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Chemical Composition and Efficiency of Five Essential Oils against the Pulse Beetle *Callosobruchus maculatus* (F.) on *Vigna radiata* Seeds

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Abstract: Cowpeas (*Vigna radiata*) are highly infested by the cowpea beetle (*Callosobruchus maculatus*) in the store. Bio effect of five plant essential oils namely lemongrass (*Cymbopogon citratus*), apple mint *(Mentha rotandifolia),* dragonhead (*Dracocephalum moldavica*), pennyroyal (*Mentha pulegium*) and yarrow (*Achillea millefolium),* were tested as seed protectants at concentrations of (1.0, 0.5 and 0.25%) for this serious pest. The chemical composition of the essential oils has been determined by gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC-MS). The basic components of lemongrass oil were neral, geranial and citronellol which represented about 80 % of the oil. In apple mint oil were linalool, ρ-minth-1en-8-ol, (s)-(-) - and geranyl acetate. Pulegone was the main component of pennyroyal oil. The main compounds of yarrow were β-pinene, chamazulene, sabinene, β-caryophyllene, germacrene-D, caryophllene oxide and 1, 8-cienole. The major components of dragonhead essential oil were geranyl acetate, geranial, followed by neral. Apple mint and pennyroyal essential oils at the highest concentration (1.0%) showed a highly significant effect in reducing egg laying of *C. maculatus* on the cowpea seeds to 10.2 and 12.4 eggs/2females, respectively. And also have a drastic effect on percentage hatchability recorded only (14.77 and 15.28%) compared with 94.95% in the control. All the tested essential oils elongated the larval and pupal periods. Apple mint, pennyroyal and lemongrass essential oils caused a very high mortalities between larvae and pupae of *C. maculates* (98.12, 97.73 and 92.32 %), respectively. The toxic and developmental inhibitory effects of essential oils may be attributable to their composition which may cause suffocation and inhibition various biosynthetic processes of the insects at their different developmental stages.

Key words: Essential oils · *Cymbopogon citratus* · *Mentha rotandifolia* · *Dracocephalum moldavica* · *Mentha pulegium* · *Achillea millefolium* · *Callosobruchus maculatus* · Insecticidal activity

INTRODUCTION

Several bruchid species attack cereals and pulses in the store and cause a loss of 10-15% with a germination loss ranging from 50- 92% [1]. About 100% loss of pulse seeds was found due to infestation by the pulse beetle [2]. *Callosobruchus maculats* is one of the major pests of stored cowpea in the tropics [3]. Although synthetic organic chemicals have been used as an effective means of stored-product pest control for many years, many compounds have been and will be phased out because of their toxicity to humans, resistance problems in insects and environmental concerns. Bioactive compounds of plant origin having insecticidal ovicidal properties are being used as grain protection agents against beetles in storage [4-7] tested five essential oils and suggest that plants of the genus *Ocimum* can be used as an alternative to synthetic insecticides. Kim *et al.* [8] showed the potent insecticidal activity of extract from cinnamon (*Cinnamomum cassia*) bark and oil, horseradish (*Cocholeria aroracia*) oil and mustard (*Brassica juncea*) oil against *C. chinensis*. Mahfuz [9] studied the toxicity of five essential oils against cowpea weevil. The effective against stored grain pests because of their slippery and/or oily property. It could also have a repellent character whereby the insect can not come in contact of the grain [10, 11].

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Many plant secondary metabolites, such as alkaloids, monoterpenoids or phenylpropanoids are toxic to insects; in addition, essential oils extracted from plants have been widely investigated for pest control properties [12],with some proving to be toxic [13]. The toxic and repellant properties of monoterpenoids have been studied by Pascual-Villalobos and Ballesta-Acosta [14] Garcı´a *et al.* [15], antifeedant, ovicidal, or oviposition inhibitors in insect pests [16, 17]. In recent work some monoterpenoids were shown to be possible alternatives to synthetic insecticides against stored-product pests [18].

The Essential Oils Used Were:

- Lemongrass (*Cymbopogon citratus* Hort.), family, Poaceae, a perennial herb widely cultivated in the tropics and subtropics. The quality of *Cymbopogon citratus* oil is generally determined by the content of citral [19]. Some other constituents of the essential oils are geranyl acetate, linayl acetate, geranial, neral, limonene, myrcine, β-Caryophyllen [20]. It is used in cases of acne, althea's foot, excessive perspiration, flatulence, insect repellent, muscleaches, oily skin, scabies; stress [21]. As a medicinal plant, *Cymbopogon citratus* has been considered a carminative antimicrobial [22], anti-oxidant [23], CNS depressant, has antibacterial, antifungal and antiviral activities [24].
- *Mentha rotundifolia* (L.) Huds. is a hybrid between *M. longifolia* (L.) and *M. suaveolens* Ehrh [25,26]. Previous studies on the essential oil of *M. rotundifolia* revealed the existence of chemotypes with different major components, as menthyl acetate [25]. Piperitenone oxide 23.5-38.6%) and cis-piperitone oxide (28.1-30.5%) were the two main constituents of the oils [27]. An oxygenated monoterpene whose biological effects (cardiovascular effects, CNS activity, antibacterial and antifungal properties, toxic, repellent and reproduction retardant toward malarial vector Anopheles stephensi) have been investigated by Dorman *et al.* [28] and Tripathi *et al.* [29].
- Dragonhead (*Dracocephalum moldavica*, Lamiaceae) is an annual plant native in Central Asia and naturalized in eastern and Central Europe [30]. The volatile oil content and its composition showed great variation due to plant origin. Citral was the major components of the oil [31]. The major compounds were geranial, geranyl acetate, neral and geraniol [32]. El-Gengaihi and Wahba, Galambosi *et al.* [33], Kakasy *et al.* [30] and Aziz and El-Sherbeny [34]. Moreover, Shatar and Altantsetseg [35] reported that linalool was the major constituents of the oil of the plants cultivated in Mangolia. It has been used in folk medicine as painkiller and for treatments of kidney complaints, against toothache and colds as well as antirheumatism [36], antitumor [37], antioxidant, antiseptic and stimulant [30].
- *Mentha pulegium* L., popularly known as pennyroyal, is highly aromatic, perhaps even more so than any other mint. The composition of *M. pulegium* has been the subject of a great number of studies and three chemotypes have been established, pulegone-type, piperitenone/piperitone-type and isomenthone/neoisomenthol-type [38]. The major components of Bulgaria pennyroyal oil were pulegone (42.9- 45.4%), piperitenone (21.7-23.1%), isomenthone (11.3-12.8%) [39].It is consumed mainly for its antiseptic, insect repellent, carminative, antispasmodic, diaphoretic and anti-inflammatory properties [40]. Traditionally, total decoction of this herb was used for the treatment of fibrosis and cervical tumors [41]. Essential oil might be considered as a potentially toxic agent on human cancer cell lines and a possible candidate for human cancer chemotherapy. However, further biological tests on the efficacy and side effects of this plant are necessary before its use in human [42].
- Yarrow, *Achillea millefolium* L. (Fam. Asteraceae) is a very hardy perennial that's native to Europe and Western Asia and widely naturalized in temperate regions [43]. The main compounds of the distilled oil were α-pinene, β-pinene, sabinene, 1, 8-cienole, camphor, linalool, β-caryophyllene, borneol, germacrene-D, caryophllene oxide, oxidized farnesene derivatives and viewed as the most important chamazulene [44, 45]. It has been used as spasmolytic, aromatic bitter, hemastatic, hypastensive and emmenagogie activities Antimicrobial activity against a range of bacteria anti-inflammatory and antispasmodic [46]. It has been used as diaphoretic and antipyretic [47] as well as act as antifungal [48] and antihyperglyceamic agent [49].

This work aimed to study the chemical composition and the efficiency of five essential oils against *Callosobruchus maculatus* (F.) on *Vigna radiata* seeds.

MATERIALS AND METHODS

Production of these species as a source of their essential oil was conducted in newly reclaimed soils at Nubaria, Behaira province west of Nile Delta of Egypt using drip irrigation system. This area is a desert region and the soil of the experimental site was deep, well-drained sandy composing with an alkaline pH 8.2, EC 2.87 dS/m, $CaCO_3$ 1.4%, O.M 0.25%. The average water content at field capacity from surface soil layer down to 60 cm depth was 12%. During the soil preparation for cultivation, Organic manure (40 m^3 h^{-1}), calcium superphosphate (300 kg ha^{-1}) and potassium sulphate (150 kg ha^{-1}) were added 15 days before sowing as was customary for the region. On 15 August, 2007, Healthy, 45 day old seedlings of lemongrass, *Cymbopogon citratus* Hort. (small shoots with roots) were transplanted into experiment farm at Nubaria. Leaving one plant with 50 cm in between and 100 cm between rows. At the end of May, 2008 the foliage (leaves and stems) was harvested. Apple mint *(Mentha routandifolum* L.) and pennyroal (*Mentha pulegium* L.) rhizomes were obtained from the Medicinal Plant Program in the Department of Plant, Soil and Insect Sciences at the University of Massachusetts, Amherst, U.S.A. In February first, in the greenhouse of NRC, cuttings (8 cm high) were rooted in potting media (sand) contained in 25cm diameter plastic pots for harding before transplanting in the field. After 45 days, (March 15, 2007), healthy rooted seedlings were transplanted into the experimental field in the Nubaria region. At the end of May, 2007 the foliage (leaves and stems) was harvested.

Certified seeds of dragonhead plant were obtained from Saturei-Ka Zahradni Bohnankraut in Poland and propagated under Egyptian conditions. The seeds were sown directly in the field on 15 October during 2007 season, after two weeks from sowing the plants were thinned twice, leaving one plant with 50 cm in between and 100 cm between rows. Drip irrigation was supplied at 2 L h^{-1}. The plants were collected at full flowering stage in May during the 2008 season. The rhizomes of *Achillea millefolium* were obtained from the Experimental Farm of the Faculty of Pharmacy, Cairo University, Egypt and were planted at 15th October, 2007 with 50 cm in between and 100 cm between rows. Flowering heads were collected three times during flowering stage 15th Febrwary, 15th March and 15th April and as well as the total yield of flowering heads were collected.

Extraction and Chemical Analysis of the Essential Oils: The fresh plant material (100 g of each species) was subjected to hydrodistillation using a modified Clevenger-type apparatus for 3 h to determine the essential oil percentage. The resulted essential oil was dried over anhydrous sodium sulphate and kept in refrigerator till GLC analysis. Chemical analysis of the oils were achieved by GC-MS on a HP 5890 II gas chromatograph coupled to a HP 5972 mass selective spectrometer using a DB wax fused silica capillary column. Gas chromatography coupled with mass spectrometry was used to identify the main volatiles released by each essential oil.

Insect Culture: Laboratory stock culture of *Callosobruchus maculatus* was started by collecting infested cowpea seeds from the Ministry of Agriculture. For purification of the culture, pairs of differentiated males and females of emerging adults were kept at 29±1C° and 75±5 R.H in plastic containers of 1 liter capacity, each containing about 200 gm of sterilized cowpea seeds and covered with muslin. Five generations were produced under those conditions before starting the different experiments. Three concentrations (1.0, 0.5 and 0.25%) of the five selected essential oils were prepared using water and two drops of tween 80 (0.01%) was added as emulsifier. One hundred grams of cowpea seeds were dipped in each concentration for ten seconds and the seeds were allowed to dry leaving a thin film from the essential oils used.

Treatment Procedure: The efficiency of five essential oils against *C. maculatus* on seeds of *Vigna radiate* was studied. Two pairs of newly emerged adults were offered option to mate and lay eggs on the treated seeds which kept in plastic containers of 1 liter capacity, cover with muslin. The control seeds were treated with water and tween 80 (0.01%). Each experiment was replicated four times. The different biological parameters were taken in consideration as follows:

Effect of the Essential Oils on the Fecundity of the Females and the Percentage Reduction of Eggs Was Recorded:

$$\% \text{ Reduction} = \frac{\text{No. of eggs laid on control - No. of eggs laid on treated}}{\text{No. of eggs on control}} \times 100$$

Effect of the Essential Oils on the Resulting Progeny:
On the Egg Stage: The incubation period (in days) was recorded for each treatment. The number of the hatched eggs was calculated from the empty egg shells found in the surface of the seeds. The percentage of the total output of eggs was calculated using the following equation.

Effect of Essential Oils on the Larval and Pupal Stages:

- The larval and pupal durations in the treated seeds as well as in the control according to Abbass [50].
- Percentage mortality between larvae and pupae (the number of weevils which bred and emerged from seeds were recorded to calculate the percentage mortality between larvae and pupae).

Statistical Analysis: The whole biological data was subjected to analysis of variance (ANOVA), using SPSS computer program and the means were compared using Duncan´ Multiple Range Test.

RESULTS AND DICUSSION

The data in Tables 1-5 indicated that the basic components of *Cymbopogon citratus* oil were neral, geranial and citronellol which represented about 80 % of the oil. In *Mentha rotandifolia* were linalool, ρ-minth-1en-8-ol, (s)-(-) - and geranyl acetate. The major components of *Dracocephalum moldavica* essential oil were geranyl acetate, geranial followed by neral. Pulegon was the main component of *Mentha pulegium* oil. The main compounds of *Achillea millefolium* oil were β-pinene, chamazulene, sabinene, β-caryophyllene, germacrene-D, caryophllene oxide and 1, 8-cienole. [51, 52].

Table 1: Essential oil composition of *Cymbopogon citratus*

Essential oil composition	Relative (%)
Myrcene	0.22
Limonene	4.25
1,8 cineole	1.12
Linalool	1.14
Citronellal	1.25
Neral	35.24
Geranial	29.82
Citronellol	23.25
Geranyl acetate	0.25
Neryl acetate	1.45
Caryophyllene oxide	0.18

Table 2: Essential oils composition of *Mentha rotandifolia*

Essential oil composition	Relative (%)
α- Thujene	0.11
Sabinene	0.11
β- Pinene	0.32
Myrcene	5.31
ρ- Cymene	0.02
1,8-Cineole	8.46
trans-β- Ocimene	0.39
Linalool oxide	0.12
Linalool	35.32
Linalyl propionate	0.38
ρ-Menth-1-en-8-ol,(s)-(-)-	11.08
Nerol	1.31
Linalyl acetate	0.12
Geraniol	2.72
Citral	0.78
Citronellyl acetate	0.31
Neryl acetate	6.11
Geranyl acetate	10.86
trans-Caryophyllene	0.08
Germacrene D	0.09

Sources: Aziz and Craker [40]

Table 3: Essential oil composition of *Dracocephalum moldavica*

Essential oil Composition	Relative (%)
α-Pinene	1.02
β- pinene	0.31
Sabinene	0.12
α- phellandrene	1.30
Limonene	0.22
1,8-cineole	1.21
Linalool	0.85
Neral	19.84
Geranial	30.63
Nerayl acetate	2.82
Geranyl acetate	37.53
Nerol	0.06
Geraniol	0.69
Caryophyllene oxide	0.29

Sources: Aziz *et al.* [1]

Table 4: Essential oils composition of *Mentha pulegium*

Essential oil composition	Relative (%)
α- Thujene	0.25
α –Pinene	0.05
Camphene	0.03
Sabinene	0.02
β- Pinene	0.42
Limonene	0.2
ρ-Menthone	0.18
Iso Menthone	5.75
Neo-Menthol	-
(-)-Isopulegone	1.08
Pulegone	88.05

Sources: Aziz and Craker [40].

Table 5: Essential oil composition of *Achillea millefolium*

Essential oil composition	Relative (%)
α-pinene	4.75
β-pinene	21.22
Sabinene	13.44
Myrcene	0.45
α –Terpinen	0.75
Limonene	2.66
1,8-cineole	4.72
ρ -Cymine	0.84
Camphor	0.97
Bornyl acetate	0.21
β-Caryophllene	10.25
α-Humulene	2.51
Germacrene -D	9.22
Caryophllen oxid	4.54
Chamazulen	20.11

The results in Table 6 showed that *Mentha rotandifolia, Mentha pulegium* and *Cymbopogon citratus* essential oils have the highest effect in decreasing the fecundity of *Callosobruchus maculatus* (10.2, 12.4 and 18.62 eggs, respectively) at the highest concentration used followed by *Achillea millefolium* oil (33.5 eggs). *Dracocephalum moldavica* oil showed the lowest effect on the *Callosobruchus maculatus* fecundity of all the tested oils (50.3 eggs).

Table 7 indicated that *Mentha rotandifolia, Mentha pulegium* and *Cymbopogon citrates* oils at the concentration of 1.0% recorded the highest percentage reduction of the total output of eggs/2 females (90.3, 82.4 and 88.2%, respectively). These results may be due to the main component of the essential oil especially the oxygenated compounds. The major components of *Mentha rotandifolia* were linalool (35.32%), ρ-minth-1en-8-ol, (s)-(-) - (11.08%) and geranyl acetate (10.86%). The toxic and repellant properties of monoterpenoids have been studied by several authors, El-Sayed *et al.* [53]. Chapman *et al.* [54] who reported that linalool is recognized as an insect repellent. Moreover, pulegone (88.05%) was the main component of *Mentha pulegium* oil, has been considered effective as a defensive chemical because of its repellency [55]. The major components of dragonhead essential oil were geranyl acetate, geranial, followed by neral. Geraniol inhibited settling of the aphid *Myzus persicae* in choice leaf-disk assay [56] and oviposition of *Prays citri*.

Table 8 showed that *Mentha pulegium* oil elongates the incubation period of the eggs laid of *Callosobruchus maculatus* females at concentrations of 1.0%. A similar but lower effect occurred for *Mentha rotandifolia* and *Cymbopogon citratus* oils. *Mentha pulegium* oil recorded the lowest percentage hatchability of *Callosobruchus maculatus* eggs 15.28, 20.94 and 25.84 at concentrations of 1.0, 0.5 and 0.25 % respectively. *Mentha rotandifolia* oil has a similar effect only at concentration of 1.0%. All the oils used have a significant effect on the percentage hatchability of *Callosobruchus maculatus* eggs especially at the highest concentration used. These results agreed with El-Sayed *et al.* [53],

Table 6: Effect of seed treatment with five essential oils on the fecundity of *Callosobruchus maculatus* females

	Total number of eggs / 2 females		
	Oil concentrations %		
Essential oils	1	0.50	0.25
Cymbopogon citrates	18.62±4.6 [a]	21.5±4.6 [a]	22.0±2.6[ab]
Mentha rotundifolia	10.2±3.4 [a]	22.7±8.6 [a]	37.4±7.4 [bc]
Dracocephalum moldavica	50.3±10.7 [c]	70.2±9.4 [c]	80.5±7.9 [d]
Mentha pulegium	12.4±3-3 [a]	17.8±3.7 [a]	18.3±4.2 [a]
Achillea millefolium	33.5±8.9 [b]	38.9±4.4 [b]	50.1±6.3 [c]
Control	105.5±2.6 [d]	105.5±2.6 [d]	105.5±2.6 [e]
F- value	51.302**	45.829**	44.345**

Table 7: Effect of seed treatment with five essential oils on the percentage reduction of the total output of eggs of *Callosobruchus maculatus*

	% Reduction of the total output of eggs		
	Oil concentrations %		
Essential oils	1	0.50	0.25
Cymbopogon citrates	82.4	79.6	79.1
Mentha rotundifolia	90.3	78.5	64.5
Dracocephalum moldavica	52.3	33.5	23.7
Mentha pulegium	88.2	83.1	82.7
Achillea millefolium	68.2	63.1	52.5

Table 8: Effect of seed treatment with five essential oils on the resulting progeny

	Incubation period (in days)			% Hatchability		
	Oil Concentration %					
Essential oils	1	0.50	0.25	1	0.50	0.25
Cymbopogon citrates	5.11±0.61	4.82±0.13	4.81±0.03	35.92	39.52	54.89
Mentha rotundifolia	5.31±0.3	5.23±0.62	4.82±0.10	14.77	28.65	69.74
Dracocephalum moldavica	4.79±0.78	4.87±0.28	4.81±0.01	68.91	70.54	74.21
Mentha pulegium	5.56±0.58	5.42±0.17	4.98±0.21	15.28	20.94	25.84
Achillea millefolium	4.84±0.49	4.88±0.25	4.79±0.04	69.55	71.93	75.33
Control	4.81±0.96	4.81±0.96	4.81±0.96	-	-	-
F- value	0.799	0.698	0.48	94.95	94.95	94.95

Table 9: Effect of seed treatment with five essential oils on the resulting progeny of larval and pupal durations

	Duration (in days)					
	Larval			Pupal		
	Oil concentrations %			Oil concentrations%		
Essential oils	1	0.50	0.25	1	0.50	0.25
Cymbopogon citrates	23.1±0.75	22.7±0.91	22.8±0.74	5.11±0.21	4.75±0.28	4.65±0.25
Mentha rotundifolia	24.5±0.79	22.9±1.08	22.1±0.82	5.56±0.24	5.21±0.23	4.74±0.32
Dracocephalum moldavica	20.7±0.84	20.1±1.38	19.5±0.92	4.21±0.27	3.81±0.31	3.52±0.35
Mentha pulegium	23.4±0.44	22.6±1.36	22.8±0.47	5.31±0.13	4.58±0.15	4.55±0.18
Achillea millefolium	21.5±1.33	21.3±0.95	20.8±0.81	4.34±0.42	4.21±0.37	4.35±0.51
Control	18.8±1.09	18.8±1.09	18.8±1.09	3.55±0.22	3.55±0.22	3.55±0.22
F-value	0.435	0.582	0.751	0.608	0.107	1.078

Table 10: Effect of seed treatment with five essential oils on the resulting progeny of mortality between larvae and pupae

	% Mortality (Larvae and pupae)		
	Oil concentrations%		
Essential oils	1	0.50	0.25
Cymbopogon citrates	92.32	87.63	86.98
Mentha rotundifolia	98.12	95.53	91.47
Dracocephalum moldavica	48.52	39.46	33.92
Mentha pulegium	97.73	95.72	94.67
Achillea millefolium	58.77	44.59	35.49
Control	14.84	14.84	14.84

Chapman *et al.* [54] and Mason [55]. Meanwhile, Raja and William [57] stated that the highest mortality and ovicidal activity against *Callosobruchus maculatus* was recorded in citrodora oil followed by lemon grass oil. Neral, geranial and citronellol represented about 80% of the *Cymbopogon citratus* oil. Leal and Uchida, [58] identified geranial as the repellent active compound of *Cymbopogon citratus* against mosquitoe

Data in Table 9 showed that larval duration of *Callosobruchus maculatus* increased for all tested oils, reached 24.5 days for *Mentha rotandifolia* oil at concentration of 1.0%. The lowest larval duration was for *Dracocephalum moldavica* oil at all concentrations used. Pupal durations of the pest were elongated by all the tested oils at all the concentrations used, the highest duration was observed with *Mentha rotandifolia* and *Mentha pulegium* oils at the 1.0% concentration. Pulegone interferes with insect development and reproduction [59].

Data in Table 10 showed that using *Mentha pulegium* oil gave 97.73, 95.72 and 94.67 % mortalities in larvae and pupae of *Callosobruchus maculatus.*

The lowest mortalities in the larval and pupal were resulted from the different concentrations of *Dracocephalum moldavica* oil (48.52, 39.46 and 33.92%). Acute toxicity of pulegone to variegated cutworm was also demonstrated by Harwood *et al.* [60]. Cineole, l-fenchone and pulegone at 50 mg/ml air caused 100% mortality of *Sitophilus oryzae, Tribolium castaneum* and *Oryzaephilus surinamensis* [61]. The insecticidal activity of many plant-products has been reported extensively against stored-product pests Lale and Mustapha [62], Cox [63], Han *et al.* [64] and Rozman *et al.* [65].

The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids. Monoterpenoids are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions [66]. Due to their high volatility, they have fumigant and gaseous action and might be of importance for stored-product insects [67].

CONCLUSION

The Aforementioned Results Indicate That:

- *Mentha rotandifolia*, *Mentha pulegium* and *Cymbopogon citrates* essential oils proved to be the most effective in reducing the population of *Callosobruchus maculates*.
- The toxicity and insecticidal activity of the essential oils are attributable to their components.
- Essential oils could be used as a biodegradable and natural bioprotectant for controlling stored product pests.

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