Biological activities of *Thymus vulgaris*, *Petroselinum sativum* and *Stachys lavandulifolia* extracts against adult *Callosobruchus maculatus* (Coleoptera: Bruchidae)

Fahimeh HAMIDI[1], Ali MEHRVAR[1,*], Naser EIVAZIAN KARY[1], Hassan VALIZADEH[2]

(1. Department of Plant Protection, Faculty of Agriculture, Azarbaijan Shahid Madani University, P. O. Box 5375171379, Tabriz, Iran; 2. Department of Chemistry, Faculty of Basic Sciences, Azarbaijan Shahid Madani University, P. O. Box 5375171379, Tabriz, Iran)

Abstract: 【Aim】 Plants secondary metabolites are potentially considered as suitable alternatives for chemical pesticides if their extents of impacts were well studied earlier on different groups of pests. This study aims to evaluate the possible impacts of solvent polarity on the insecticidal activity of different plant extracts. 【Methods】 The extraction solvents were selected from a wide range of polarities, *n*-hexane, ethyl acetate and methanol. The lethal effects of *Petroselinum sativum* seed extracts as well as *Thymus vulgaris* and *Stachys lavandulifolia* shoot extracts on *Callosobruchus maculatus* adults at 28 ± 2℃, 50% ± 5% RH and a photoperiod of 16L: 8D were evaluated, and their ovicidal and oviposition deterrence effects were also evaluated. 【Results】 The lowest LC_{50} value for contact toxicity to *C. maculatus* adults was associated with the *n*-hexane extract of *T. vulgaris* (0.05 g/mL) following with the ethyl acetate and methanol extracts, respectively. The ethyl acetate extract of *T. vulgaris* had the lowest LT_{50} value (10.04 h), with the highest relative speed of kill index (42.23%) among all the extracts. All of the extracts at LC_{20} and LC_{50} concentrations resulted in 100% ovicidal activity in contact toxicity to *C. maculatus* adults. In terms of oviposition deterrence, the methanol extract of *S. lavandulifolia* had the highest deterrence activity (97.54%). 【Conclusion】 Based on the results, it is concluded that *T. vulgaris*, *P. sativum* and *S. lavandulifolia* extracts show significant performances in terms of insecticidal and ovicidal toxicities as well as oviposition deterrence against *C. maculatus* adults. However, non-polar (*n*-hexane) extracts of the studied plants show better performances in comparison with other extracts.

Key words: *Callosobruchus maculatus*; plant-derived insecticides; solvents; crude extract; biological activity

1 INTRODUCTION

Callosobruchus maculatus, is one of the main insect pests of legumes especially the cowpea, in Asia, Africa and throughout tropical and semi-tropical regions (Talukder and Howse, 1995; Lee *et al.*, 2001). This polyphagous pest feeds on the seeds of various legumes and its larvae reduce the marketability and germination ability of the damaged seeds. However, over 90 percent loss of cowpea seeds has been caused by this pest in the world (Ogunwolu and Odunlami, 1996; Pascual-Villalobos and Ballesta-Acosta, 2003). Application of pesticides is one of the major challenges we are faced with today. The use of methyl bromide and phosphine has been limited in silos and warehouses due to the severe hazards it may cause to humans and the environment (Haque *et al.*, 2000). Today, use of plant secondary metabolites and compounds against stored product pests is considered as one of the best alternatives for controlling them. The insecticidal properties of essential oils of Lamiaceae and Apiaceae plants have been evaluated in several studies (Chaubey, 2008; Ilboudo *et al.*, 2010). In the present study, extracts of the plants *Thymus vulgaris*, *Lavandula officinalis*, *Ocimum basilicum* and *Stachys lavandulifolia* from the Lamiaceae family and *Petroselinum sativum* from the Apiaceae family have been extracted with three solvents *i. e.*, *n*-hexane, methanol and ethyl acetate and their toxicities against *Callosobruchus maculatus* were evaluated. Also the ovicidal and oviposition deterrence effects were studied for each of the extracts.

* Corresponding author, E-mail: alimehrvar7061@gmail.com

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2 MATERIALS AND METHODS

2.1 Insect culture

The laboratory culture of *C. maculatus* was obtained from Department of Plant Protection, Faculty of Agriculture, Azarbaijan Shahid Madani University, and the colony was maintained at 28 ± 2℃, 50% ±5% RH and a photoperiod of 16L∶8D. The insects were reared on cowpea seeds in 1 L plastic containers. In each container, over 150 g of cowpea seeds were poured and 100 pairs of male and female adults were released in it. In order to obtain a population of the same age, the adults were removed from the containers after 24 h and the oviposited seeds were moved into the rearing containers.

2.2 Preparation of plant extracts

Since the active ingredients of the plants might have different polarities, the solvents were selected in the study from a wide range of polarities, *viz.*, *n*-hexane, ethyl acetate and methanol with the relative polarities of 0.009, 0.228, and 0.762, respectively. The plants were collected from North West of Iran, especially from East-Azarbaijan province. In the experiments, seeds of *P. sativum* and *O. basilicum* and shoots (leaves, stems and flowers) of *L. officinalis*, *T. vulgaris* and *S. lavandulifolia* were used. The shoots of the plants were washed and shade dried at room temperature. Dried leaves, stems, and flowers were submerged completely in *n*-hexane, methanol, and ethyl acetate separately in 1 L sterile glass jars for 5 days. The powdered seeds of *P. sativum* and *O. basilicum* were treated in the same manner. For extraction of each plant material an equal ratio (1∶1) of each solvent was used.

The obtained solution was filtered through a Whatman No. 1 filter paper. The filtrate of each extract was then concentrated in a rotary evaporator apparatus at 40℃ with 200 r/min. The concentrated extracts were kept separately in suitable and sealed glass jars and also labeled. The jars were kept at a refrigerator with 3 ± 1℃ (Ravindran *et al.*, 2012).

2.3 Bioassay of plant extracts

A concentration of 0.2 g/mL of each compound (15 solvent-extract) were poured into 9 cm petri plates lined with Whatman No. 1 filter paper. In the control group, only the relevant solvent was used. The petri plates were air dried for 2 h and then 15 adults of 1 – 3 d old were released in each plate and the lids were put back (Mahdavi Arab *et al.*, 2008). The plates were kept in an incubator at 28 ± 2℃ and a relative humidity of 50% ± 5% with a photoperiod of 16L∶8D. The mortality data were recorded regularly at 3, 6, 9, 12 and 24 h after the treatments. The experiment was repeated three times. However, those extracts whose mortality value was less than 50% were removed from the rest of the experiments.

Biological activity (lethal concentrations and time) of the selected extracts were then determined. To this end, the range of concentrations for each of the extracts with five-fold dilution was determined using logarithmic intervals. The study was conducted in a factorial experiment (plant-solvent) using the completely randomized design (CRD) with three replications. All the experimental conditions were the same as mentioned earlier and then the LC_{50} and LT_{50} values were calculated. In order to determine the LT_{50} values, the highest concentration of each solvent-extract treatment was used.

2.4 Ovicidal bioassays

In the study, the LC_{20} and LC_{50} concentrations of the 15 plant extracts in contact toxicity to adults were used. Sixty pairs of male and female adults of 1 – 3 d old were released on 200 g of untreated cowpea seeds and were allowed to oviposit for 24 h. Then, all of the adults were removed and the cowpea seeds which contained two eggs were collected. In case the number of eggs on a seed exceeded two, the extra eggs were removed with a forceps under a stereomicroscope. Five of the egg-bearing seeds were treated with determined concentrations of the extracts and then placed in 27 mL (2.2 cm in diameter and 7 cm in height) sealed glass jars after the solvent evaporated. For proper ventilation, the lids of jars were covered with lace and then were kept for 31 d in experimental conditions until the adults were emerged. Then the numbers of hatched and unhatched eggs were recorded. In the control group, only the solvent was used. The experiment was replicated three times.

2.5 Oviposition deterrent responses

To this end, the LC_{25} concentrations of each extract in contact toxicity to adults were used. The petri containers (9 cm) were divided into three sections using thick white cardboard in a way that a pathway was formed in the middle of the container for the insect to move and choose its oviposition location freely. In one section, the treated cowpea seeds and on the other side the control seeds (soaked with the relevant solvent) were placed, and in the third section, five pairs of newly emerged male and female adults were released using a soft brush (Fig. 1). All the experiments were conducted in an incubator maintaining temperature 28 ± 2℃, and relative humidity of 50% ± 5% with a photoperiod of 16L∶8D and were replicated three

times. After four days, the number of eggs on each of the seeds was recorded using the stereomicroscope and the oviposition deterrent index (ODI) was calculated using the following formula:

$$\text{ODI}(\%) = \left(1 - \frac{NEt}{NEc}\right) \times 100.$$

Where, *NEt* is the number of eggs laid on the treated seeds, and, *NEc* is the number of eggs laid on the control seeds in each replication.

Fig. 1　Designed petri dish to evaluate the oviposition deterrence of plant extracts against *Callosobruchus maculatus* adults

The petri container (9 cm) was divided into three sections: A: Adults (five pairs of newly emerged male and female adults), B: Treated cowpea seeds, and C: Control seeds (soaked with the relevant solvent), with a pathway in the middle to perform free choice by the insects for oviposition.

2.6　Data analysis

The data were analyzed using the MSTAT-C software, and Duncan's Multiple Range Test (DMRT) was used in order to compare the means. Before the analyses, the normality test was conducted and the data were transferred where necessary. To evaluate the toxicity of the extracts, SPSS version 22 was used. In all the experiments, mortality values were corrected using Abbott's formula (Abbott, 1925). For a better and informal comparison of time-lethality reactions of the extracts, the relative speed of kill index (RSK) was used as follows:

$$\text{RSK}(\%) = 100 - (\text{LT}_{50}t \times 100)/\text{LT}_{50}h.$$

Where, $\text{LT}_{50}t$ is the LT_{50} value of each solvent-extract treatment, and $\text{LT}_{50}h$ is the highest amount of LT_{50} value among the experiment.

3　RESULTS

3.1　Concentration-response effects of the extracts on adults

The results of insecticidal activity of 15 plant materials extracted by various solvents from different parts of five plants at a concentration of 0.2 g/mL against *C. maculatus* adults are presented in Table 1. Among them, *n*-hexane, methanol, and ethyl acetate extracts of *T. vulgaris*, *P. sativum*, and *S. lavandulifolia* resulted in over 50% mortality against the pest and were chosen for the next round of experiments while *Lavandula officinalis* and *Ocimum basilicum* extracts were removed from the rest of the experiments.

Table 1　Mean of corrected mortality rates of different plant extracts at the concentration of 0.2 g/mL against *Callosobruchus maculatus* adults

Plant	Solvent	Corrected mortality after 24 h (%)
Thymus vulgaris	*n*-Hexane	100
	Methanol	100
	Ethyl acetate	100
Petroselinum sativum	*n*-Hexane	79
	Methanol	85
	Ethyl acetate	79
Stachys lavandulifolia	*n*-Hexane	82
	Methanol	50
	Ethyl acetate	66
Lavandula officinalis	*n*-Hexane	15
	Methanol	3
	Ethyl acetate	5
Ocimum basilicum	*n*-Hexane	16
	Methanol	12
	Ethyl acetate	5

The LC_{50} values resulted from contact toxicity of *n*-hexane, methanol, and ethyl acetate extracts of *T. vulgaris*, *P. sativum*, and *S. lavandulifolia* are presented in Table 2. These values showed that *C. maculatus* adults were the most sensitive to the *n*-hexane extract of *T. vulgaris* and the least sensitive towards the methanol extract of *S. lavandulifolia*. Moreover, the difference among the various samples of extract for each plant was not significant. Based on the obtained results, the *T. vulgaris* solvent-extracts were more toxic than *P. sativum* and *S. lavandulifolia* solvent-extracts against *C. maculatus* adults and the toxicity of *P. sativum* and *S. lavandulifolia* seeds decreased in various solvents, respectively. However, low LC_{50} values in *C. maculatus* adults showed the high sensitivity of the pest towards these extracts.

3.2　Time-mortality responses of adults to the extracts

The results suggested that the ethyl acetate extract of *T. vulgaris* and *P. sativum* had the lowest LT_{50} values among the three extracts but for *S. lavandulifolia* the lowest value was associated with the *n*-hexane extract (Table 3). The variation range of LT_{50} values was obtained between 10.04 h (related to the ethyl acetate extract of *T. vulgaris*) and 17.38 h (for the ethyl acetate extract of *S.*

lavandulifolia). In other words, among all the extracts, the ethyl acetate extract of *T. vulgaris* had the highest RSK (42.23%), which was followed by the methanol and *n*-hexane extracts of the same plant (Table 3).

Table 2 Probit analysis of concentration-mortality response of *Callosobruchus maculatus* adults to *n*-hexane, methanol and ethyl acetate extracts of *Thymus vulgaris*, *Petroselinum sativum* and *Stachys lavandulifolia* after 24 h

Plant	Solvent	β ± *SE*	χ^2	LC_{50} (g/mL) (Fiducial limit)	Relative activity *
Thymus vulgaris	*n*-Hexane	1.99 ± 0.37	9.01^{ns}	0.05(0.04 – 0.07)	5.00
	Methanol	2.59 ± 0.48	18.4^{ns}	0.08(0.07 – 0.12)	3.13
	Ethyl acetate	2.32 ± 0.38	15.43^{ns}	0.06(0.05 – 0.07)	4.17
Petroselinum sativum	*n*-Hexane	4.39 ± 0.77	12.04^{ns}	0.17(0.16 – 0.19)	1.47
	Methanol	6.43 ± 1.03	20.86^{ns}	0.13(0.12 – 0.14)	1.92
	Ethyl acetate	4.68 ± 0.77	6.22^{ns}	0.16(0.15 – 0.18)	1.56
Stachys lavandulifolia	*n*-Hexane	6.35 ± 1.02	5.14^{ns}	0.13(0.12 – 0.14)	1.92
	Methanol	3.22 ± 0.55	16.28^{ns}	0.25(0.22 – 0.29)	1.00
	Ethyl acetate	3.80 ± 0.62	10.67^{ns}	0.16(0.14 – 0.18)	1.56

^{ns}Non-significant at $P > 0.05$ with one-way analysis of SPSS 22. * All lines were compared with the highest level of LC_{50} value (Shapiro and Argauer, 2001).

Table 3 Probit analysis of time-mortality responses of *Callosobruchus maculatus* adults to *n*-hexane, methanol and ethyl acetate extracts of *Thymus vulgaris*, *Petroselinum sativum* and *Stachys lavandulifolia*

Plant	Solvent	Concentration (g/mL)	β ± *SE*	χ^2	LT_{50} (h) (Fiducial limit)	RSK (%)
Thymus vulgaris	*n*-Hexane	0.10	2.41 ± 0.35	3.64^{ns}	13.51(11.28 – 17.10)	22.27
	Methanol	0.11	2.95 ± 0.38	4.49^{ns}	11.92(10.29 – 14.17)	31.42
	Ethyl acetate	0.10	2.71 ± 0.35	4.12^{ns}	10.04(8.59 – 11.91)	42.23
Petroselinum sativum	*n*-Hexane	0.22	2.74 ± 0.39	23.76*	16.65(12.87 – 25.80)	4.20
	Methanol	0.18	5.11 ± 0.63	4.70^{ns}	15.92(14.26 – 18.18)	8.40
	Ethyl acetate	0.22	3.69 ± 0.48	20.58^{ns}	15.79(13.09 – 20.61)	9.15
Stachys lavandulifolia	*n*-Hexane	0.18	2.98 ± 0.41	24.35*	16.22(12.74 – 24.05)	6.67
	Methanol	0.36	3.91 ± 0.51	52.87**	16.87(12.60 – 29.93)	2.93
	Ethyl acetate	0.26	4.30 ± 0.56	18.68^{ns}	17.38(14.49 – 22.11)	0.00

RSK: Relative speed of kill index. ^{ns}Non-significant at $P > 0.05$, *Significant at $P \leqslant 0.05$, **Significant at $P \leqslant 0.01$ with one-way analysis of SPSS 22.

3.3 Ovicidal activity of the extracts

Ovicidal toxicity of the 15 plant extracts showed that all of the extracts at their LC_{20} and LC_{50} concentrations caused 100% adult mortality in contact toxicity. Therefore, it is possible that these extracts have significant ovicidal toxicity even at lower concentrations.

3.4 Oviposition deterrence of the extracts

Based on the obtained data in the study, all of the extracts had a desirable level of oviposition deterrence (Table 4). The methanol extract of *S. lavandulifolia* was the most effective and the ethyl acetate extract of *T. vulgaris* showed the least deterrence activity on the oviposition of *C. maculatus* (Fig. 2). In addition, *n*-hexane, methanol, and ethyl acetate extracts of *S. lavandulifolia* had higher levels of oviposition deterrence compared with the extracts of *P. sativum* and *T. vulgaris* (Fig. 2).

Table 4 Oviposition deterrent index (ODI) of *n*-hexane, methanol and ethyl acetate extracts of *Thymus vulgaris*, *Petroselinum sativum* and *Stachys lavandulifolia* against *Callosobruchus maculatus* adults after four days

Plant	Solvent	LC_{25} concentration against the adults (g/mL)	ODI (%)
Thymus vulgaris	*n*-Hexane	0.02	85.78
	Methanol	0.05	75.33
	Ethyl acetate	0.03	70.34
Petroselinum sativum	*n*-Hexane	0.12	82.98
	Methanol	0.10	75.00
	Ethyl acetate	0.12	93.26
Stachys lavandulifolia	*n*-Hexane	0.10	91.32
	Methanol	0.15	97.54
	Ethyl acetate	0.11	87.09

ODI: Oviposition deterrent index.

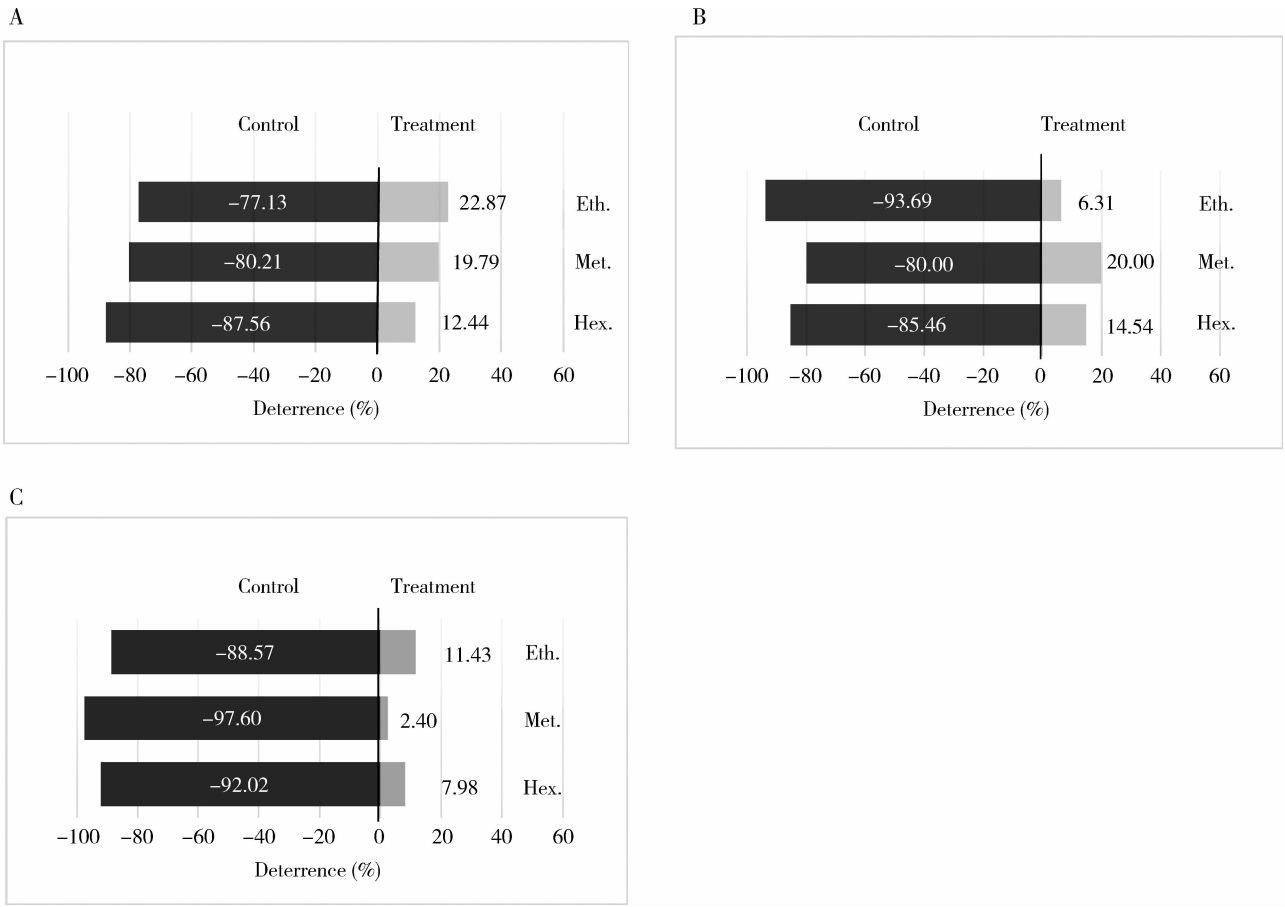

Fig. 2　Deterrence of plant extracts in solvents, *n*-hexane (Hex.), methanol (Met.) and ethyl acetate (Eth.), from oviposition of *Callosobruchus maculatus* adults after four days

A: *Thymus vulgaris*; B: *Petroselinum sativum*; C: *Stachys lavandulifolia*. All the solvent-extracts show oviposition deterrence in the study; *n*-hexane extract of *T. vulgaris*, ethyl acetate extract of *P. sativum*, and methanol extract of *S. lavandulifolia* show more oviposition deterrence activity as compared to other solvent-extracts, respectively.

3.5　Concentration-exposure time interactions

The result of mean comparison suggests that the interaction effect between concentration and time is significant only for the *n*-hexane extract of *T. vulgaris*, and it is insignificant for other solvents and extracts. Thus, Table 5 presents only the values related to this solvent-extract treatment. Mortality at the highest concentration (0.1 g/mL) started within the first three hours and the lowest concentration (0.02 g/mL) resulted in a bit mortality (2.22%) after 9 h. Based on the data as concentration increases, mortality enhances as well (Table 5).

Table 5　Interaction effects of different concentrations and exposure time against *Callosobruchus maculatus* adults with *n*-hexane extracts of *Thymus vulgaris*

Exposure time (h)	Mean percent mortality of adults exposed to different extract concentration (g/mL)				
	0.02	0.04	0.06	0.08	0.1
3	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 e	0.00 ± 0.00 e	8.89 ± 0.11 d
6	0.00 ± 0.00 e	2.22 ± 0.03 e	2.22 ± 0.03 e	2.22 ± 0.03 e	17.77 ± 0.34 bcd
9	2.22 ± 0.03 e	8.89 ± 0.11 d	13.33 ± 0.33 cd	24.44 ± 0.67 abcd	31.11 ± 0.43 abcd
12	8.89 ± 0.11 d	22.22 ± 0.34 bcd	20 ± 0.17 bcd	33.33 ± 0.45 abc	40 ± 0.73 abc
24	20.00 ± 0.16 bcd	40.00 ± 0.34 abc	44.44 ± 0.51 abc	55.55 ± 0.67 ab	77.77 ± 0.67 a

Data (mean ± *SE*) with different letters have significant difference by Duncan's multiple range test ($P \leqslant 0.01$).

4　DISCUSSION AND CONCLUSION

4.1　Impacts of solvent polarity on the insecticidal activity of different plant extracts

Attempts were conducted to exploit novel molecules with highly precise targets for sustainable insect pest management in stored grain. Based on studies, *T. vulgaris*, *P. sativum*, and *S. lavandulifolia* contain high levels of secondary compounds such as alkaloids, terpenoids and

limonoids as well as phenols and ether compounds which are probably the origin of insecticidal properties in these plants (Shakarami *et al.*, 2004). Using 50 plant species on *Tribolium castaneum* (Tenebrionidae) with three solvents including *n*-hexane, methanol, and acetone, Pascual-Villalobos and Robledo (1999) realized that extracts prepared using the methanol solvent had a higher level of contact toxicity. They argued that methanol is better at solving the secondary metabolites of the plants. The results of the present study were in line with that study for methanol extracts of *P. sativum* and *T. vulgaris*, yet the results differed for the other extracts (Table 1). As seen in Table 1, insect reactions to extracts of different plants extracted with different solvents were not the same, meaning that the toxicity of the extracts of the same plant depended on the solvent with which it was extracted. That is, the toxicity of the extract of the plant with one solvent would be different from the extract of the same plant with another solvent. The reason for the significant difference among various solvents (non-polar to polar) is their different abilities in solving plant compounds.

Studies have shown that the active compounds of *S. lavandulifolia* have biological traits. These compounds include phenylethanoids, terpenoids, and flavonoids. Also, myrcene (20%), alpha-pinene (18%), gamma-muurolene (13.2%), and eugenol (7%) can be mentioned as the other compounds of the plant (Babakhanlo *et al.*, 1998). Myrcene and alpha-pinene by suppressing acetylcolinesterase enzyme have insecticidal activity on the pest (Yaghoutnejad *et al.*, 2013). Studies suggest that thymol and carvacrol are the most important components of *T. vulgaris* essential oil. By affecting the permeability of the axonal membrane, carvacrol disrupts the balance of potassium and phosphorus (Yaghoutnejad *et al.*, 2013). This plant also contains phenylpropene-derived compounds which make its extract effective against viruses, bacteria, and fungi (Dholwani *et al.*, 2008). The main monoterpene hydrocarbons of *Satureja sahendica* oil are γ-terpinene (42.2%), p-cymene (6.5%), α-terpinene (5.1%), myrcene (2.3%), limonene (1.1%) and α-thujene (1%). Carvacrol (31.9%) is the predominant representative of oxygenated monoterpene components which was identified in *S. sahendica* (Hassanpouraghdam *et al.*, 2009). These studies show that the same chemical compounds from different plants may play an identical role against various insects (Senrung *et al.*, 2014; Venditti *et al.*, 2017). Correspondingly, *P. sativum* seeds and fruits have oily substances, aleurone, tannins, and a phenylpropene which was known as apiole. Its chemical name is 1-allyl-2,5-dimethoxy-3,4-methylenedioxybenzene and has been found in the essential oil of celery and all parts of parsley (Parthasarathy, 2008). However, the toxic effects of apiole are worthy of further researches (Haghighi *et al.*, 2011). *Petroselinum hortense* fruits were identified comprising 28 components (Hassanpouraghdam, 2010). Hassanpouraghdam (2010) suggested that phenylpropanoids (70.7%) were the main group of volatile oil components of *P. hortense* fruits, which were followed by monoterpene hydrocarbons (26.1%). These compounds are known to act as the chemical groups affecting insect nervous system (Hosseinzadeh *et al.*, 2015).

4.2 Biological responses of *C. maculatus* adults to different solvent-extracts

A look at the variation range of LC_{50} values and also the relative activity of each of the solvents with *T. vulgaris* suggests that the lowest LC_{50} value is associated with the *n*-hexane extract of this plant. However, this value was not significantly lower than the ethyl acetate extract, but both extracts showed significantly lower LC_{50} values than the methanol extract which set the highest LC_{50} value for *T. vulgaris*. Things were the same for *S. lavandulifolia*, yet *P. sativum* yielded different results as its methanol extract had the lowest LC_{50}, followed by *n*-hexane and ethyl acetate extracts with a significant difference. The highest relative activity is associated with the *n*-hexane extract of *T. vulgaris* which is five-fold higher than the methanol extract of *S. lavandulifolia* (as the weakest extract), and followed by the ethyl acetate and methanol extracts of the plant, respectively (Table 2).

Based on the obtained results, it is concluded that using *n*-hexane, methanol, and ethyl acetate extracts of *T. vulgaris* in management programs of this pest is more effective than using those of both *P. sativum* and *S. lavandulifolia* and can yield desirable results. However, before using any of these extracts, it is recommended that their side effects on non-target organisms and the extract persistence in nature be considered.

The same trend was also observed with LT_{50} values. For this reason, all the studied extracts were able to kill 50% of the adults of the pest until 17.4 h after the application. So, this can be considered as an important factor in the pest management.

4.3 Ovicidal and oviposition activities

The LC_{20} and LC_{50} concentrations of all the plant extracts resulted in 100% ovicidal activity against

C. maculatus adults. Plant extracts are likely to have several chemical compounds which block egg aeropils and prevent gas exchange and cause the death of the embryo. In addition, it has been revealed that disruption in the egg cytoplasm causes the formation of a dead egg with a black spot caused by the dead embryo (Deepa and Remadevi, 2011). This is because of penetration of plant extracts through the egg chorion and causing the death of the growing embryo (Singh *et al.*, 1978; Senrung *et al.*, 2014). However, mortality depends on the type, concentration, and timing of the extract. Similarly, the ethanol extract of *Tithonia diversifolia* (Asteraceae) seeds had a significant ovicidal toxicity against *Sitotroga cerealella* (Gelechiidae) eggs (Fouad *et al.*, 2014). The ovicidal toxicity of the extracts of *Melia azedarach*, *Lantana camara*, *Artemisia annua*, and *Cannabis sativa* on *Plutella xylostella* (Plutellidae) eggs showed that alcoholic extracts cause more death in eggs than aqueous extracts and the mortality enhances as concentration increases. The weak effect of the aqueous extract is perhaps due to the lower extraction of active compounds by water (Kumar *et al.*, 2009). Looking at previous findings and the results obtained from the present study, it seems that the extracts used in the research can act as effective pesticides for controlling and managing *C. maculatus*.

Also, suitable oviposition deterrence was revealed by all of the plant extracts at their LC_{25} in contact toxicity (Table 4). The oviposition deterrent index due to application of the methanol extract of *S. lavandulifolia* was initially revealed more effective than the other treatments. But, taking a look at the applied concentration of the methanol extract of *S. lavandulifolia* (0.15 g/mL) and comparing with the ethyl acetate extract of *T. vulgaris* (0.03 g/mL), it is shown that although both the concentrations are equivalent to their LC_{25} value in contact toxicity, the concentration value of the methanol extract of *S. lavandulifolia* is five-fold bigger than the applied concentration of the ethyl acetate extraction of *T. vulgaris*. Consequently, in pest management programs, paying attention to the recommended concentrations of each plant extract is necessary for achieving desirable economic results.

4.4 Conclusion

It could be concluded that, all the studied plant extracts showed desired effects against the insect as a delegate of stored grain pests. However, an overview of the results from polar and non-polar extracts shows that the *n*-hexane (non-polar) extracts of *T. vulgaris* and *S. lavandulifolia* performed better activity against *C. maculatus* adults. This is perhaps due to the suitable penetration of non-polar compounds of these plants through the integument, respiratory system, alimentary canal, and other parts of the body of adults. Also, it is revealed that the difference among the toxicity of plant extracts is not only due to the type of effective compounds in each of the plants but also due significantly to the type of the solvents used for the extractions.

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百里香、欧芹和熏衣草水苏提取物对四纹豆象成虫的生物活性

Fahimeh HAMIDI[1], Ali MEHRVAR[1,*], Naser EIVAZIAN KARY[1], Hassan VALIZADEH[2]

(1. Department of Plant Protection, Faculty of Agriculture, Azarbaijan Shahid Madani University, Po. Box. 5375171379, Tabriz, Iran; 2. Department of Chemistry, Faculty of Basic Sciences, Azarbaijan Shahid Madani University, Po. Box. 5375171379, Tabriz, Iran)

摘要:【目的】植物次生代谢物对不同类害虫的影响程度如得以详细研究,则它们可能有望成为化学杀虫剂的合适替代物。本研究旨在评价溶剂极性对不同植物提取物杀虫活性的可能影响。【方法】从极性不同的化合物正己烷、乙酸乙酯和甲醇中选择提取溶剂。于 28±2℃、相对湿度 50%±5%、光周期 16L∶8D 条件下评价欧芹 *Petroselinum sativum* 种子提取物以及百里香 *Thymus vulgaris* 和熏衣草水苏 *Stachys lavandulifolia* 茎叶提取物对四纹豆象 *Callosobruchus maculatus* 成虫的致死效果,并评价其杀卵和产卵忌避效果。【结果】在对四纹豆象成虫的触杀毒性中,百里香正己烷提取物(0.05 g/mL)的 LC_{50}值最低,其次是乙酸乙酯和甲醇提取物。百里香的乙酸乙酯提取物对四纹豆象成虫的 LT_{50}值最低(10.04 h),在所有提取物中相对击倒速度指数最高(42.23%)。在对成虫的触杀毒性中,LC_{20}和 LC_{50}浓度的所有提取物都具有 100% 的杀卵活性。熏衣草水苏的甲醇提取物对四纹豆象成虫具有最高的产卵忌避活性(97.54%)。【结论】本研究结果得出,百里香、欧芹和熏衣草水苏提取物对四纹豆象成虫具有明显的杀虫和杀卵毒性,并对其具有产卵忌避性。不过,非极性(正己烷)提取物的性能更佳。

关键词: 四纹豆象; 植物源杀虫剂; 溶剂; 粗提物; 生物活性

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