than 1000 plant species, including economically important agricultural and ornamental plants (Attia, S. et al., 2013; Papapostolou, KM., et al, 2021). Spider mites use the perforated parts of

their mouths to suck sap from plants, leaving spots or wrinkles called spots. Mites feeding will reduce chlorophyll and water content, resulting in reduced yields due to reduced photosynthetic capacity (Cullen, E., et al, 2009).

It is common practice that, rotation of several insecticides with different modes of action to reduce the development of resistance against the abamium, aquinocil, bifenthrin, bifenazate, ethoxazole, fenpiroximate, hexythiazox and spirodiclofen that are regular sprayed for mites control (Piraneo, TG., et al., 2015; Wu, M. et al., 2019). However, some studies have reported that, the development of resistance to the pesticides is through target site sensitivity and/or high detoxification enzyme activity and some cases of complex resistance have also been observed (Adesanya, AM., et al., 2021; Cagaty, NS., et al., 2018; Xue, W., et al. ., 2020). Growers have no choice but to increase the concentration of active ingredients or use pesticides more frequently than before to keep the pest population below the level of economic injury, which can inevitably lead to unintended problems, including adverse effects on non-target organisms and the environment (Attia, S., et al., 2013).

The environmental problems caused by the overuse of pesticides have attracted the attention of scientists and the public in recent years. It is estimated that approximately 2.5 million tons of pesticides are applied to crops each year and global pesticide damage is estimated at $100 billion annually. The cause of this problem is the high toxicity and non-biodiversity nature of pesticides and residues in soil, water sources and plants that affect public health (Benelli, G., et al., 2017).

The risks associated with the use of synthetic insecticides have led to the development of an environmental movement seeking sustainable alternatives to pesticides. This evidence has led to the proliferation of organic agricultural products now found in major supermarkets and the banning of synthetic pesticides in many local areas (Christie, M., 2010). Among pest control alternatives, the global market for biopesticides in 2010 was around $1 billion. This market is expected to grow to $ 3.3 billion in 2014. The EO market has experienced the strongest growth of all botanical pesticide markets in recent years. Safety and regulatory issues have played an important role in this development. Its widespread use as an herbal remedy in Europe, Japan and North America has increased confidence in its safety. However, EOs, like many natural products, is not always rigorously tested or officially registered (Thakur, AK., et al., 1992).

Botanical pesticides are considered as a good alternative to synthetic pesticides because of their lethal and sub lethal effects, such as repellent, miticidal and oviposition deterrent activities to numerous pests and low mammalian toxicity (Benelli and Pavela, 2017b; Bougherra, HH., et al., 2015; Pavela, R., 2014). Plant essential oils generally consist of complex mixtures of mono and sesquiterpenoids and biogenetically related phenols such as 1, 8-cineole, eugenol, thymol and menthol (Isman, MB., et al, 2006).

Essential oils include lemongrass (*Cimbopogon winteriana*), Eucalyptus globulus, rosemary (*Rosemarinus officinalis*), vetiver (*Vetiveria zizanoides*), clove (*Eugenia caryophyllus*) and thyme (*Timus vulgaris*). Peppermint (*Mentha piperita*) repels ants, flies, fleas and ticks; pennyroyal (*Mentha pulegium*) repels ants, fleas, gnats, ants and caterpillars. Spearmint (*Mentha spicata*) and basil (*Ocimum basilicum*) are also effective in repelling mosquitoes. Likewise, essential oil plants such as *Artemesia vulgaris, Melaleuca* leucadendron, *Pelargonium rhododendron, Lavandula angustifolia, Mentha piperita and Juniperus virginiana* are effective against various insect and fungal pathogens (Kordali, S., et al., 2005).

Dabrowski, ZT. and Seredynska, U. (2007) revealed the acaricide properties of Allium aqueous extract on *T. urticae*. Garlic extract was tested at concentrations of 12.5, 25 and 50 g/l and showed that garlic treatment increased insect mortality. It is also effective against the establishment of insects, feeders and permanent feeders on the host plant. Garlic also showed inhibitory effects against human ticks (*Ixodes ricinus*) and *T. urticae* mites (Hincapie, CAL., et al., 2008). Various studies have shown that garlic can be useful in controlling the population of T. utricae in rice fields. Essential oils are effective against two spotted spider mites. This oil contains several chemicals that are toxic against *T. utricae* (Singh, UP., et al., 2001).

The present study was carried out to evaluate, the toxic effects of different essential oils against the adults of *T. Utricae* and to comparison of level of toxicity among essential oils at different level of concentration.

Materials and Methods

The experiments were carried out in the department of Plant protection, Institute of Agronomic Sciences and Slovak University of Agriculture, Nitra, Slovakia.

Insect rearing

Two spotted spider mites were collected from the agricultural fields in Slovak university of Agriculture, Nitra and reared in laboratory under controlled condition (RH: 55-80% and temperature 25 ℃).

Plants material

Beans plants were grown in pots in laboratory. Then these pots with healthy plants were transferred to rearing closed cage of mites for breeding and more population.

Essential oils

Four essential oils of Citronella Java (*Cymbopogan winterianus*), Lavender (*Lavendula officianlis*), Clove leaf (*Syzygium Aromaticum*) and Cardamom (*Elettaria cardamomum*) were tested.

Methodology

Leaf disc method was adopted to evaluate the impact of different Essential oils against two spotted spider mites. For this purpose, Leaf discs of 1.5 cm were prepared from healthy bean plants leaves. Then these leaves discs were dipped in solution of oils for 10 seconds. After this, leaf discs were kept in Petri dish at room temperature for 10 minutes for dry. After dry these leaves discs were kept on wet cotton foam, which was placed in petri dish to ensure no escaping. Wet cotton water soaked foam with water were considered as barrier for *T. utricae*. Mites were collected from breeding culture and placed on leaves discs with the help of camel hair brush. Each leaf disc had 5 adult mites. The experiment was replicated four times and data was recorded on hour interval basis after 2hrs, 4hrs, 6hrs, 8hrs and 12 hrs till 24 hours. Toxicity was observed on the basis of mortality. After specific hours the mites was gently touched with fine needle and no movement or slight movements was the indication of toxic effects of essential oil. Results were subjected through standard statistical software analysis.

Results

After all, finally the Experiments results showed that all essential oils showed toxicity toward the two spotted spider Mites. In case of citronella essential oil (*Cymbopogan winterianus*), the maximum toxicity was observed at high concentration 2.00% after 24 hours. It is also observed that mortality was started after 0.6% concentration but the mortality was not too much. So after increase the concentration percentage up to 2.00% leading toward the 0.8%, 1.00% maximum mortality was observed at 2.00%. It was 90% after 24 hours of application by using leaf disc method (Table 1).

Table 1. Mortality caused by Citronella (*Cymbopogan winterianus*) essential oil on *Tetranychus utricae* when applied at different concentrations in leaf disk bioassays.

Treatment	**Mean Mortality (%) ± SE**					
EO (v/v)	**2 h**	**4 h**	**6 h**	**8 h**	**12 h**	**24 h**
0.2%	0.0 ± 0.0b	0.0 ± 0.0d	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0d	0.0 ± 0.0d
0.4%	0.0 ± 0.0b	0.0 ± 0.0d	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0d	0.0 ± 0.0d
0.6%	0.0 ± 0.0b	0.0 ± 0.0d	5.0 ± 0.21c	10.0 ± 0.24c	15.0 ± 0.24c	15.0 ± 0.24cd
0.8%	15.0 ± 0.24a	15.0 ± 0.24c	20.0 ± 0.31b	30.0 ± 0.37b	30.0 ± 0.37b	30.0 ± 0.37bc
1.0%	15.0 ± 0.24a	25.0 ± 0.31b	25.0 ± 0.31b	30.0 ± 0.37b	35.0 ± 0.40b	40.0 ± 0.40b
2.0%	20.0 ± 0.31a	40.0 ± 0.40a	45.0 ± 0.48a	65.0 ± 0.67a	70.0 ± 0.73a	90.0 ± 0.73a

In the columns, the data followed by the same letter are not significantly different (P 0.05, LSD test).

After experiment, it was concluded that lavender Essential oil (*Lavendula officianlis*) start showing mortality at 0.6% concentration but after long time of application. During experiments 45% mortality was observed at 1.00% concentration. While maximum mortality 95% was observed at 2.00% concentration of lavender oil (Table 2). Clove leaf (*Syzygium Aromaticum*) essential oil have very low mortality at 0.6% and 0.8% concentrations. During experiments 40% mortality was observed at 1% while at 2% maximum

mortality up to 70% was observed (Table 3). Cardamom (Elettaria cardamomum) essential oil final results showed that, at the concentration of 0.4%, 0.6%, 0.8% mortality was not observed. While at 0.8% and 1.00% concentration mortality started and at 2.00% Concentration maximum mortality up to 65% was observed (Table 4). In case of comparison, it is concluded by the final results that, Lavender Essential oil showed maximum toxicity 95% against the two spotted spider mites. After the lavender essential oil maximum toxicity 90% was observed by citronella essential oil. In case of Clove leaf and cardamom E'o, it can be concluded clearly they showed mortality but Clove leaf essential oil showed high mortality than Cardamom E'o. Final results also concluded that, during experiments maximum toxicity 95% was observed by Lavender essential oils and minimum mortality 65% was observed by Cardamom essential oils.

Table 2. Mortality caused by Lavender (*Lavendula officianlis*) essential oil on *Tetranychus utricae* when applied at different concentrations in leaf disk bioassays.

Treatment	**Mean Mortality (%) ± SE**					
EO (v/v)	**2 h**	**4 h**	**6 h**	**8 h**	**12 h**	**24 h**
0.2%	0.0 ± 0.0b	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0c	0.0 ± 0.0d	0.0 ± 0.0d
0.4%	0.0 ± 0.0b	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0c	0.0 ± 0.0d	0.0 ± 0.0d
0.6%	0.0 ± 0.0b	0.0 ± 0.0cd	5.0 ± 0.20cd	10.0 ± 0.24c	15.0 ± 0.24c	15.0 ± 0.24cd
0.8%	15.0 ± 0.24a	15.0 ± 0.24bc	20.0 ± 0.37bc	30.0 ± 0.37b	30.0 ± 0.37b	30.0 ± 0.37bc
1.0%	15.0 ± 0.24a	30.0 ± 0.37b	30.0 ± 0.37b	30.0 ± 0.37b	35.0 ± 0.40b	45.0 ± 0.58b
2.0%	20.0 ± 0.31a	40.0 ± 0.40a	50.0 ± 0.54a	60.0 ± 0.60a	85.0 ± 0.87a	95.0 ± 0.96a

In the columns, the data followed by the same letter are not significantly different (P 0.05, LSD test).

Table 3. Mortality caused by Clove Leaf (*Syzygium aromaticum*) essential oil on *Tetranychus utricae* when applied at different concentrations in leaf disk bioassays.

Treatment	**Mean Mortality (%) ± SE**					
EO (v/v)	**2 h**	**4 h**	**6 h**	**8 h**	**12 h**	**24 h**
0.2%	0.0 ± 0.0b	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
0.4%	0.0 ± 0.0b	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
0.6%	0.0 ± 0.0b	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
0.8%	0.0 ± 0.0a	0.0 ± 0.0c	0.0 ± 0.0c	5.0 ± 0.24c	10.0 ± 0.24c	15.0 ± 0.24bc
1.0%	15.0 ± 0.24a	20.0 ± 0.31b	20.0 ± 0.37b	30.0 ± 0.37b	35.0 ± 0.40b	40.0 ± 0.40b
2.0%	20.0 ± 0.31ba	40.0 ± 0.40a	45.0 ± 0.48a	55.0 ± 0.60a	65.0 ± 0.73a	70.0 ± 0.73a

In the columns, the data followed by the same letter are not significantly different (P 0.05, LSD test).

Table 4. Mortality caused by Cardamom (*Elettaria cardamomum*) essential oil on *Tetranychus utricae* when applied at different concentrations in leaf disk bioassays.

Treatment	**Mean Mortality (%) ± SE**					
EO (v/v)	**2 h**	**4 h**	**6 h**	**8 h**	**12 h**	**24 h**
0.2%	0.0 ± 0.0b	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
0.4%	0.0 ± 0.0b	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
0.6%	0.0 ± 0.0b	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c
0.8%	0.0 ± 0.0a	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0c	5.0 ± 0.20c	10.0 ± 0.24bc
1.0%	15.0 ± 0.24a	10.0 ± 0.21b	15.0 ± 0.24b	30.0 ± 0.37b	30.0 ± 0.37b	35.0 ± 0.40b
2.0%	25.0 ± 0.31a	40.0 ± 0.40a	40.0 ± 0.40a	45.0 ± 0.48a	55.0 ± 0.58a	65.0 ± 0.67a

In the columns, the data followed by the same letter are not significantly different (P 0.05, LSD test)

Discussion

Plant-derived essential oils as active ingredients for botanical insecticides and repellents have attracted research interest in the past 20 years (Isman, MB., 2020), as they exhibit a variety of bioactivities, including insecticidal, insecticidal, repellent, ovicidal and growth enhancing regulatory effects against insects and arthropod pests, but many of them show relatively low mammalian toxicity (Koul, O., 2008; Prakash, A., et al., 2014).

In the present study, the toxicity of four oils against the two-spotted spider mite was examined, the essential oils tested included citronella java (*Cymbopogon winterianus*), lavender (*Lavendula officinalis*), clove leaf (*Syzygium aromaticum*) and cardamom (*Elettaria cardamomum*) showed comparable toxicity. Previous studies have shown that *T. urticae* reported the toxic activity of these essential oils. Out of the four oils mentioned, two essential oils lavender and citronella had higher toxicity as previously reported (Roh, HS., et al., 2011).

Various biotic and abiotic factors such as weather, altitude, genotype, interaction with surrounding organisms and extraction conditions are known to affect the chemical composition of essential oils resulting in different bioactivities (Isman, MB., et al., 2008).

Many essential oils show insecticidal activity not only against spider mites but also against insects and other arthropod species. Lemongrass oil has insecticidal and acaricidal activity against the African fever fly, Anopheles gambiae and the diamond fly, *Plutella xilostella* (Phukhahad, S., et al., 2021) and clove oil against the vector-carrying roundworm. This was also reported that not only the bioactivity of plant essential oils, but also the lower legal restrictions compared to synthetic pesticides are favorable for the development of botanical pesticides.

In this study, lavender essential oil showed potent contact toxicity against Two Spotted spider mite. In the past few decades, great efforts have been made to test new and more active repellents, but compared to the number of successes in identifying new candidates, efforts to explain the underlying mechanisms of behavior are rarely explored (Haddi, K., et al., 2020) .

Compared to conventional and synthetic pesticides, essential oils tend to evaporate and degrade quickly. Due to their volatile nature, controlling the volatility to increase the intrinsic repellent activity, as well as prolonging the protection time, will be beneficial for the better use of essential oils in pest control applications. Meanwhile, in the principle of Integrated Pest Management (IPM), the predatory beetle, *Scolothrips longicornis* and two phytoseiid species, *Neoseiulus californicus* and *Typhlodromus bagdasarjani*, are used for acaricide application (Farazmand, A., et al., 2015).

To successfully use a push (or pull and kill) strategy using attractive oil for mites, the active oil against the bio-control agent must be repellent and/or potentiating. From an industrial point of view, one of the main challenges in the commercialization of pesticides is financial pressure, mainly because of expensive safety tests for toxicological and environmental tests to meet regulatory standards and requirements (Swale, DR., 2019). Tests are increasing and safety standards are getting stricter these days. Some essential oils, including cedarwood, cinnamon, lemongrass, clove, geranium and lemongrass, due to their natural origin, long history of human use, and, more importantly, their mammalian toxicity (Regnault-Roger, C., et al., 2012); oil is exempt from the requirements of the Federal Insecticide, Fungicide and Rodenticide Act under a minimum risk exemption. This will provide a great opportunity to develop new strategies against pests (Isman, MB., 2020; Madreseh-Ghahfarokhi, S., et al., 2019).

Conclusion

After the experiments it was concluded that high level of toxicity 95% was showed by lavender (*Lavendula officianlis*) essential oils against *Tetranychus urticae*. After this citronella Java (*Cymbopogan winterianus*) essential oil showed 90% toxicity against two spotted spider mite. It is also concluded that clove leaf (*Syzygium Aromaticum*) showed more toxicity against the two spotted spider mite (*Tetranychus urticae*) as compared to Cardamom (*Elettaria cardamomum*) essential oils.

Acknowledgement

This research was financially supported by the Operational Program Integrated Infrastructure within the project: Sustainable smart farming systems taking into account the future challenges 313011W112, co-financed by the European Regional Development Fund.

Conflict of Interest

The authors declare no conflict of interest.

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Citation:

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