

The efficacy of some medicinal plant extracts and essential oils against potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae)

Hooshang Rafiee-Dastjerdi^{1*}, Fereshteh Khorrami¹, Jabraeil Razmjou¹, Behrouz Esmailpour², Ali Golizadeh¹ and Mehdi Hassanpour¹

1. Department of Plant Protection, Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran.

2. Department of Horticulture science Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, Iran.

Abstract: The potato tuber moth (PTM), *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae), is an important pest of potato and other solanaceous plants. It is cosmopolite and oligophage that exists in most countries where potatoes are grown. Because of chemical treatment adverse effects, there is a necessity to find safe alternatives to synthetic insecticides. So, medicinal plants are promising since they are safe, inexpensive and effective. In this direction, potato tubers were dipped in 1 ml of 5% methanolic extracts of fumitory, licorice, lavender and oregano. The percentage of first larval penetration to tubers and oviposition-preference of mentioned medicinal plants was investigated on PTM. Subsequently, fumigant toxicity of essential oils of basil, european pennyroyal, lavender, mint and savory were investigated on *P. operculella*. Treated potato tubers by methanolic extract of lavender elicited the lowest percentage (19.3) of first larval penetration. Studying of oviposition-preference demonstrated that the largest number of eggs were laid on control and fumitory with 28 and 10 eggs after three days, respectively. Probit analysis of essential oils showed that the most effective oil was savory which exhibited LC₅₀ value on adults of PTM equivalent to 0.048 µL/L air.

Keywords: *Phthorimaea operculella*, oviposition-preference, fumigant toxicity, medicinal plant

Introduction

The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae) is one of the most destructive pests of potato in both fields and storage (Ferreire *et al.*, 1994). Damage of the pest in suitable conditions in storage is more than in the field (Das, 1995). The PTM larvae cause severe damage to stored

potatoes through mining into tubers that lead to rotting by penetration of fungal and bacterial agents. Furthermore, high cost and hazardous effects of insecticides make us seek and use other safe means such as medicinal plants for pest control. Essential oils (EOs) are volatile and natural compounds characterized by a strong odor and are formed as plant secondary metabolites by plants belonging to botanical families, like Myrtaceae, Lauraceae, Lamiaceae, Asteraceae. These compounds have functions in chemical defense, acting as repellents, insecticides, acaricides, and by attracting natural enemies of herbivores (Karamanoli, 2002; Karamanoli *et al.*, 2005;

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* **Corresponding author**, e-mail: rafiee@uma.ac.ir

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Bakali *et al.*, 2008). Usually they are extracted by hydro-distillation and they comprise terpenes and terpenoids and other aromatic and aliphatic constituents. EOs affect several targets at the same time, because of their great number of constituents; this fact decreases the target organisms' resistance or adaptation. Also, EOs induce cytotoxicity, damage the cellular membranes, act as prooxidants on proteins and DNA and produce reactive oxygen species. Such activity is mostly induced by phenols, aldehydes and alcohols. In some cases, essential oils and their components have demonstrated nuclear and cytoplasmic mutagenicity, acting on mitochondria and the respiratory system (Bakkali *et al.*, 2008). The biological activity of EOs and their components on pest insects comprise behaviour and feeding deterrence effects, fumigant toxicity, knockdown activity and lethal toxicity via contact. These substances are generally active against a broad spectrum of pests. Perhaps the most favorable aspect of using EOs and their constituents in pest management is their non-mammalian toxicity and their non-persistence in the environment (Isman, 2000). Some researches show the role of some plant oils as control agents against different insect pests (Al-Dosary, 2007). There are many researches to show the role of some plant oils as control agents against storage pests but there is less knowledge about their effects on PTM, for example Moawad (2000) reported that applying the 1% oils of *Mentha citrata* (Ehrh.), *Cymbopogon citratus* (DC.) Stapf and *Myristica fragrans* Houtt. decrease larval penetration of *P. operculella*. Moawad *et al.*, (2007) showed ovicidal activity of essential oils of margorum, cardamom, rosemary and terpinin on PTM. In present study efficacy of methanolic extracts from *Fumaria officinalis* L., *Lavandula angustifolia* L., *Glycyrrhiza glabra* L. and *Origanum vulgare* Mill were evaluated on oviposition-preference activity and larval penetration of *P. operculella*. Also, the effect of essential oils of *L. angustifolia*, *Mentha longifolia* L., *Mentha spicata* L., *Ocimum basilicum* L. and *Satureja hortensis* L. were studied on adults of the pest.

Materials and Methods

Insect

The Colony of potato tuber moth was reared on Agria Cultivar of potato, an important host of PTM in Ardabil province (Iran). Experiments were carried out under laboratory conditions at 26 ± 1 °C, $60 \pm 5\%$ RH and photoperiod of 16: 8 (L: D).

Plant material

O. basilicum, *M. longifolia*, *M. spicata* and *S. hortensis* were provided from green house of department of horticulture at University of Mohaghegh Ardabili. *F. officinalis* and *G. glabra* were collected during summer of 2011 from environs of college of agriculture at University of Mohaghegh Ardabili. These materials were dried at room temperature for three weeks in darkness. *O. vulgare* was purchased from local market of Khomein city. Essential oils were extracted from flowers of *L. angustifolia* and leaves of *O. basilicum*, *M. longifolia*, *M. spicata* and *S. hortensis* and they were subjected to hydro distillation using a modified cleverger-type apparatus. The obtained oils were stored in vials coated with Aluminum paper at 4 °C. Methanolic extracts were obtained from flowers of *L. angustifolia*, leaves of *O. vulgare* and aerial parts of fully flowered *F. officinalis* and *G. glabra* by using soxhlet extractor. Excess methanol was evaporated in a rotary evaporator.

Bioassays

Larval penetration

To examine the percentage of larval penetration of *P. operculella*, the first larval instar was used because it searches and mines into the host (Varela and Bernays, 1987). At first, each potato tuber was dipped in 1 ml of 5% methanolic extracts of fumitory, licorice, lavender or oregano. When solvent was evaporated and tubers were dried, they (three potato tubers) per treatment were transferred into plastic boxes with ventilated lids kept at 26 ± 1 °C, $60 \pm 5\%$ RH and photoperiod of 16: 8 (L: D). Then 20 newly hatched larvae were

introduced on each potato by fine hair brush and the number of individuals moving into potatoes was recorded after 24 h. Each experiment was replicated four times.

Oviposition-preference activity

In this investigation, methanolic extracts 5% were obtained from flowers of *L. angustifolia* and leaves of *O. vulgare*, *F. officinalis* and *G. globra* by using soxhlet extractor. Then excess methanol was evaporated in a rotary evaporator. These extracts were evaluated for oviposition-preference activity of *P. Operculella*. Three tubers were dipped in 1ml of 5% botanical extracts and the fourth one (control) was dipped in 1ml of solvent (methanol) alone, after solvent evaporating, these tubers were put in four sides of exposure cage (32*32*32cm). Fifteen virgin pairs of one-day old adults (15 males & 15 females) were transferred into each exposure cage and their oviposition rates was recorded up to 24, 48 and 72 h. Experiment had three replicates in laboratory conditions at 26 ± 1 °C, $60 \pm 5\%$ RH and photoperiod of 16: 8 (L: D).

Fumigant toxicity

Bioassay trials were carried out following Rahman and Schimdt technique (1999) and Negahban *et al.*, (2007). The ranges of concentrations for different compounds were determined by preliminary dose setting experiments. The ranges for *L. angustifolia*, *M. logifolia*, *M. spicata*, *O. basilicum*, and *S. hortensis* were 0.8- 1.2 (1.2, 1.08, 0.96, 0.84, 0.8), 0.04- 0.12 (0.12, 0.08, 0.06, 0.44, 0.04), 0.04- 0.2 (0.2, 0.12, 0.08, 0.052, 0.04), 0.08- 0.2 (0.2, 0.16, 0.12, 0.96, 0.08) and 0.04- 0.12 (0.12, 0.08, 0.06, 0.044, 0.04) $\mu\text{L/L}$ air, respectively. Filter papers were impregnated with the required concentration of essential oils and they were placed in the cap of glass vials and covered with Parafilm. Virgin male and female moths (five males and five females each one-day old) were placed in glass vials and were exposed to vapors of Essential oils of basil, european

pennyroyal, lavender, mint and savory before the vials were capped. These vials were transferred to growth chamber at 26 ± 1 °C, $60 \pm 5\%$ RH and photoperiod of 16L: 8D. Each experiment was replicated five times. Mortality was counted after 24 h of exposure to plant essential oils.

Data analysis

In order to determine LC_{50} 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. The relationship between data was examined by analysis of variance (ANOVA) and correlation analysis. The means were separated by using the Tukey test.

Results and Discussion

Results given in Table 1 demonstrate that lavender, oregano and licorice extracts had the highest preventive effect on PTM larval penetration, compared with 90.22% and 82% penetration for the control and fumitory extract, respectively ($F = 23.6$; $df = 4,15$; $P < 0.05$; $CV = 1.62$). Moawad (2000) noted that the dusting on potato tuber by 1% natural oils of *Mentha citrae*, *Cymbopogon citrates*, *Myristica fragrans* and α -ionon reduced the percentage of larval penetration of *P. operculella*. Rama (1989) reported that dusting Neemrich oil (*Azadirachta indica*) on potato tubers had larvicidal and ovicidal effects against PTM. Moawad *et al.*, (2007) showed that dusting potato tuber by natural Cardamon oil (1.5%) mixed with talcum powder was the most repellent against PTM (larval penetration was 13.3%) while the penetration of larvae (%) by dusting of 1.5%, 1% and 1% concentrations of Rosemary, Cardamon and Margorum were 23.3, 23.3 and 26.7%, respectively. They also noted that no adults could emerge from larvae feeding on tubers treated with 1.5% concentration of Cardamon.

Table 1 Effect of methanolic extracts on percentage of first larval penetration of *Phthorimaea operculella* into potato tubers.

Tested plant extracts	Larval penetration (%) \pm S. E.
Fumitory	82 ^a \pm 2.31
Licorice	34 ^b \pm 2.08
Lavander	19.3 ^b \pm 2.30
Oregano	24.6 ^b \pm 2.43
Control	90.22 ^a \pm 1.88

* Means in column with the same letter are not significantly different ($p < 0.05$)

According to Table 2, applying 5% extracts of four medicinal plants on potato tubers reduced egg laying of the *P. operculella* whereas the pest preferred to lay eggs on un-treated tubers ($F = 11.26$; $df = 3, 32$; $P < 0.05$; $CV = 19.60$). The number of laid eggs on tubers treated with licorice, lavender and oregano were 0, therefore the pest preferred to oviposit on non-treated tubers. These results demonstrated that *F. officinalis* (Fumariaceae) had rather low inhibitory effects against the pest but more researches are needed. Ajamhassani and Salehi (2004) examined the effect of leaf powder and 5% extract of *Sambucus ebulus* (L.), *Artemisia annua* (L.) and *Pterocarya fraxinifolia* (Lam) on oviposition rate of potato tuber moth. They reported that either leaf powder or extracts of mentioned plants had deterrent activity but the extracts were more deterrent than their powders. Shelke *et al.*, (1987) assayed ovicidal action of some vegetable oils and extracts in the storage pest of potato, *P. operculella* and demonstrated that the oil from the exterior leathery rind of lime (*Citrus aurantifolia*) had up to 60% deterrent activity.

Table 2 The mean number of laid eggs of *Phthorimaea operculella* on treated and non- treated tubers after 24, 48 and 72 hours (oviposition-preference).

Tested plant extracts	The mean number of eggs \pm S. E.		
	24 h	48 h	72 h
Fumitory	0 ^b	10 ^b \pm 1.32	10 ^b \pm 1.03
Licorice	0 ^b	0 ^c	0 ^c
Lavander	0 ^b	0 ^c	0 ^c
Oregano	0 ^b	0 ^c	0 ^c
Control	13 ^a \pm 2.09	22 ^a \pm 1.03	28 ^a \pm 1.66

* Means in columns with the same letter are not significantly different ($p < 0.05$)

Results presented in Table 3 show that according to LC_{50} values and their confidence limits the most effective oils were savory (LC_{50} value 0.048 μ L/L air) and European pennyroyal ($LC_{50} = 0.056$ μ L/L air). Savory has anti-dermatitis, antimicrobial and antifungal activities, so it could be recommended for application as a pest control agent. (Djenane *et al.*, 2011), it could be very suitable for controlling the pest. Lavender essential oil presented rather low activity (LC_{50} value 0.91 μ L/L air), but in comparison with hazardous insecticides, it may be very safe and suitable compound for controlling *P. operculella*. Papachristos and Stamopoulos (2002) investigated repellent, toxic and reproduction inhibitory effects of essential oil vapors on *Acanthoscelides obtectus* (Say) and they found that LC_{50} value of lavender EO on its adults was high which is in contradiction with our results. The differences observed in toxicity of plant EO between our results and theirs could be due to a number of causes: chemical compositions of plant that can be affected by planting and growing region, weather conditions, insect species tested and its sensitivity to compounds extracted from the plant. Shaaya *et al.*, (1997) found that vapor of *Labiatae* sp. oil (ZP51) at higher concentrations (1.5-4.5 μ L/L air) caused 90% mortality of major stored product insects.

Eventually, based on our results it could be concluded that the treatment of potato tubers with plant oils and extracts can help in having healthy product. Whereas the pest is active in tubers and the farmers cannot see the pest except at pre pupating and adult stages, it is better and wiser that farmers be recommended them to manage the pest at this stage

(emerging of adults) because they can observe them and will more easily accept recommendations by the farm advisors. According to our results it is suggested that all essential oils and plant extracts except fumitory could be effective against the pest.

Table 3 Probit analysis of fumigant toxicity of some essential oils to one-day-old adults of *Phthorimaea operculella* after 24 h.

Essential oil	LC ₅₀ (μL/L air) (95% confidence limits)	LC ₉₀ (μL/L air) (95 % confidence limits)	Slope ± S. E.	Chi-square
Basil	0.108 (0.096-0.12)	0.24 (0.20-0.34)	5.59 ± 0.84	2.42
European pennyroyal	0.056 (0.048-0.06)	0.13 (0.108-0.18)	6.36 ± 0.87	3.17
Lavander	0.912 (0.87-0.94)	1.23 (1.16-1.36)	6.19 ± 0.79	1.74
Mint	0.076 (0.06-0.08)	0.26 (0.2-0.44)	3.96 ± 0.55	0.34
Savory	0.048 (0.044-0.052)	0.1 (0.08-0.12)	7.74 ± 1.00	1.96

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کارایی برخی عصاره‌ها و اسانس‌های گیاهان دارویی روی بید سیب‌زمینی *Phthorimaea operculella* (Lepidoptera: Gelechiidae)

هوشنگ رفیعی دستجردی^{۱*}، فرشته خرمی^۱، جبرائیل رزمجو^۱، بهروز اسماعیل‌پور^۲، علی گلی‌زاده^۱ و مهدی حسن‌پور^۱

۱- دانشگاه محقق اردبیلی، دانشکده کشاورزی، گروه گیاه‌پزشکی

۲- دانشگاه محقق اردبیلی، دانشکده کشاورزی، گروه باغبانی

* پست الکترونیکی نویسنده مسئول مکاتبه: rafiee@uma.ac.ir

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چکیده: بید سیب‌زمینی با نام علمی *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) یکی از آفات مهم سیب‌زمینی و دیگر گیاهان خانواده سولاناسه می‌باشد. این آفت همه جازی و الیگوفاز بوده و خسارت آن در اکثر کشورهایی که سیب‌زمینی کاشت می‌گردد، مشاهده می‌شود. به‌خاطر اثرات سوء مصرف آفت‌کش‌های شیمیایی، نیاز به یافتن روش‌های امن جایگزین احساس می‌شود. گیاهان دارویی به‌خاطر امن بودن، ارزان بودن و تأثیر مناسب جایگزین خوبی به‌نظر می‌رسند. در این راستا، غده‌های سیب‌زمینی با یک میلی‌لیتر از عصاره‌های متانولی ۵٪ شاه‌تره، شیرین بیان، اسطوخودوس و مرزنجوش آغشته شده و درصد نفوذ لاروهای سن اول به غده و ترجیح تخم‌ریزی حشرات کامل بید سیب‌زمینی محاسبه شد. همچنین سمیت تدخینی اسانس‌های ریحان، پونه، اسطوخودوس، نعناع و مرزه روی بید سیب‌زمینی بررسی شد. کمترین درصد نفوذ لارو بید سیب‌زمینی در غده‌های تیمار شده با عصاره متانولی اسطوخودوس به‌میزان ۱۹/۳ درصد مشاهده شد. مطالعه ترجیح تخم‌ریزی نشان داد که بیشترین تخم گذاشته شده در شاهد و عصاره شاه‌تره پس از سه روز، به‌ترتیب، ۲۸ و ۱۰ عدد انجام شد و در بقیه تیمارها هیچ تخمی مشاهده نشد. تجزیه پروبیت اثر اسانس‌ها نشان داد که اسانس مرزه با LC_{50} معادل ۰/۰۴۸ میکرولیتر بر لیتر هوا روی حشرات کامل بید سیب‌زمینی بیشترین تأثیر را داشته است.

واژگان کلیدی: بید سیب‌زمینی، گیاهان دارویی، سمیت تدخینی، درصد نفوذ لارو، ترجیح تخم‌ریزی