Journal of Medicinal Plants and By-products (2019) 2: 163-169

Original Article

Effect of Essential Oils from *Nepeta crispa, Anethum graveolens* and *Satureja hortensis* Against the Stored-product Insect "*Ephestia kuehniella* (Zeller)"

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Article History: Received: 11 October 2018/Accepted in revised form: 08 June 2019

Abstract

Essential oils of medicinal plants have insecticidal properties as bio pesticides. In this study fumigant effect of three plant essential oils extracted from *Nepeta crispa* Willd (Lamiaceae), *Satureja hortensis* L. (Lamiaceae) and *Anethum graveolens* L. (Apiaceae) were investigated against third instar larvae of the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Pyralidae), during 2017 at Urmia University, Iran. Extraction of essential oils was carried out by hydrodistillation using a Clevenger-type apparatus. All of the essential oils were highly effective to *E. kuehniella* larvae. The highest mortalities produced by essential oil of *N. crispa* with LC_{50} value of 6.846 µl L^{-1} air followed by *A. graveolens* and *S. hortensis* with LC_{50} values of 18.001 and 30.088 µl L^{-1} air, respectively. There was a direct relationship between insecticidal activity and oils concentration. The results revealed that essential oils from the three aromatic plants have a potential insecticidal activity against the third larvae of the stored-product insect "Zeller" under laboratory conditions and might have paved an effective and friendly environmental treatment technique in Integrated Pest Management (IPM) programs.

Keywords: Medicinal plants, Bio pesticides, Insecticidal activity, *Ephestia kuehniella*

Introduction

Over the past decades, the stored products pests control measures have been one of the major tasks for conservators because the damage inflicted to foodstuff is irreversible. Several insect species pose a potential threat to variety of stored products [1]. The Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) is recognized as one of the most cosmopolitan pests attacking stored products and causes serious losses both in quantity through feeding damage and quality by contaminating the product with cast skin [2,3]. In Iran this pest habitats are flour and corn mills. Larvae reduce product quality by feeding. Apart from direct infestation of *E. kuehniella*, the faeces and webbings of larvae spoil the product [4]. According to earlier experiences, fumigants are commonly utilized for control of stored-products pests. Concerns about the development of insect resistance to phosphine have made the search for new alternatives imperative [5-8]. Natural insecticides such as essential oils would be applied as alternatives to conventional insecticides which are environmentally sustainable [9].

The genus *Nepeta* (Lamiaceae), with 280 species, is grown in Europe, Asia and a few parts of Africa [10]. Some of the species are used as traditional medicine for cancer, toothache, colds, anemia, headache, diarrhea, indigestion, tuberculosis, and other various ailments [11,12]. This genus contains 67 species in Iran [13]. Zhu *et al*. [14] and Ali *et al*. [15] stated essential oils of some species of *Nepeta* have insecticidal effect, but the review of the

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literature revealed the scarcity of information over the toxicity of *Nepeta crispa* Willd essential oil. The plants savory, *Satureja hortensis* L., belongs to the Lamiaceae family, has been used as medicinal herb anti-inflammatory properties [16]. Sahin *et al*. [17] reported antimicrobial properties of *S. hortensis*. Insecticidal activities of savory have been indicated [2,18]. *Anethum graveolens* L. (dill) (Apiaceae) is an annual medicinal plant vegetates in Middle East, Russia and Egypt [19]. The plant's seeds have a potent spicy odor and are therefore used as a seasoning agent in various foods. *Anethum* has sedative effect of intestinal spasms and griping, colic pain, liver diseases, etc. [20]. *A. graveolens* essential oil possesses varying degrees of insecticidal and antibacterial activities [21,22]. A large number of essential oils from herbal medicines have been demonstrated the insecticidal activity against a number of stored product insects [2,23,24]. Aslan *et al*. [25] reported insecticidal activity of essential oil of *N. racemosa* (Lamiaceae) on third instar larvae of *E. kuehniella*. Insecticidal effect of the essential oil from *S. hortensis* on *Lipaphis pseudobrassicae* Davis (Aphididae: Homoptera) has been documented [26].

Botanical insecticides are renewable, environment compatible and relatively safe enough to natural enemies [27]. The objective of this research was investigating the toxicity of essential oils of *N. crispa*, *S. hortensis* and *A. graveolens* against one of the most important stored-product insect "*E. kuehniella* (Zeller)".

Material and Methods

Rearing of Insects

The experiments were carried out at Department of Medicinal Plants, Higher Education Center Shahid Bakeri Miyandoab and Department of Plant Protection, Faculty of Agriculture, Urmia University, Iran, during 2017. The Mediterranean flour moth, *E. kuehniella*, was collected from the Department of Plant Protection. Stock cultures of *E. kuehniella* was established and maintained on a mixture of 600 g wheat flour containing 200 g yeast. The insects were kept in a temperature-controlled chamber with 25±5 °C and 65±5% R.H. and photoperiod of 14:8 (L:D) h.

Plant Material

Aerial parts of *Nepeta crispa*, *Satureja hortensis* and *Anethum graveolens*at at flowering stages were harvested from the mountain areas of West-Azerbaijan province (Northwestern Iran) in a middle of August 2016. Plant materials were dried at room temperature and cut into small pieces. The essential oils were extracted from the samples using a Clevenger-type apparatus for 4 h. The obtained essential oil was maintained in a refrigerator at 4°C for subsequent experiments. The essential oil was analyzed using GC/MS [28].

Determination of concentrations range

The appropriate range of essential oils concentrations was prepared in accordance with the procedure described by Moradeshaghi and Pourmirza [29] with some modifications.. Based on standard dose fixing procedure preliminary bioassays were conducted with different doses. Mortality data from all bioassays were analyzed with SPSS software [30] and LD_{25} and LD_{75} were determined. Then, the logarithms of these concentrations were calculated and the logarithmic interval between doses was determined as follows;

$$d = \frac{X5 - X1}{n - 1}$$

d: logarithmic distance between two concentrations

X1: The logarithm of the concentration caused 25% of mortality

X5: The logarithm of the concentration caused 75% of mortality

n: The number of concentrations

The three concentrations between the first and fifth concentrations were calculated by estimating the logarithmic distance (d).

X2: The logarithm of the concentration of oil at logarithmic distance multiplied by one X2= X1+d

X3: The logarithm of the concentration of oil at logarithmic distance multiplied by two X3= X1+2d

X4: The logarithm of the concentration of oil at logarithmic distance multiplied by three X4= X1+3d

Next, the antilogarithms of these numbers were obtained and subsequently main concentrations were determined.

Bioassays

The fumigant toxicity assay of *N. crispa, S. hortensis* and *A. graveolens* essential oils conducted against *E. kuehniella*. Fumigation experiments carried out using the method of Aslan *et al*. [25] with some modifications. Ten third instar larvae of the insect were placed in the 100 ml glass jars as experiment units. The jars were worn with muslin cloth for inhibition of larvae contact to filter paper [31]. The larvae of *E. kuehniella* were exposed to essential oil vapors (2.5, 3.98, 6.32, 10 and 16 µl L^{-1} air for *N. crispa*,17, 23.28, 31.91, 43.75 and 60 µl L^{-1} for *S. hortensis* and 8, 11.95, 17.84, 26.65 and 40 µl L^{-1} air for *A. graveolens*). The oils were pipetted onto filter paper (Whatman No.1) and appended to the under surface of the jar's lid. The mortality rate was recorded after 24 h and those insects that did not move when lightly probed or shaken in the mild heat were considered dead. Each treatment was replicated six times. The bioassay was performed at 25±5 °C and 65±5% R.H. and photoperiod of 14:8 (L:D) h. In each trial, the control groups were treated except that no essential oils were employed.

Data Analysis

Data were analyzed using ANOVA and treatment means were compared by Tukey's test at α=0.05 level [30]. Probit analysis was conducted to estimate LC_{25}, $LC_{50,}$, LC_{75} and LC_{95} values [32].

Results

GC/MS analysis of the essential oils of *N. crispa* showed that 1,8-Cineole (57.69%), Beta-pinene (6.53%) and Alpha Terpineol (4.44%) were the predominant components. The components of Carvacrol (34.74%), Gamma-terpinene (34.27%) and Para-cymene (16.96%) in *S. hortensis*and the components of L-Phellandrene (34.19%), Carvone (23.67%), Limonene (21.47%), alpha-Terpineol (5.58%), and Para-cymene (5.50%) were the most abundant components in *A. graveolens* (Table 1).

Insecticidal activity of essential oils against *E. kuehniella* larvae

The results revealed that all essential oils had lethal effects to the tested insects. Probit analysis data (P<0.0001) revealed that *N. crispa* essential oil (LC_{50}=6.846 µl L^{-1} air) possessed stronger fumigant toxicity against third instar larvae of *E. kuehniella* compared with those of essential oils of *A. graveolens* (LC_{50}=18.001 µl L^{-1} air) and *S. hortensis* (LC_{50}=30.088 µl L^{-1} air) (Table 2). In a bioassay of the essential oils of *N. crispa* (F= 55.56; df= 4, 25; P<0.001), *S. hortensis* (F= 56.24; df= 4, 25; P<0.001) and *A. graveolens* (F= 59.44; df= 4, 25; P<0.001) against third instar larvae of *E. kuehniella* mortality varied significantly with increasing concentration of essential oils. Mortalities of the larvae were 89.7, 88.3 and 86.7 % after 24 h exposure to the highest concentrations of *N. crispa*, *S. hortensis* and *A. graveolens* essential oils, respectively (Table 3).

Table 1 Predominant the essential oils components of the studied plants

Medicinal Plant	Chemical group	Component	*RI	Composition %
N. crispa	Monoterpenoid	1,8-cineole	1032	57.69
N. crispa	Monoterpene	beta-pinene	978	6.53
N. crispa	Monoterpene	alpha terpineol	1192	4.44
S. hortensis	Monoterpenoid	carvacrol	1305	34.74
S. hortensis	Monoterpene	gamma-terpinene	1060	34.27
S. hortensis	Alkylbenzene related to a monoterpene	para-cymene	1025	16.96
A. graveolens	Monoterpene	l-phellandrene	1005	34.19
A. graveolens	Terpenoids	carvone	1249	23.67
A. graveolens	Monoterpene	limonene	1030	21.47
A. graveolens	Monoterpene	alpha-terpineol	1188	5.58
A. graveolens	Alkylbenzene related to a monoterpene	para-cymene	1025	5.50

*RI= Retention Index.

Table 2 Probit analysis data for *Nepeta crispa* Willd. , *Satureja hortensis* L. and *Anethum graveolens* L. essential oils against the third instar larvae of *Ephestia kuehniella* Zeller

Essential oil	n	LC_{50} (µl L^{-1})	*CI (µl L^{-1})	LC_{95} (µl L^{-1})	*CI (µl L^{-1})	Slope ± SE	** χ^2
N. crispa	10	6.846	6.004–7.845	26.427	20.037–40.052	2.803± 0.331	0.616
S. hortensis	10	30.088	27.194–33.146	82.953	67.702–113.559	3.734±0.441	0.392
A. graveolens	10	18.001	15.94–20.342	62.888	48.694–93.026	3.029± 0.35	0.537

* CI: confidential interval; ** χ^2: chi-squared value

Table 3 Fumigant activity of *Nepeta crispa* Willd., *Satureja hortensis* L. and *Anethum graveolens* L. essential oils against the third instar larvae of *E. kuehniella*

Pest species	Concentration(µl L^{-1} air)	Mortality (%) mean±SE
N. crispa	2.5	11.7 ± 3.07 a
	3.98	25.0 ± 5.63a
	6.32	46.7 ± 3.33b
	10	65.0 ± 4.28c
	16	89.7 ± 3.33d
S. hortensis	17	16.7 ± 3.33 a
	23.28	36.7± 3.33 b
	31.91	53.3 ± 4.22 c
	43.75	70.0 ± 2.58 d
	60	88.3 ± 4.77 e
A. graveolens	8	13.3 ± 2.11 a
	11.95	31.7 ± 4.77 b
	17.84	50.0 ± 2.58 c
	26.65	66.7 ± 4.22 c
	40	86.7 ± 4.22 d

Means within column with the same letter(s) are not significantly different (P>0.05) according to Tukey's test.

Discussion

Strong insecticidal activities of essential oils derived from many species of Lamiaceae and Apiaceae have been indicated on stored-product pests [36-39]. Lolestani and Shayesteh [40] investigated and showed relatively bioactivity of *Ziziphora clinopodioides* Lam. (Lamiaceae) and Chaubey *et al*. [33,37] reported insecticidal effect of *Trachyspermum ammi*, *Anethum graveolens* and *Cuminum cyminum* against stored product insects.

The insecticidal activity of essential oils depends on their chemical constituents, application rates, extension of exposure times and experimental conditions [41,42]. Monoterpenes exert toxic impact through different ways such as penetrating the insect body via the respiratory system (fumigant effect), cuticle (contact effect) or digestive system (ingestion effect) [43]. Sahaf *et al*. [28] stated that *Carum copticum* toxicity is related to its major components which are monoterpenoids. Monoterpenoids may act against insects on the nervous system, including c-aminobutyric acid (GABA)-gated chloride channels, acetylcholine esterase, sodium channels, octopamine receptors, tyramine receptors, nicotinic acetylcholine receptors (nAChR) and possibly other targets [44]. In this study, 1,8-cineole (57.69%), carvacrol (34.74%) and L-phellandrene (34.19%), were found to be the major constituent of *N. crispa*, *S. hortensis* and *A. graveolens* respectively. Hence, high fumigant toxicity of the essential oils to *E. kuehniella* may be due to the presence of the monoterpenoids as a major constituent of them.

Based on the LC_{50} values, *N. crispa* was more toxic to the larvae compared to the other plants. Our results are in conformity with those of Plata-Rueda [45] who reported diallyl disulfide was more toxic than diallyl sulfide, principal constituents of *Allium sativum* L., for pupa of *Tenebrio molitor*. Slope value expresses rate of change in the mortality in relation to a unit change in concentration of toxicants [46,47]. According to our results, the steep slope of the dose–response line of *E. kuehniella* larvae exposed to *S. hortensis* indicated that a small increasing in essential oil concentration would cause substantial mortality which agrees with other similar studies [18,48].

The mortality rates for third instar larvae of *E. kuehniella* introduced to *N. crispa* were 89.7%. The toxic effect of *Nepeta* species have been showed against *Tribolium castaneum* [15]. Moderate to strong insecticidal activity have reported for *Nepeta* species [23]. 1,8-cineole (dominant composition of *Nepeta* oil) has been indicated as a toxic volatile against insect pests [41,49,50]. *S. hortensis* essential oil caused 88.3% mortality in *E. kuehniella* larvae. Heydarzade and Moravvej [18] indicated similar results who evaluated insecticidal effects of *Foeniculum vulgare* Mill., *Teucrium polium* L. and *S. hortensis* essential oils on

Callosobruchus maculatus. The essential oil of *S. hortensis* has been reported to possess high toxic effect against *E. kuehniella* and *Plodia interpunctella* [51]. Carvacrol and Thymol have been revealed as the major constitutes of *S. hortensis* [18]. These compounds have been reported to possess toxic effects against agricultural, stored-product and medical arthropod pests [28,34,52]. The exposure of *E. kuehniella* larvae to essential oil of *A. graveolens* created 86.7 %mortality. Babri *et al*. [21] reported the toxic effect of *A. graveolens* against *Periplanata americana*, *Musca domestica* and *Tribolium castaneum*. The fumigant activity of *A. graveolens* seed essential oil has been demonstrated against two stored product insects *T. confusum* and *C. maculates* [53]. Carvone and limonene have been identified as the main components of *A. graveolens* [35,54]. Insecticidal activity of *A. graveolens* may be attributed to appreciable concentration of the composition [21,38,55]. Control of stored-products pest insects is essential wherever foodstuff quality is to be maintained. Plant products have been reported to possess insecticidal or deterrent activity against stored product pests [56].

Conclusion

According to our results, the essential oil of *N. crispa* was found to be the most effective against *E. kuehniella*. However the findings of this study revealed the fumigant activity of *N. crispa*, *S. hortensis* and *A. graveolens* essential oils to control *E. kuehniella* larvae that could be considered as potential agents which help to reduce stored-products insect populations in IPM programs. Nevertheless further studies are required to obtain toxicity data for other stored-product insects, the residues of the oils in food, flavor quality of food and persistence experiments.

Acknowledgements

We thank Department of Medicinal Plants, Higher Education Center Shahid Bakeri Miyandoab, and Department of Plant Protection, Faculty of Agriculture, Urmia University, Iran, for financial support and providing the facilities.

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