herba polonica
International journal edited by the Institute of Natural Fibres and Medicinal Plants
Vol. 65 No. 2 2019

Received: 2017-11-21
Accepted: 2019-04-25
Available online: 2019-06-30

DOI: 10.2478/hepo-2019-0009

EXPERIMENTAL PAPER

Fumigate efficacy of *Juniperus foetidissima* essential oil and two terpenes against *Phthorimaea operculella*

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Summary

Introduction: Potato (*Solanum tuberosum* L.) is an important vegetable crop in Syria. Potato tuber moth *Phthorimaea operculella* is the main damaging pest of this crop. Many approaches were tried to control it.

Objectives: This study evaluates the insecticidal activity of *Juniperus foetidissima* essential oil and two monoterpenes against potato tuber moth using fumigation bioassays.

Methods: Essential oil was extracted from new plant branches of *Juniperus foetidissima* using Clevenger-type apparatus. Potato tuber moth *Phthorimaea operculella*, (adults, eggs, larvae, and pupae) were exposed to different concentrations of the essential oil of *Juniperus foetidissima* for various periods. The significance of differences between treatments at $p<0.05$ were evaluated by one-way analysis of variance using the mortality percentages data. The LC_{50} and LC_{90} were calculated by Probit analysis.

Results: Essential oil vapor with LC_{50}: 0.3 μl/l air was very effective against potato tuber moth at the adult stage. However; adults sensitivity to monoterpenes varied: Nerol caused a 100% mortality at 0.025 μl/l air after 6 h, while the same concentration of citronellol caused 98% mortality. The 0.125 μl/l air concentration of the two compounds caused 100% mortality after 6 h.

Conclusion: Results suggest the possibility of using *Juniperus foetidissima* essential oil as a fumigant to control potato tuber moth infestation in stores.

Key words: ***Phthorimaea operculella, Juniperus foetidissima, essential oil, nerol, citronellol***

Słowa kluczowe: ***Phthorimaea operculella, Juniperus foetidissima, olejek eteryczny, nerol, citronelol***

INTRODUCTION

Cultivated potato (*Solanum tuberosum* L.) is a crucial food crop in Syria. It is estimated that, (2017 data), the cultivated area in Syria is about 24376 hectares, with a total production of 562416 tons [1]. Many pests attack potato plants under field conditions. The most destructive pest for this crop is potato tuber moth, *Phthorimaea operculella*. This insect also attacks some other *Solanaceae* and *Piperacae* species in tropical, subtropical, and Mediterranean agro-zones [2- 4]. Many practices used by farmers to minimize the damage to tubers caused by potato tuber moth are useless. The use of synthetic organic pesticides against crops pests, although showing immediate and spectacular results, increases environmental pollution and causes health problems to humans as well as animals. Moreover, insects have developed resistance against synthetic insecticides and made them less effective [5]. Hence, there is a need for environment friendly and safer alternatives to control these pests.

Essential oils maintain protection for plants against bacteria, plant viruses, fungi, insects and herbivores. Those oils reduce herbivores appetite for such plants. Essential oils repel some insects and attracts some others for pollination or seeds dispersal. Plants essential oils have been suggested as an alternative source for insect control products [6, 7]. Monoterpenoids extracted from essential oils have a role in defending plants. Some monoterpenoids are very toxic to insects, while others are repellent or have inhibitory effects [8-10]. Most of botanical insecticides have minor toxicity to non-target organisms, and rapidly biodegrade [6, 11]. They also provide an alternative for resistance management as many botanical insecticides are highly effective against insecticidal-resistant pests [12-14].

Plant essential oils were suggested as appropriate agents in controlling pest infestation in stored products [15-16]. Monoterpenoids found in essential oils are volatile lipophilic compounds that can penetrate insects' bodies and disrupt their physiology. Because essential oils are highly volatile, they have fumigant action and are potential insecticides that might be used for controlling insects damaging stored crops [17-19].

The effect of seven vegetable oils against PTM adults were experimentally studied by Shelke *et al.* [20]. They found that neem oil (*Azadirachta indica*) in concentrations ranging between 0.05 and 0.1% had a deterrent effect on the PTM oviposition. Sharaby [21] reported that potato tuber moth reproduction was significantly reduced when males or females were exposed to orange peel oil vapor. That effect increased with the increase in oil concentration and exposure time. Moawad [22] reported an ovicidal activity of essential oils extracted from margorum, cardamom, rosemary and terpintin to PTM. Rafiee-Dastjerdi *et al.* [23] studied the insecticidal activity of the essential oil extracted from five different plant species, namely: basil, European pennyroyal, lavender, mint and savory on potato tuber moth. Sharaby *et al.* [24] used the bulb powder of *Allium cepa* to dust potato tubers and found that it was highly effective in reducing of the number of eggs deposited as well as reducing adult emergence. *Juniperus foetidissima* Willd. (*Cupressaceae*) grows in Syria. It has many uses because its wood resists damages caused by insects [25]. The components of its essential oil was previously reported by many authors [17, 26]. The fungal growth inhibition activity of *J. foetidissima* and *J. oxycedrus* wood extracts was investigated by Balaban *et al.* 27].

The aim of this work is to evaluate the insecticidal effects of *Juniperus foetidissima* essential oil and two commercial monoterpenes on potato tuber moth infestation during storage period.

MATERIALS AND METHODS

Essential oil extraction

Newly branched sprouts of planted *Juniperus foetidissima* Willd were collected at the flowering period in May 2015, from Yafour area (Rif-Damascus, Syria) and the species was identified by Prof. M. Oudat (plant taxonomist, AECS). Plant material were initially air dried at room temperature for 7 days, then powdered. Clevenger-type apparatus was used to extract the essential oil by hydrodistillation for 3 h [28]. Essential oil yield was 1.4% w/w (dry weight).

Gas chromatography analysis showed the presence of 22 compounds in *Juniperus foetidissima* essential oil. Main compounds were: citronellol (22.3%), cadalene (19.1%), and bornyl acetate (14.3%) [17].

Monoterpenoids

A 95% pure citronellol (the main constituent of *J. foetidissima)* and 99% pure nerol (a well known monoterpen) standard material were purchased from Sigma–Aldrich.

Insects

Adults, eggs, larvae, and pupae of potato tuber moth *Phthorimaea operculella* used in fumigation experiments were from a laboratory stock culture originally attained from natural field. Insect larvae and adults were fed and reared in jars under optimal culture conditions, (temperature, humidity and day-night cycle) [29].

Fumigation experiments

Three replicates of fumigation experiments for each concentration, life stage, and exposure time were conducted to test the insecticidal effect of the essential oil and the two monoterpenes: citronellol and nerol against potato tuber moth. The applied oil concentrations were chosen to identify the one that achieve a 100% mortality. Fumigation experiments were designed as reported in a previous study [10]. Adults and larvae were fumigated in jars, (1 liter air volume), while eggs and pupae, (3–5 days old), were fumigated in Petri dishes,(160 cm^3 air volume). The tested doses of extracted oil on adults were: 0.15, 0.25, 0.50, 0.75, 1 and 2 μl/l air, on eggs were: 0.05, 0.25, 0.50, 0.75 and 1 μl oil/160 cm^3 air, on larvae were: 0.05, 0.10, 0.50, 1 and 3 μl/l air, and on pupae were: 0.05, 0.25, 0.50, 1, 2 and 6 μl/160 cm^3 air. Adults mortality was determined 24 h post exposure. Hatched eggs percentage was calculated seven days post treatment. Larvae development was monitored for 40 days. Pupae mortality was determined by adult emergence after 7 days. Parallel control groups were also included.

The toxic effects of citronellol and nerol against adult insects (one day old) were investigated as reported in a previous study [10]. Adults were considered dead when no leg or antennal movements were observed.

Experiments mimicking the current situation in storing potatoes were conducted. Plastic barrels of 50 liters capacity were filled with twenty five kilograms of sound and fresh potato tubers. Slices of paper filters carrying insects eggs aged 24 and 48 h (792 eggs) were distributed within each barrel and on potato tubers. Filter papers measuring 3 x 3 cm containing the concentration of plant essential oil which gave 100% mortality (1 ml/50 l air), were placed within each barrel, then the barrels were closed tightly. The barrel was left for a mating period, (the period of hatching eggs), which lasted 10 days. The barrels were then opened and hatched eggs counted. Incubated tubers were observed six weeks later, (for one generation period), under laboratory conditions. Numbers of pupae and adults resulting from the first generation were recorded in kind and account for three independent experiments, in addition to the non-treated control. Statistical analysis and calculation of LC_{50} and LC_{90} were made according to our previous study [10].

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS

Vapor of the essential oil extracted from the dried new leafy branches of *J. foetidissima* showed variable toxicity against potato tuber moth. This toxicity is associated with insect developmental stage, oil concentration and exposure time. Mortality significantly increased with increasing essential oil concentration. Exposure of adults to the highest dose of 2 μl/l air caused a 100% mortality after 24h exposure period. Whereas, the lowest concentration used, i.e. 0.15 μl/160 cm^3 air resulted in only 2% death after one day of exposure (fig. 1). LC_{50} for *J. foetidissima* essential oil on adults was 0.3 μl/l air, whereas the LC_{90} was 0.9 μl/l air (tab. 1). In the case of pupae, exposure to 6 μl/ 160 cm^3 air (37.5 μl/l air) of the essential oil vapor resulted in 100% death of pupae after seven-day exposure period. The lowest concentration of 0.05 μl//160 cm^3 air (0.31 μl/l air) to which the pupae were exposed resulted in a 13% mortality after 24 h exposure period, (fig. 1). The LC_{50} of the essential oil on pupae was 4.97 μl/l air at 24 h

Table 1

Calculated LC_{50} and LC_{90} values for *Juniperus foetidissima* essential oil against some different developmental stages of potato tuber moth (*Phthorimaea operculella*)

P . operculella	LC_{50} μl/l air	LC_{90} μl/l air	Slope±SE	F_{cal}	F_{05}	d.f	χ2	P
Adults	0.3	0.9	2.2±0.4	35.9	7.7	5	9.5	0.0001
Eggs	2.9	8.4	2.4±0.6	14.97	10.13	4	7.8	0.0001
Pupae	4.97	36.2	1.2±0.3	19.8	6.6	6	11.07	0.0001

Units: LC_{50} and LC_{90} – μl/l air, applied at 25±1°C, d. f. – degrees of freedom

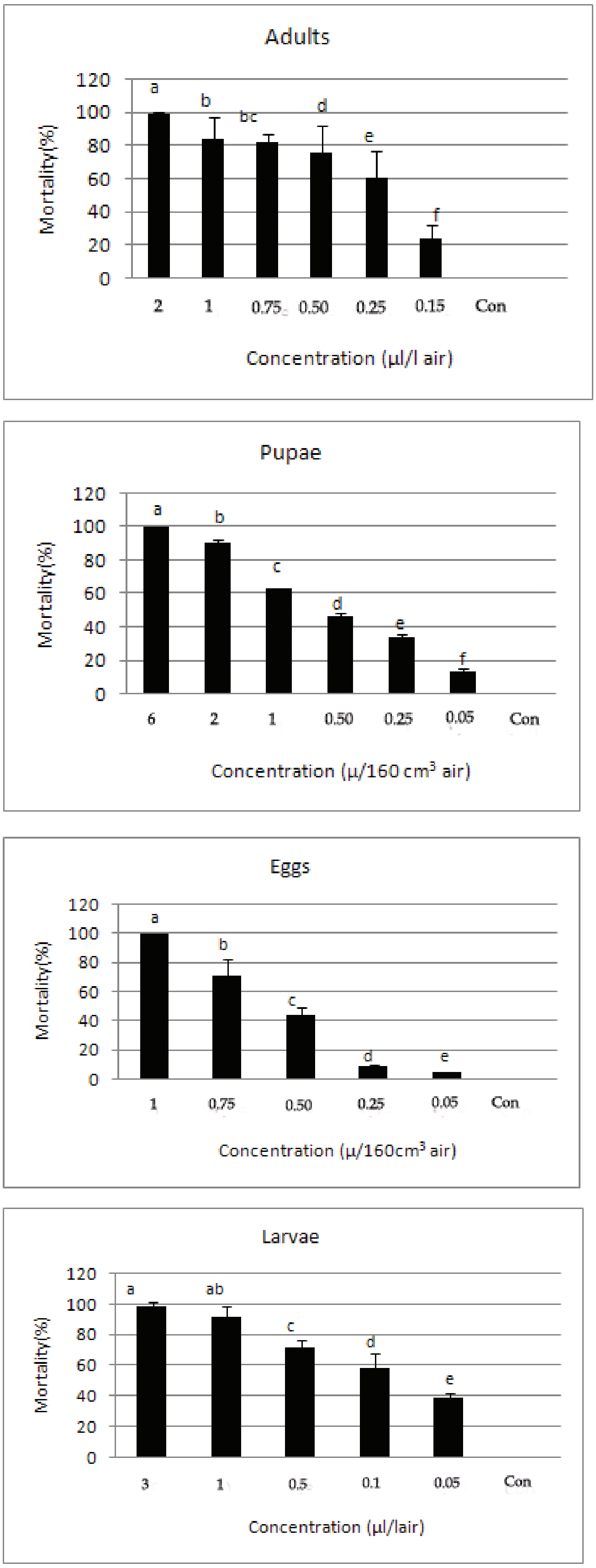

Figure 1

Mortality percentages of potato tuber moth (*Phthorimaea operculella*) stages due to treatment with different concentrations of *Juniperus foetidissima* oil. Means with same letters do not differ significantly at $p<0.05$

exposure period (tab. 1). Eggs appeared to be highly sensitive to toxic effect of *J. foetidissima* essential oil vapor; a 1 µl/160 cm^3 air caused death of eggs by 100% after 24 h exposure period (fig. 1), with LC_{50} of 2.4 µl/l air (tab. 1). Larvae were highly affected with 3 µl/l air dose (fig. 1), potato tubers were free from infection. The 0.05 µl/l air dose showed a fumigant activity against PTM larvae but potato tubers were infected. This indicates for the need to apply higher doses to efficiently control this insect.

The two monoterpenes also showed insecticidal activity against adults of potato tuber moth with strong differences in mortality percentages depending on exposure time and concentration. As it was shown in figure 2, nerol was relatively more toxic than citronellol. The nerol caused 97% mortality at 0.025 µl/l air concentration after 3 h of treatment. This mortality reached 100% when insects were exposed to nerol for 6 hours. The same concentration of citronellol caused a 91% and 98% mortality after 3 h and 6 h of exposure, respectively (fig. 2). However, at a dose of 0.125 µl/l air, each of the two compounds caused a 100% mortality 6 h of exposure (fig. 2).

Results obtained from treatment of PTM eggs in a plastic barrel with this essential oil showed that the oil vapor killed the eggs in a dose-dependent manner. At a concentration of 1 ml/50 l air, the average number of hatching eggs was zero. None of the developed embryos inside the eggs was able to reach the last stage of development. All tubers were completely intact compared to the control (average number of hatching eggs 100%). The concentration used to achieve this level of protection gives an idea of the importance of calculating the appropriate dose to protect potato tubers from this scourge during the storage of the crops.

DISCUSSION

The essential oil of *J. foetidissima* plants showed a high toxic effect on all developmental stages of PTM. The most sensitive life stage of PTM to *J. foetidissima* oil vapors was the adult and the larvae were the most tolerant. All adult insects were killed (100% mortality), when they were exposed to 3 µl/l air concentration for 48 h. The major compounds found in the essential oils of *J. foetidissima* have been reported previously to have a fungal growth inhibition [27], and an insecticidal activity

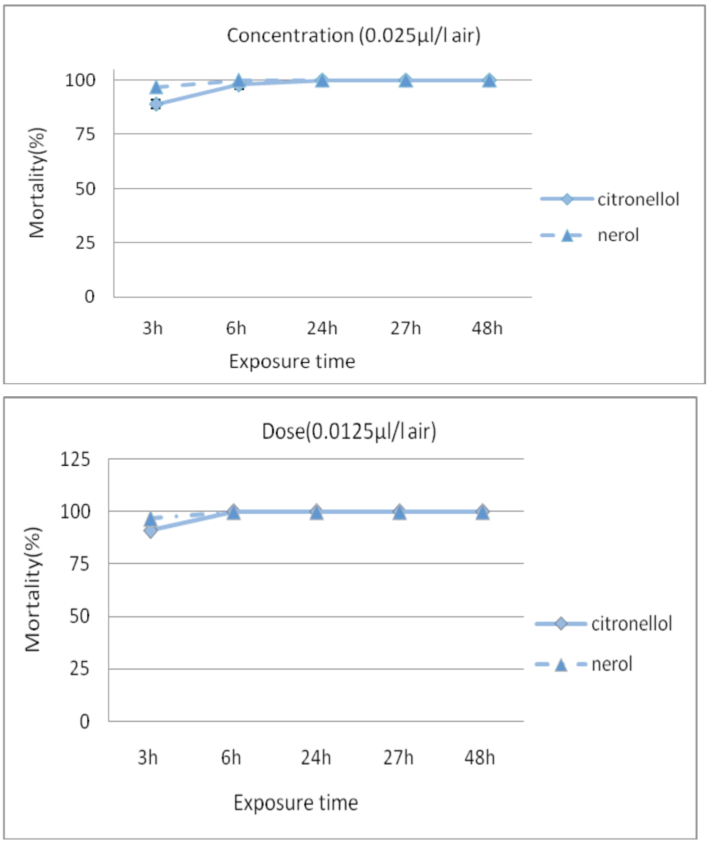

Figure 2

Mortality percentages of potato tuber moth (*Phthorimaea operculella*) adults exposed to monoterpenoids (citronellol, nerol) vapors for different periods against *T. granaruim* larvae [17]. Our results are ± in agreement with results published by Tayoub *et al.* [10], Rafiee-Dastjerdi *et al.* [23], Sharaby *et al.* [24], Moawad & Ebadah [30] who wrote that essential oils and their monoterpene components protect potato tubers against potato tuber moth infestation during storage.

On the basis of calculated LC_{50} values, *J. foetidissima* oil was substantially more toxic than lavander essential oil, the LC_{50} for *J. foetidissima* was 3 times lower. *J. foetidissima* oil was more toxic to one-day-old adults of *P. operculella* after 24 h (LC_{50} values were 0.3 and 0.9 µl/l air, for *J. foetidissima* and lavander, respectively). The *J. foetidissima* essential oil toxicity was almost similar to basil oil toxicity which has LC_{50} value of 0.108 µl/l air. However, mint and savory European pennyroyal oil have higher toxicity than *J. foetidissima* essential oil on PTM adults (LC_{50} values were 0.048 and 0.065 µl/l air for mint and savory European pennyroyal oil, respectively) [23]. Differences observed in toxicity of plant essential oil between our results and Rafiee-Dastjerdi *et al.* [23], could be due to a number of reasons: the plant oil chemical composition that can be affected by season, growing region and weather conditions. Sensitivity of the insect species to the compounds found in the essential oil, the monoterpenes in particular, which represent a 51.4% of the total compounds found in the *J. foetidissima* essential oil [17]. Citronellol, the major compound in *J. foetidissima* (22.3%) essential oil was used in perfumes and insect repellents [31], and as mosquito repellnt [32], and in biopesticides according to a document registered in the United States [33]. Exposure of *Oryzaephilus surinamensis* to citronellol for 14 h caused 100% mortality [34]. Bornyl acetate, the second most abundant compound of this essential oil tested (14.3%) has shown fumigant toxicity against stored-product insects such as *Sitophiles oryzae* (L.) and *Callosobruchus chinensis* (L.) [35]. α-Terpeneol and terpinene-4-ol have fumigant toxicity to *S. oryzae* [19]. The essential oils and their constituent monoterpenoids might act as neurotoxins [36]. Although the toxic effects of oil constituents used in the study have not been thoroughly investigated against humans and other mammals, available data for the use of plant materials containing monoterpenoids as pharmaceuticals and flavourings indicated no health side effects of their use. Therefore they are considered less harmful to humans than most conventional pesticides. In conclusion treatment of the potato tuber moth with *J. foetidissima* essential oil can safely protect potato tubers during storage period. Isolation of the active constituents of this essential oil will enables a detailed investigation of their toxic effects on insect pests.

ACKNOWLEDGEMENTS

The authors would like to express their thanks and gratitude to Professor Ibrahim Othman, Director General of Atomic Energy Commission of Syria and Professor Nizar Mir Ali for support and encouragement.

Conflict of interest: Authors declare no conflict of interest.

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