

## BIOACTIVITY OF *MARRUBIUM VULGARE* AND *ACHILLEA MILLEFOLIUM* LEAF EXTRACTS ON POTATO TUBER MOTH *PHTHORIMAEA OPERCULELLA* ZELLER

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**ABSTRACT:** The potato tuber moth *Phthorimaea operculella* Zeller (Lep., Gelechiidae) is one of the most important pests of potato worldwide. Plants are a rich source of novel natural substances that can be used to produce safe materials in IPM. In this study, ovicidal, oviposition deterring and fumigant activity of hexane, ethyl acetate, methanol and aqueous extracts of *Achillea millefolium* and *Marrubium vulgare* on different developmental stages of PTM have been investigated. The results indicate that maximum ovicidal activity was observed in hexane extract in both plants with LC<sub>50</sub> values of 6.55 and 8.03 mg/l. All tested concentrations of *M. vulgare* and *A. millefolium* crude extracts caused great reductions in the number of eggs deposited. Among the tested extracts, except hexane extract of *M. vulgare* and ethyl acetat extract of *A. millefolium* remains induced the greatest antioviposition deterring effect, with no eggs oviposited. The fumigant toxicity of the *M. vulgare* and *A. millefolium* crude extract against 1<sup>st</sup> larval instar and adults of PTM was different. Among tested extracts only Hexane extract of *M. vulgare* had fumigant activity on adults of PTM.

**KEY WORDS:** Ovicidal activity, Fumigant activity, Plant extract, Hexane, Ethyl acetat, Methanol.

The potato tuber moth (PTM), *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae), is an oligophagous and serious pest of the solanaceous plants such as potato, tomato, tobacco and egg-plant worldwide (Fenemore, 1988; Rondon, 2010). Larvae bores into the potato tubers, leaves and stems in the field and storage. Excreta deposited in the feeding channels increases the risk of infection by plant pathogens (Koul et al., 2008; Moawad & Ebadah, 2007; Fathi & Shakarami, 2014). The PTM originated in southern and central America but now it can be found in almost all potato production areas worldwide and recently emerged as a potential economic pest of potatoes in the most parts of Iran. The control of this pest is based on the application of wide spectrum insecticides. Chemical insecticides cause health hazards to human beings, natural enemies and environment (Ishaaya & Horowitz, 2009; Relyea, 2005). Plants are a rich source of novel natural substances that can be used to develop environmentally safe materials (Scott et al., 2003).

Zoubiri & Baaliouamer (2014) in their studies found various plant species with insecticidal potential. Insecticidal activity of many plants against several insect pests has been investigated (Sharaby et al., 2009). The effects of plant extracts on insects can be manifested in several manners including toxicity, mortality, antifeedant, growth inhibitor, suppression of reproductive behavior and reduction of fecundity and fertility (Keita et al., 2000; Niroula & Vaidya, 2004; Rakesh, 2009; Nerio, 2010; Bokaeian et al., 2013; Adlin et al., 2015).

Plants of the Asteraceae and lamiaceae family contain effective secondary metabolites that could affect insect's behavior and biology (Abd El-Aziz 2011). *Achillea* contains various species of perennial plants found worldwide. The member of *Achillea* genus contained terpenoids, lignans, flavonoids and other

compounds in its foliage and flowers with different biological activity against insects and microorganisms (Vitalini et al., 2011; Zhiani & Moradi, 2014).

The lamiaceae plants were considered as one of the large plant families that was evaluate the occurrence of typical secondary metabolites. The genus *Marrubium* comprises different species, which are found wildly in many regions of Azarbaijan province in Iran. Among them, *Marrubium vulgare* L. is a perennial plant that its foliage and flowers contains aromatic compounds with biological activity (Kadri et al., 2011; Zawislak, 2012; Abdi & Hassani, 2013; Hamedeyazdan et al., 2013). Aromatic plants, and their essential oils, are among the most efficient botanicals that could induce fumigant and topical toxicity as well as antifeedant or repellent effects. They are toxic to different developmental stage of insects (Regnault-Roger, 1997). For example, oviposition deterrent and ovicidal activity was found in crude extracts of *Syzigium lineare* leaves against *Spodoptera litura* Fab. (Jeyasankar et al., 2013) and the maximum oviposition deterrent and ovicidal activity were observed in ethyl acetate extract. In *Mentha citrata* essential oil containing linalool and linalyl acetate exhibit significant fumigant toxicity to the rice weevils *Sitophilus oryzae* and *Marrubium persicum* contains higher proportions of non-terpenoid keton, namely acetophenone (Hamedeyazdan et al., 2013). Acetophenone was demonstrated to cause acute and delayed types of insecticidal and ovicidal activities (Zohair, 1995; Liu et al., 2014). Some plant's volatiles contain compounds with fumigant toxicity against insects. The fumigant toxicity of plants extracts from aianthus was investigated by Lu & He (2010). All the plant extracts had potent fumigant activities against *O. surinamensis* and *S. oryzae* adults. Lu et al. (2012) reported that *A. officinarum* rhizome extract exhibited strong fumigant, repellent activity in a dosage-dependent manner against *T. castaneum* adults.

Other aspects of plant derived compounds were ovicidal activity that was efficient on different insect species. Adline et al. (2015) reported that hexane, chloroform and ethyl acetate extracts of *Glinus lotoides* have ovicidal potential against the *Corcyra cephalonica* eggs. The maximum egg mortality was caused by ethyl acetate extract. All the concentrations of the extracts applied were able to cause ovicidal activity against the *C. cephalonica*. Studies on ovicidal effects of aqueous and alcoholic extracts of different plants were carried out against the diamondback moth, *Plutella xylostella* (L.) results revealed that extracts of all plants had significant ovicidal activity (Kumar et al., 2009). Ovicidal activity of acetone extracts of some plant species were evaluated. *Murraya Tabernaemontana*, *Chenopodium* and *Lantana camara* showed ovicidal activity against *C.cephalonica* (Dwivedi & Venugopalan, 2001).

In addition with ovicidal activity some plants showed oviposition deterring activity which is so important in depressing the population of insects and good equipment in IPM. Singh (2011), Arivoli & Tennyson (2013) and Rana et al. (2013) reported some aspects of oviposition deterring activity of different plant species and their potential on population of insects in field and storage. Oviposition deterrent and ovicidal activity of crude extracts of *Syzigium lineare* leaves, were tested against *Spodoptera litura* Fab. The maximum oviposition deterrent and ovicidal activity were observed in ethyl acetate extract (Jeyasankar et al., 2013).

All of studies on efficiency of plants extracts on biology and behavior of insects shows potential of them in controlling insects in IPM programs. The aim of this study was to evaluate the insecticidal activity of the hexane, ethyl acetat, methanol and aqueous extracts from *Marrubium vulgare* and *Achillea millefolium* against eggs, larvae and adults of *P. operculella* in laboratory condition.

## MATERIAL AND METHODS

**Insects rearing:** The adults of PTM were obtained from the laboratory colony maintained at the plant protection department of Azarbaijan Shahid Madani University. Larvae were reared on potato tubers in controlled condition of  $26\pm 2^{\circ}\text{C}$  and a photoperiod of 16:8 (L: D) h in  $50\pm 10\%$  relative humidity. The bottoms of rearing cages were furnished with a thin layer of soft sand as a pupation substrate (Maharjan and Jung 2011).

**Collection and processing of plants:** Aerial parts of the plants studied in this investigation were collected from different localities in north-western regions of Iran, before flowering period, dried in shadow and room temperature, then powdered. About 1000 gr of each plant material was sequentially extracted with n-hexane, ethyl acetat, methanol and distilled water for a period of 3 days and then filtered. The filtered contents were subjected to rotary evaporator until solvents were completely evaporated and solid crude extracts collected in vials for proper assays.

**Ovicidal bioassay:** The ovicidal activity of plants extracts was examined with contact method. Egg batches of 1 day-old and 6 hour old were collected, numbered and divided into treatment and control groups. In order to test the contact toxicity of extracts, the first group of eggs was dipped in different concentrations of test extracts diluted in water. Aqueous solution was used only for control (second) group. After drying for 20 minutes, egg batches were inserted in Petri dishes and subsequently covered. Number of eggs hatched in control and treatments were recorded and the corrected percentage of ovicidal activity was calculated using Abbott's formula. The ranges of concentrations for different compounds were determined by preliminary dose setting experiments (Arivoli and Tennyson, 2013).

$$\text{Abbot corrected mortality (\%)} = \frac{(\text{unhatched eggs in treatment} - \text{unhatched eggs in control})}{(100 - \text{unhatched eggs in control})} \times 100$$

**Oviposition deterrence assay:** The oviposition deterrent activity was assessed using methods used by Arivoli and Tennyson (2013), with slight modifications. To study the oviposition deterrence effect and the number of eggs deposited in the presence of different extracts of experimental plants, a multiple concentration test was carried out (50, 25 and 12.5 g/l). Adults were provided continuously with 10 percent sucrose solution with a filter paper. The same Potato slices provided (1cm thickness), and then each slice sprayed with extracts served as treated while those sprayed with solvent and water acted as negative and positive control respectively. Five pairs of newly emerged adult (male and female) moths were introduced into a cage with treated potato slices and control. After 48 hours the number of eggs laid by the females was recorded on treated and control potato slices. A total of eight trials with three replicates per trial were carried and percent oviposition deterrent activity calculated according to Arivoli and Tennyson (2013) and Abd-el Aziz (2011) method with modifications.

$$\text{Oviposition deterring activity \%} = \frac{(\text{number of eggs in control} - \text{number of eggs in treatment})}{(\text{number of eggs in control} + \text{number eggs in treatment})} \times 100$$

**Fumigant toxicity:** The fumigant activity of tested extracts was determined according to the method described by Abd El-Aziz (2011). The fumigant toxicity

experiments carried out on first larval instars and adults of PTM. The concentrations loaded on an exact surface of filter paper then attached to the cap of vials (20 ml) then covered with organza cloth to prevent direct contact of insects to extracts. Five first larval instars (24h old) introduced to each vials and percent of mortality recorded after 48 hrs in treated and control vials.

About adult insect's fumigant bioassay, filter papers were impregnated with the required concentration of extracts then were placed underside surface of the screw caps of the glass jars after solvent evaporation (10 min). The inner surface of caps covered with organza cloth to prevent direct contact of insects to extracts. After introducing the adults (5 adults per jar) to the jars, the lids covered with parafilm. The adults were provided with 10% honey solution. These jars were transferred to growth chamber at  $26\pm 2^{\circ}\text{C}$ ,  $50\pm 10\%$  RH and photoperiod of 16:8 (L: D) h. Each experiment was replicated three times. Mortality was counted after 24 h of exposure to plant crude extracts.

**Statistical Analysis:**  $\text{LC}_{50}$  value of extracts was determined according to Hong et al (1988) for the contact method. Corrected percentage mortality was calculated using Abbott's formula. From the corrected mortality larval  $\text{LC}_{10}$ ,  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values were calculated using the computation program of probit analysis using SPSS software. The ovicidal, fumigant and oviposition deterring activity were analyzed using one-way ANOVA. Significant differences between treatments were determined using Duncan's multiple-range test ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

### Ovicidal activity of plants crude extracts

Results of ovicidal activity of *M. vulgare* and *A. millefolium* crude extracts on 6 and 24 h old eggs of PTM are presented in Table 1 and 2. Maximum ovicidal activity was observed in hexane extract in both plants with  $\text{LC}_{50}$  values of 6.55 and 8.03 mg/l respectively. Methanol and ethyl acetate extracts of *M. vulgare* showed similar activity with  $\text{LC}_{50}$  values of 10.7 and 11.5 mg/l in 6 h old eggs and 10.7 and 12.79 mg/l in 24 h old eggs respectively. The least ovicidal activity was determined in aqueous extract of both studied plants with  $\text{LC}_{50}$  values of 19.4 and 19.95 mg/l in 6h old eggs and 13.54 and 20.71 mg/l in 24 h old eggs respectively. The obtained  $\chi^2$  values were non-significant for all the tested extracts. The probit analysis clearly indicates that the hexane extract of both plants has the potential to kill the eggs of PTM at different embryogenesis periods.

### Oviposition deterring activity results of plants extracts

Oviposition deterrent activity normally indicates deterrent activity potential of plant extracts (Table 3). All tested concentrations of *M. vulgare* and *A. millefolium* crude extracts caused sharp reductions in the number of eggs deposited. Among the tested extracts and concentrations, except Hexane extract of *M. vulgare* and Ethyl acetate extract of *A. millefolium* remains induced the greatest antioviposition effect, with no eggs laid at treated potato slices. And about two exceptions these treatments also reduced the number of eggs laid in comparison with control. Oviposition deterring activity increased with concentration dependent manner.

### Fumigant toxicity results

The fumigant toxicity of the *M. vulgare* and *A. millefolium* crude extract against 1<sup>st</sup> larval instar and adults of PTM is shown in Tables 4. Plants extracts showed strong fumigant activity against PTM in a concentration-dependent manner. Among tested extracts only Hexane extract of *M. vulgare* had fumigant activity on adults of PTM. About 1<sup>st</sup> larval instar, the  $\text{LC}_{50}$  values of hexane extract of *M. vulgare* were more than other extracts (17.27 mg/l). Aqueous Extract of both plants had no fumigant toxicity against PTM. Also ethyl acetate and methanol extract of *A. millefolium* had no fumigant activity against studied stages

of PTM. It seems that 1<sup>st</sup> larval instar of PTM are susceptible to extracts than adults.

## DISCUSSION

The most effective botanical extracts would be those offering a broad spectrum of activity against various life stages of the pest. The effective control agent should reduce the insect population at all life stages. This study provides evidence that *M. vulgare* and *A. millefolium* crude extracts have toxic effects against studied stages of *Phthorimaea operculella*.

Pesticides based on plant metabolites have demonstrated efficacy against a range of stored product pests. They may be applied as fumigants, or direct sprays with a range of effects from lethal toxicity to repellence or oviposition deterrence in insects. These features indicate that pesticides based on plants metabolites could be used in a variety of ways to control a large number of pests (Duke, 1990; Ishaaya & Horowitz, 2009; Nerio et al., 2010). In this study except aqueous extract of both plant species, remained extracts was more toxic on eggs of PTM. Hexane with a non-polar property, extracted compounds affects eggs in both plants, in some cases non-polar solvents extracts more efficient compounds that are more effective against insects. For example Ho et al. (1995) reported that non-polar extracts of *Illicium verum* completely suppressed F1 adult emergence in *Tribolium castaneum* and *Sitophilus zeamais* while polar extracts only caused a significant reduction in F1 adult emergence. In other study the effects of four natural plant oils were tested against eggs of PTM. The cardamon oils exhibited the best reduction in percentage of eggs hatchability (Moawad & Ebadah, 2007). Studies on ovicidal effects of aqueous and alcoholic extracts of four different plants against the diamondback moth, *Plutella xylostella* (L.) revealed that plants extracts had significant effect on the mortality of eggs. However, the alcoholic extracts were found to be better than the aqueous extracts (Kumar et al., 2009).

Different chemical compounds from plants containing, carvacrol, carveol, geraniol, linalool, menthol, terpineol, thymol, verbenol, carvones, fenchone, menthone, pulegone, thujone, verbenone, cinnamaldehyde, citral, citronellal, and cinnamic acid have ovicides activity against *M. domestica* (Rice & Coats, 1994). Phytochemical screening on *Glinus lotoides* showed a varied composition of secondary metabolites including flavonoids, tannins, terpenes, sterols, coumarins and saponins that may be responsible to ovicidal activity of this plant on *Corcyra cephalonica* eggs.

Dwivedi & Garg (2003) reported ovicidal activity of flower extract of turmeric and *Lantana camara* against *C.cephalonica*. They reported that ovicidal effect which may be due to its easy penetration through delicate covering of vitellin and chorion membrane thereby increasing the mortality rate. High percentage of egg mortality caused by the extract is assumed to be caused by the active ingredients present in them which might have disrupted blastokinesis and induced impaired larval hatching.

Furthermore, PTM adults showed high susceptibility to the fumigation by hexane extract of *M. vulgare*. But other extracts had no effect on adults while first larval instar of PTM strongly affected by Hexane, ethyl acetat and methanol extract of this plant and hexane extract of *A. millefolium*. Results shows that first larval instar of PTM are susceptible to *M. vulgare* fumigant toxicity. The fumigant toxicity of three plant extracts from Ailanthus against *Oryzaephilus surinamensis*, *Sitophilus oryzae* and *Liposcelis paeta* adults were investigated. All the plant extracts had potent fumigant activities against *O. surinamensis* and *S. oryzae* adults. Similar to present results, the fumigant toxicity significantly increased with the increasing concentration (Lu & He, 2010). Lu et al. (2012) reported that *A. officinarum* rhizome extract exhibited strong fumigant, repellent

activity in a dosage-dependent manner against *T. castaneum* adults. In different studies it has been demonstrated that chemical compounds with high repellent activity include:  $\alpha$ -pinene, limonene, citronellol, citronellal, camphor and thymol. Although synthetic chemicals are still more frequently used as repellents than plant extract materials, these natural products have the potential to provide efficient, and safer repellents for humans and the environment (Nerio et al., 2010).

Oviposition deterring, ovicidal and fumigant activity of plants extract are in relation with chemical composition of them. The Lamiacea family has been reported to have insecticidal activities due to presence of phytochemicals. Hamedeyazdan et al. (2013) reported that *Marubium persicum* contains higher proportions of non-terpenoid ketone, namely acetophenone. Acetophenone was demonstrated to cause acute and delayed types of insecticidal and ovicidal activities (Zohair, 1995; Liu et al., 2014). These studies reveal that ketones were more effective as fumigants. Trans-anethole, thymol, 1,8-cineole, carvacrol, terpineol, and linalool have been evaluated as fumigants against *T. castaneum*. The major components of *Marrubium vulgare* were eudesmol, citronellol, citronellyl formate (Kadri et al., 2011; Zawislak, 2012) and Tetramethyl heptadecan, Germacrene, Pinene, Phytol, Dehydro-sabina Ketone, Piperitone, Cadinene, Octen and Benzaldehyde (Abadi & Hassani, 2013). Secondary metabolites such as piperine, caryophyllene and limonene are reported act as insecticide. Many insecticidal components of plant extracts are mainly monoterpenes, such as limonene which have been shown to be toxic to PTM (Fang et al., 2010; Wang et al., 2014). Studies on extracts of *M. vulgare* had indicated that piperine and other active piperamides were responsible for the toxicity of these extracts to the *Callosobruchus chinensis* L. (Tavares et al., 2011; Scott et al., 2003).

Ateyyat & Abu-Darwish (2009) revealed that *A. millefolium* contains compounds such as flavonoids which are soluble in polar solvents such as acetone and ethanol. Flavonoids have a catecholic B-ring that seems to be responsible for the toxicant activity to insects (Onyilagha et al., 2004). Nadim et al. (2011) reported that, the predominant constituents of *A. millefolium* were sabinene, cineole, borneol, bornyl acetate, pinene, pinene, terpinene and chamazulene. Effects of *A. millefolium* extracts on different developmental stages of insects were investigated and in some cases the compounds showed a acceptable control on pest insects (Conti et al., 2010; Zoubiri & Baaliouamer, 2014). Dehghan & Elmi (2014) reported that chemical compounds of essential oils of *Achillea* species were highly variable, which may be due to the differences in their chemical polymorphic structure and environmental conditions. Difference on type and composition of metabolites is responsible to insecticidal activity of extracts, thus we suggest determining the chemical composition of studied plants extracts to identify potent insecticidal metabolites in these native plants. Plant species of the families Asteraceae and Labiatae are known for their content in diterpenes and sesquiterpenes. Sesquiterpenes display extensive structure variety and have been reported to serve as toxic or feeding deterrents to herbivore insects (Fraga, 2004).

The results suggest that extracts of both the species have a potential to act as ovipositional deterrent and can be employed against PTM in stored condition. Studies showed that secondary metabolites such as monoterpenes volatiles are more effective as insect fumigants. Pulegone, linalool and limonene are known effective fumigants against *Sitophilus oryzae*. While *Mentha citrata* oil containing linalool and linalyl acetate exhibit significant fumigant toxicity to these rice weevils (Singh, 2011; Aryvoli & Tennyson, 2013; Rana et al., 2013; Jeyasankar et al., 2013).

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Table 1. Ovicidal activity of *M. vulgare* and *A. millefolium* crude extracts on 6 h old eggs (mg/l)

Plant	Extract	Slop±SE	$\chi^2$	LC <sub>10</sub> (CL 95%)	LC <sub>50</sub> (CL 95%)	LC <sub>90</sub> (CL 95%)
<i>M. vulgare</i>	Hexane	1.63±0.33	7.83 <sup>ns</sup>	1.06 (0.2-2.28)	6.55 (3.57-9.6)	40.15 (24.92-103.06)
	Metanol	1.99±0.4	6.19 <sup>ns</sup>	2.44 (0.72-4.32)	10.7 (6.95-14.97)	47.20 (30.20-114.90)
	Etyl acetat	1.28±0.35	8.30 <sup>ns</sup>	1.15 (0.05-3.02)	11.5 (5.75-19.1)	49.114 (50.27-1336.99)
	Aqueous	1.42±0.4	12.61 <sup>ns</sup>	2.43 (0.2-5.37)	19.4 (11.61-35.53)	155.12 (65.65-2365.31)
<i>A. millefolium</i>	Hexane	1.62±0.43	5.35 <sup>ns</sup>	1.3 (0.09-3.14)	8.03 (3.48-12.13)	49.61 (28.95-207.67)
	Metanol	1.66±0.4	15.31 <sup>ns</sup>	1.98 (0.28-4.13)	11.72 (6.8-16.93)	69.45 (39.39-290.13)
	Etylacetat	1.00±0.37	11.22 <sup>ns</sup>	1.01 (0.0-3.64)	19.30 (9.05-48.99)	367.34 (95.95-882425.22)
	Aqueous	1.33±0.37	12.71 <sup>ns</sup>	2.16 (0.16-4.91)	19.95 (12.43-35.05)	183.69 (75.54-3018.18)

Table 2. Ovicidal activity of *M. vulgare* and *A. millefolium* crude extracts on 24 h old eggs (mg/l)

Plant	Extract	Slop±SE	$\chi^2$	LC <sub>10</sub> (CL 95%)	LC <sub>50</sub> (CL 95%)	LC <sub>90</sub> (CL 95%)
<i>M. vulgare</i>	Hexane	1.78±0.35	7.52 <sup>ns</sup>	1.52 (0.4-2.88)	8.0 (5.01-11.13)	42.1 (26.85-101.42)
	Methanol	1.22 ± 0.35	4.64 <sup>ns</sup>	0.95 (0.03-2.73)	10.7 (4.89-18.02)	120.0 (50.66-1863.0)
	Ethyl acetate	2.19 ± 0.48	8.22 <sup>ns</sup>	3.32 (0.9-5.85)	12.79 (8.04-18.12)	49.21 (31.50-127.45)
	Aqueous	1.61±0.44	4.58 <sup>ns</sup>	2.17 (0.19-4.85)	13.54 (7.13-21.86)	84.30 (42.65-620.41)
<i>A. millefolium</i>	Hexane	2.17±0.52	23.89 <sup>ns</sup>	3.33 (0.07-7.18)	12.96 (4.71-21.73)	50.37 (27.75-711.66)
	Methanol	1.75±0.39	8.79 <sup>ns</sup>	2.45 (0.51-4.72)	13.26 (8.39-18.56)	71.64 (42.62-235.8)
	Ethyl acetate	1.58±0.44	12.02 <sup>ns</sup>	2.07 (0.15-4.67)	13.45 (7.32-19.97)	87.22 (45.45-647.82)
	Aqueous	1.25±0.39	8.49 <sup>ns</sup>	1.95 (0.05-4.91)	20.71 (12.05-42.76)	219.94 (78.48-12371.02)

Table 3. Oviposition deterring activity of *M. vulgare* and *A. millefolium* crude extracts (% of total eggs oviposited)

Concentration (gr/l)		<i>A. millefolium</i>				<i>M. vulgare</i>			
		Hexane	Ethyl acetate	Methanol	aqueous	Hexane	Ethyl acetate	Methanol	aqueous
0.02	Treatment	0	0	0	0	26.31	0	0	0
	Control	100	100	100	100	73.69	100	100	100
0.015	Treatment	0	2.5	0	0	23.32	0	0	0
	Control	100	97.5	100	100	76.68	100	100	100
0.01	Treatment	0	6.12	0	0	12.62	0	0	0
	Control	100	93.88	100	100	87.38	100	100	100

Table 4. Fumigant toxicity of *M. vulgare* and *A. millefolium* crude extracts on PTM (mg/l)

Plant	Extract	Stage	Slop±SE	$\chi^2$	LC <sub>10</sub> (CL 95%)	LC <sub>50</sub> (CL 95%)	LC <sub>90</sub> (CL 95%)
<i>M. vulgare</i>	Hexane	L	3.62±0.92	2.81 <sup>ns</sup>	1.87 (0.8-2.58)	4.22 (3.28-5.51)	9.53 (6.81-23.13)
	Hexane	A	6.46±1.99	7.02 <sup>ns</sup>	10.94 (6.01-13.14)	17.27 (14.82-22.48)	27.29 (21.44-65.44)
	Ethyl acetate	L	2.08±0.57	7.25 <sup>ns</sup>	0.76 (0.10-1.47)	3.14 (1.74-4.66)	12.94 (7.69-55.32)
	Methanol	L	2.69±0.62	7.67 <sup>ns</sup>	1.01 (0.35-1.59)	3.03 (2.09-4.23)	9.07 (5.99-23.41)
<i>A. millefolium</i>	Hexane	L	3.83±0.61	3.20 <sup>ns</sup>	0.61 (0.10-1.12)	2.15 (1.21-3.08)	7.59 (4.87-23.29)

L: First larval instar, A: Adults. ns: non-significant  $\alpha=0.5$