Efficacy of Some Medicinal Plant Essential Oils, Extracts and Powders Combined with Diatomaceous Earth on Cowpea Weevil, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae)

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Abstract: *Background*: *Cowpea weevil, Callosobruchus maculatus F. (Coleoptera: Bruchidae) is an important storage pest around the world. Material and Methods: In this research, we were examined the synergistic interaction between Satureja hortensis L., Trachyspermum ammi I. Sprague, Ziziphora tenuior* L.*, Cuminum cyminum* L. *and Foeniculum vulgare Mill. essential oils, ethanolic extracts and powders alone and with Diatomaceous earth (DE) against C. maculatus adults under laboratory condition. Principle results: Results showed that DE had high toxicity to the pest (LC_{50}= 0.15 g/kg). Probit analysis showed that the most effective essential oil, extract and powder were T. ammi, Z. tenuior* and *C. cyminum which exhibited LC_{50} values equivalent to 0.64 (µL/L air), 578.5 (µL/kg of grain) and 0.45 (g/kg of grain), respectively. Essential oils, ethanolic extracts and powders of mentioned plants synergized the performance of DE sample. Hence, combination of these plants with DE could have potential for use in integrated stored product pest-management programs. The results of this research revealed the high repellency of savory essential oil for controlling the pest. But other EOs showed weak repellency. The results demonstrated the average repellency of mentioned extracts.*

Keywords: *Callosobruchus maculatus*, Diatomaceous earth, synergistic effect, medicinal plant, extract

1. Introduction

Cowpea weevil, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) is one of the most destructive pests of cereals particularly cowpea that its larvae cause severe damage to this product. High cost and hazardous effects of insecticides make us seek and use other safe means such as medicinal plants for pest control. Essential oils are volatile and natural compounds that have functions in chemical defense, acting as repellents, insecticides, acaricides, and by attracting natural enemies of herbivores [2]. Diatomaceous earth (DE) is also an alternative to chemical insecticides. Among plant compounds, plant powders used as cereal guarders [14]. Therefore, according to high notability of plant powders in store pest control, we examined efficacy of *S. hortensis*, *T. ammi*, *Z. tenuior*, *C. cyminum* and *F. vulgare* essential oils, ethanolic extracts and powders for *C. maculatus* controlling. We also assayed synergistic/antagonistic interaction between these plant essential oils, ethanolic extracts and powders with Diatomaceous earth against *C. maculatus* adults under laboratory condition at 27±1°C, 55±5% RH and continuous darkness.

2. Literature Survey

As [23] reported antagonistic effect of *Carum copticum* Clarke and *C. cyminum* powders in combination with DE against *Sithophilus granarius* L. and *Tribolium confusum* Jacquelin du Val, we assayed synergistic/antagonistic interaction between *S. hortensis*, *T. ammi*, *Z. tenuior*, *C. cyminum* and *F. vulgare* essential oils, ethanolic extracts and powders with Diatomaceous earth (DE) against *C. maculatus* adults. The main goal of the present investigation was to show the role of medicinal plants (botanical sources) and DE in controlling pests with the lowest hazards to human and environment.

3. Materials and Methods

3.1 Insect and DE

Adults of *C. maculatus* used in the experiments were obtained from cultures maintained in the Entomology laboratory, Urmia University, Iran. The colony of *C. maculatus* was reared on cowpea, an important host of the pest in Iran. Thus, we were transmitted 50 males and females on 100 gram of cowpea. All adults used in the experiments were 1-2 day old of mixed sex.

Iran has several natural sources of DEs, of which the Maragheh (37°22′41.39″N 46°19′28.16″E) mine has been selected. The insecticidal activity and sub-lethal concentration (LC_{25}) of Iranian DE was applied for the experiments. Experiments were carried out under laboratory conditions at 27±1°C, 55±5% RH and continuous darkness. All experiments were replicated three times and the control group was used for monitoring. When no leg or antennal movements were observed, insects were counted dead.

3.2 Plant powder

Seeds of *T. ammi*, *C. cyminum* and *F. vulgare* and leaves of *S. hortensis* and *Z. tenuior* were purchased from local market of Urmia province-Iran on Aprill 2015. These dried plants were transferred to the laboratory and stored at −24 °C. At the time of conducting experiments, they were milled and sifted using 30 mesh lab sieve to obtain unit particle sizes.

3.3. Essential oil

Essential oils were extracted from seeds of *T. ammi*, *C. cyminum* and *F. vulgare* and leaves of *S. hortensis* and *Z. tenuior* and they were subjected to hydro distillation using a modified clevenger-type apparatus.

3.4. Ethanolic extracts

Ethanolic extracts were obtained from seeds of *T. ammi*, *C. cyminum* and *F. vulgare* and leaves of *S. hortensis* and *Z. tenuior* by using soxhlet extractor. Excess ethanol was evaporated in a rotary evaporator.

4. Bioassays

4.1 Plant powder

Cowpea (20 g) was poured into 250 ml glass vials and a The ranges for *S. hortensis*, *T. ammi*, *Z. tenuior*, *C. cyminum* and *F. vulgare* powders were 0.5-25, 2.5-6, 1.5-50, 0.5-6 and 4-10 g/kg of grain, respectively. Cowpea grains were treated with different concentrations of plant powders. Vial caps were screwed tightly and they were shaken manually for 5 min to distribute the powder in the grain mass. Subsequently, 20 adults of *C. maculatus* were introduced to each vial and the vials were placed in incubator set at 27±1°C, 55±5% RH and continuous darkness. Untreated cowpea served as the control treatment with three replicates for each concentration. The mortality was counted 7 days after exposure.

4.2 Essential oil

Bioassay trials were carried out following [13] and [6]. The ranges of concentrations for different compounds were determined by preliminary dose setting experiments. The ranges for *S. hortensis*, *T. ammi*, *Z. tenuior*, *C. cyminum* and *F. vulgare* were 1.6-5.6, 0.56-1.6, 0.8-5.6, 0.64-2.4 and 0.72-1.6 µL/L air.

Repellency trials were carried out following [21]. Whatman papers were cut into two equal halves. For final ranging of EOs, different concentrations of them dissolved in 150 µL of aceton. The ranges were 1, 2, 3 and 5 µL/cm^2. One half of Whatman paper was treated with different concentrations of EO solution as uniform as possible by using micro pipette and the other half of the paper was treated with aceton only (as control). After 10 minutes and evaporation of solvent, Whatman paper put on the bottom of Petri dishes (8 cm diameter and 2 cm hight). Next, 10 numbers of one-day old released on the center of Whatman paper. The number of insects was computed in each side after 24 h. Percentage of repellency was accounted by $PR=\frac{Nc-Nt}{Nc+Nt}$. In this equivalent, Nc= number of insects on control surface and Nt= number of insects on treated surface [8]. Each treatment was replicated three times.

4.3 Ethanolic extract

For evaluating toxicity of ethanolic extracts against weevils on cowpea grains, cowpea (20 g) was poured into 250 ml glass vials. The ranges for *S. hortensis*, *T. ammi*, *Z. tenuior*, *C. cyminum* and *F. vulgare* extracts were 2000-3500, 1000-12500, 500-1000, 500-7500 and 1500-4500 µL/kg of grain, respectively. When solvent was evaporated and cowpea grains were dried, 20 adults of *C. maculatus* were transferred to the glasses. Then, they were placed in incubator set at 27±1°C, 55±5% RH and continuous darkness. Each treatment was replicated three times. Insect mortality was recorded 24 h after treatment.

Repellency trials were carried out following [21]. Whatman papers were cut into two equal halves. The ranges were 6, 7, 8 and 9 µL/cm^2. One half of Whatman paper was treated with different concentrations of extract solution as uniform as possible by using micro pipette and the other half of the paper was treated with ethanol only (as control). After evaporation of solvent Whatman paper put on the bottom of Petri dishes (8 cm diameter and 2 cm height). Next, 10 numbers of one-day old released on the center of Whatman paper. The number of insects was computed in each side after 24 h. Percentage of repellency was accounted by $PR=\frac{Nc-Nt}{Nc+Nt}$. In this equivalent, Nc= number of insects on control surface and Nt= number of insects on treated surface [8]. Each treatment was replicated three times.

That is notable that in repellency trials, both essential oil and ethanolic extract, at higher concentration of given in tables 4 and 5, insects were died and this was not our purpose. So we considered these doses although they had no repellency.

4.4 DE

Cowpea (20 g) was poured into 250 ml glass vials. The range was 0.008-1 g/kg of grain. Cowpea grains were treated with get concentrations of DE. Vial caps were screwed and they were shaken manually for 3-5 min to distribute the DE in the grain mass. Subsequently, 20 adults of *C. maculatus* were introduced to each vial and the vials were placed in incubator set at 27±1°C, 55±5% RH and continuous darkness. Untreated cowpea served as the control treatment. The mortality was counted 7 days after exposure. Each treatment had three replicates.

4.5 Plant powders, EOs and ethanolic extracts combined with DE

Sub-lethal concentrations (LC_{25}) of plant powders/ essential oils or extracts were combined with LC_{25} of DE to investigate whether there was a synergistic or antagonistic interaction between plant materials with DE. Sub-lethal concentration (LC_{25}) of DE was mixed completely with the grains by manually shaking the glass vials for 5 min. Then, sub-lethal concentrations of *S. hortensis*, *T. ammi*, *Z. tenuior*, *C. cyminum* and *F. vulgare* powders/ essential oils or extracts were mixed with the treated cowpea and shaken manually for an additional 5 min. Subsequently, 20 adults of *C. maculatus* were introduced to each vial; the caps were screwed down tightly and kept in an incubator. Conditions of the experiments were the same as the previous ones and untreated cowpea served as a control. For essential oils and ethanolic extracts, mortality was recorded 2 days after exposure and for the combination of plant powders with DE, mortality surveyed 7 days from inception of exposure. Each treatment had three replicates.

5. Data Analysis

In order to determine LC_{50} values, the data were analyzed using the probit procedures with SPSS for Windows® release 16. The percentage data were transformed into arcsin√x before statistical analysis. For determining synergistic/antagonistic interactions, trials were carried out following [20]. The relationship between data was examined by analysis of variance (ANOVA) and correlation analysis. The means were separated by using the Tukey test.

6. Results

Results given in table 1 demonstrate that DE was the most effective on the pest (LC_{50}= 0.15 g/kg). Probit analyses of plant powders show that cumin had high toxicity on cowpea weevil adults. Based on LC_{50} values and slope, cumin and fennel are toxic for this pest which exhibited LC_{50} values on adults of *C. maculatus* equivalent to 0.45 and 1.40 g/kg respectively.

According to table 2, essential oils of ammi and fennel were effective on the pest which LC_{50} values equivalent to 0.64 and 0.72 µL/L air. Results presented in Table 3 show that according to LC_{50} values the most effective ethanolic extracts were ziziphora (LC_{50} value= 578.5 µL/kg) and cumin (LC_{50} = 850 µL/kg). The repellent activity of ethanolic extracts from 5 aromatic medicinal plant species against *C. maculatus* adults varied according to plant species (Table 4). At a dose of 9 µL /cm2, average repellency against cowpea weevil adults was obtained with the extracts of fennel, savory and ziziphora which exhibited 50, 52 and 53% of repellency. The other two plant extracts exhibited < 15% repellency. At all doses of savory essential oil, potent repellency against the pest was observed (mean repellency= 96.41%) (Table 5). But essential oil of ziziphora had no repellent activity. The other three EOs exhibited < 12% repellency. There was no mortality in the untreated control of all experiments. The inclusion of *T. ammi*, *C. cyminum*, *F. vulgare*, *S. hortensis* and *Z. tenuior* powders with Iranian DE appear to lead to synergistic interaction against *C. maculatus* (Table 6). Furthermore, in all cases, there were synergistic interactions in the combination of essential oils with DE (Table 7). In the case of *C. maculatus* exposed to a mixture of all ethanolic extracts and DE, the synergistic interaction was recorded (table 8).

7. Discussion

Adults of *C. maculatus* proved sensitive to plant powders, essential oils and ethanolic extracts combined with DE. However DE had the highest toxicity for *C. maculatus* adults. The diatomaceous earth has proved successful for the management of different stored grain insect pests and can be replaced with the conventional insecticides [3] but their overall efficacy depends upon different factors, such as type and concentration of DE, grain moisture content, temperature, insect species, insect density and type of grain commodity [15], [4]. In the present study, powders of *C. cyminum* and *F. vulgare*, essential oils of *T. ammi* and *F. vulgare* and ethanolic extracts of *Z. tenuior* and *C. cyminum* showed high insecticidal activity against *C. maculatus*. Application of essential oils and their formulations to grain seeds for storage is an inexpensive and effective technique, and its easy adaptability will give additional advantages leading to acceptances of this technology by farmers. A study to improve the effectiveness of botanical derivatives as insecticides will benefit agricultural sectors of developing countries, as these substance are not only of low cost, but also have less environmental impact in term of insecticidal hazards involved. [1] reported that the essential oils proved to be the most effective in reducing the population of *C. maculatus*. It has been well established that certain plant-derived extracts and phytochemicals may provide potential alternatives to the currently used insecticides. The inclusion of *T. ammi*, *C. cyminum*, *F. vulgare*, *S. hortensis* and *Z. tenuior* powders, EOs and ethanolic extracts with Iranian DE appear to lead to synergistic interaction against *C. maculatus*. Therefore, concentrations of essential oils, extracts, powders and DE seem to be practical for controlling the pest. The insecticidal efficacy of *C. copticum* essential oil has been proved against *C. maculatus* (F.), *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst) and *Plodia interpunctella* (Hübner) [17] , [18]. According to the findings, interaction of essential oil with DEs has high insecticidal effectiveness against *C. maculatus* that [23] reported same results but these researchers showed that the inclusion of *C. copticum* powder with Iranian DEs did not appear to lead to synergistic interaction against *S. granarius* and *T. confusum* that is in contrast with our results. Similar reports were given by [22]. [22] assessed the influence of garlic essential oil combined with DE, and noted that combination of oil with DE decreased the effective dose of both treatments. They speculated that this combination decreased cost of management and adverse effects of the oil. However, the mode of action for the increased activity of essential oil combined with DE has not been elucidated. We demonstrated that in the case of *C. maculatus* exposed to a mixture of plant powders and DE, the synergistic interaction was recorded. Also, there is the potential of combining *P. guineense* seed powder and diatomaceous earth for increased biological effects against *C. maculatus* infesting cowpea seed in storage [10]. [5] demonstrated that plant powders of *C. capitatum* and *P. fraternus* are toxic to *C. maculatus* and *S. zeamais* which are pests of stored cowpea and maize seeds respectively. Both powders can be used as protectants by local farmers in small farm holdings as they are easily available bio-pesticides and are environmentally friendly. Synergetic effect of powdered *P. guineense* and diatomaceous earth in imparting acute toxicity, discouraging oviposition and damage to cowpea seeds by *C. maculatus* [10]. For the first time, we assayed synergistic interactions in combination of ethanolic extracts and DE against adults of *C. maculatus* that there is no report. We know that plant-derived insect repellent agents are selective, have no or little harmful effect on non-target organisms or the environment, and can be applied as insect repellents [16]. Based on our results, essential oil of *S. hortensis* was the most repellent for *C. maculatus* adults. Hence, there are numerous reports on the insecticidal and repellent activity of the essential oils from *Satureja* species [11], [12]. [7] showed that EO of *Salvia mirzayanii* Rech had high repellent activity against *C.*

maculatus and *Tribolium confusum*. In this investigation, ethanolic extracts of fennel, savory and ziziphora had average repellency. [9] demonstrated that crude ether extract of seeds of *Monodora myristica* (Gaertner) Dunal is far more effective as legume seed protectant against *C. maculatus* infestation than the ground seeds.

8. Conclusion

Recently, post-harvest loss of grain due to insect pests has become a major concern all over the world such that demand for good quality products, which are free from chemical residues, is high and increasing rapidly. It is possible that the plant factors conferring protection on the seeds against *C. maculatus* may have repellent and toxic properties. Therefore, according to our results, *T. ammi*, *C. cyminum*, *F. vulgare*, *S. hortensis* and *Z. tenuior* powders, essential oils and ethanolic extracts can be applied to control the pest infestations in small scales or as a part of an integrated pest management (IPM) strategy combined with other reduced risk control techniques. Although combination of mentioned essential oils, extracts and powders with DE is very effective for controlling the pest. Also, Savory EO is very repellent for adults of *C. maculatus*. However, additional experimental work is required.

9. Future Scope

These compounds were very effective, but one of the most important problems for introducing and applying these compounds in stores may be low knowledge of farmers about more safety and lower costs of pest control (botanical sources). Therefore, we hope that farmers could accept and apply these compounds for remaining healthy and promoting healthy productions.

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Table 1: Probit analysis of toxicity of some plant powders and DE to one-day-old adults of Callosobruchus maculatus after 7 days

Compound	χ^2	Slope ±SE	LC_{50} (g/kg)	LC_{25}(g/kg)	LC_{90}(g/kg)
cumin	0.723	3.81 ± 0.45	0.45	0.20	2.35
fennel	2.484	4.11 ± 0.56	1.40	0.95	2.5
ziziphora	1.495	1.17 ± 0.15	5	1	20.5
savory	13.021	0.96 ± 0.13	2	0.45	12.5
ammi	0.99	4.16 ± 0.58	3.45	2	6.95
Diatomaceous earth	4.590	2.07 ± 0.26	0.15	0.05	0.60

Table 2: Probit analysis of fumigant toxicity of some essential oils to one-day-old adults of Callosobruchus maculatus after 24h

Eo	χ^2	Slope ±SE	LC_{50} (µL/L air)	LC_{25}(µL/L air)	LC_{90}(µL/L air)
ammi	2.31	4.04 ± 0.53	0.64	0.40	1.36
cumin	2.53	3.23 ± 0.43	1.52	0.64	7.44
fennel	5.17	4.75 ± 0.68	0.72	0.56	1.44
savory	2.25	3.92 ± 0.49	2.00	1.36	4.32
ziziphora	1.39	1.85 ± 0.26	1.44	0.64	1.36

Table 3: Probit analysis of toxicity of some ethanolic extracts to one-day-old adults of Callosobruchus maculatus after 24 h

Extract	χ^2	Slope ±SE	LC_{50} (µL/kg)	LC_{25}(µL/kg)	LC_{90}(µL/kg)
ammi	1.46	1.34 ± 0.20	900	625.5	1421.5
cumin	1.26	1.71 ± 0.22	850	351	1424
fennel	1.16	3.59 ± 0.51	1853	1203.5	4208
savory	2.60	7.15 ± 0.98	2266	1823.5	3423.5
ziziphora	1.59	5.46 ± 0.79	578.5	435.5	993.5

Table 4: Percentage of repellencies caused by some ethanolic extracts against adults of Callosobruchus maculatus after 24 h

Compound	Concentration (µl/cm^2)	% Repellency	Mean Repellency (%) ± S. E
ammi	6	0 ± 0	12^{c} ± 0.23
	7	0 ± 0	
	8	0 ± 0	
	9	12 ± 0.23	
cumin	6	0 ± 0	10.66^{c} ± 0.20
	7	0 ± 0	
	8	0 ± 0	
	9	10.66 ± 0.20	
fennel	6	30 ± 1.02	38.16^{b} ± 1.76
	7	32.66 ± 1.78	
	8	40 ± 1.70	
	9	50 ± 2.57	
savory	6	40.24 ± 0.78	46.05^{a} ± 4.52
	7	45 ± 1.22	
	8	47 ± 1.5	
	9	52 ± 2.02	
ziziphora	6	30 ± 1.5	39.25^{b} ± 1.99
	7	32 ± 1.70	
	8	42 ± 2.12	
	9	53 ± 2.66	

Different letters showed significant difference ($p \leq 0.01$)
(df=14, F=114)

Table 5: Percentage of repellencies caused by some essential oils against adults of Callosobruchus maculatus after 24 h

Compound	Concentration ($\mu l/cm^2$)	% Repellency	Mean Repellency (%) ± S. E
ammi	1 2 3 5	0 ± 0 0 ± 0 0 ± 0 10 ± 0.07	10 ^{b} ± 0.07
cumin	1 2 3 5	0 ± 0 0 ± 0 2.66 ± 0.04 9.33 ± 0.99	5.99 ^{c} ± 0.51
fennel	1 2 3 5	0 ± 0 1 ± 0.01 12 ± 0.1 20.66 ± 1.22	1122 ^{b} ± 0.44
savory	1 2 3 5	90 ± 4.98 95.66 ± 5.31 100 ± 5.99 100 ± 6.43	96.41 ^{a} ± 5.61
ziziphora	1 2 3 5	0 ± 0 0 ± 0 0 ± 0 0 ± 0	0 ^{d} ± 0

Different letters showed significant difference ($p \leq 0.01$)
(df=14, F=119.26)

Table 6: Synergistic interactions between some plant powders and Iranian diatomaceous earth (DE) against Callosobruchus maculatus

DE+ plant powder	%mortality ± S.E.		interaction
	Expected	Observed	
DE+ ammi p	69.62 ± 3.02	89 ± 3.14	synergism
DE+ cumin p	59.62 ± 2.98	78 ± 2.22	synergism
DE+ fennel p	70.73 ± 2.25	90 ± 4.03	synergism
DE+ savory p	59.62 ± 2.98	75 ± 2.72	synergism
DE+ ziziphora p	70.73 ± 3.25	92 ± 3.82	synergism

P: powder

Table 7: Synergistic interactions between some essential oils and Iranian diatomaceous earth (DE) against Callosobruchus maculatus

DE+ EO	%mortality ± SE		interaction
	Expected	Observed	
DE+ ammi EO	69.62 ± 3.25	90 ± 3.66	synergism
DE+ cumin EO	59.62 ± 2.66	70 ± 2.53	synergism
DE+ fennel EO	62.66 ± 2.57	70 ± 3.98	synergism
DE+ savory EO	59.62 ± 2.66	70 ± 2.93	synergism
DE+ ziziphora EO	60 ± 2.73	70 ± 3.98	synergism

EO: essential oil

Table 8: Synergistic and antagonistic interactions between some ethanolic extracts and Iranian diatomaceous earth (DE) against Callosobruchus maculatus

DE+ ethanolic extract	%mortality ± SE		interaction
	Expected	Observed	
DE+ EE of ammi	59.62 ± 2.98	60 ± 2.22	synergism
DE+ EE of cumin	76.73 ± 3.25	90 ± 4.03	synergism
DE+ EE of fennel	76.62 ± 2.25	80 ± 3.03	synergism
DE+ EE of savory	76.73 ± 3.25	90 ± 4.03	synergism
DE+ EE of ziziphora	76.62 ± 2.25	80 ± 3.03	synergism

EE: ethanolic extract

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