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Effects of Garlic Oils on the Fecundity and Hatchability of *Callosobruchus maculatus* L. (*Coleoptera: Bruchidae*)

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Abstract Laboratory experiments were conducted to evaluate the efficacy of the three types of garlic oils; Sudanese, Chinese and Egyptian on the eggs of cowpea beetle *Callosobruchus maculatus*. Volatile oils from Sudanese and Chinese garlic were obtained by steam distillation and Soxhlet extraction using ethanol correspondingly, whereas Egyptian garlic oil was bought as a ready-made product. The oils were tested at concentrations of 0.01%, 0.1%, 1%, 5% and 10%, at exposure periods ranging from 24-72 hrs. Respective average number of eggs laid after exposure to the lowest concentration of Sudanese, Chinese and Egyptian oils were; 5.4, 4.0, 4.1 after 24 hrs; 0.7, 0.1, 0.1 after 48 hrs 0.2, 0.5, 0.5 after 72 hrs. The oils from the three garlic cultivars significantly reduced number of eggs laid with the highest dose (10%) caused complete inhibition of egg laying. Average number of eggs hatched when exposed to the lowest concentration (0.01%) were; 0.25 after one day and 1.00 after 7 days of exposure to Sudanese oil vapours, 0.00 after one day and 0.75 after 7 days of exposure to Chinese oil vapour and 0.00 after one day and 0.75 after 7 days of exposure to Egyptian oil vapour.

Keywords Garlic Oils, Eggs of *Callosobruchus maculatus*, Hatchability, Inhibition

1. Introduction

Cowpea [*Vigna unguiculata* L. (Walp.)] is an important crop for many subsistence farmers in tropical areas, especially in Africa. Henriet et al. [1] reported the existence of up to 43 crop mixtures in the Sudan savanna of Nigeria with millet–cowpea mixture being predominant, representing 22% of the fields sampled. Cowpea is susceptible to many insect pests in the field [2]. To protect the beans against the store pests *C. maculatus*, many methods can be used. Traditionally the beans are mixed or covered with materials that are locally available. Recently, methods such as storage in airtight plastic or steel containers, application of chemical insecticides, gamma irradiation, freezing the beans; can be used as additional alternatives. Garlic *Allium sativum* L. is widely used as a spice for human food throughout the world which indicates its high safety margin to mammals. Moreover, garlic was also reported to have medicinal, antibacterial activity, antihelminthic, antiprotozoal properties [3], hypoglycemic action [4] and hypoledomic effects [5]. Garlic volatile oil had been shown to have insecticidal, repellent, antifeedant, antibacterial, nematicidal and acaricidal properties [4-7].

In the Sudan, Ahmed [8] reported the efficacy of garlic oil to control store insect pests. Abdallah [9-10] evaluated the action of garlic volatile oils against major store pests and insecticidal properties of volatile oil from local garlic cultivar with promising results. Khirallah [11] evaluated the efficacy of oil extract from Chinese garlic cultivar against two major bruchids. Taha [12] reported the efficacy of Egyptian garlic oil against the same pests. El-sonoussy [13] investigated the ovicidal and anti-oviposition properties of volatile oils obtained from (Sudanese, Chinese and Egyptian) garlic against *B. incarnates* with promising results. This study was initiated to cast light on the efficacy of volatile oils from (Sudanese, Egyptian and Chinese) garlic on control of the cowpea beetle *C. maculatus* (L.).

2. Materials and Methods

Experimental Insects

Callosobruchus maculatus infested cowpea seeds were collected from Omdurman and Khartoum markets. The culture was brought to the laboratory of the Department of Crop Protection; Faculty of agriculture U. of K. Culture was sieved by mesh No. 5 to remove adult insects. Adults were reared in glass jars, with a capacity of 3 kg, more than half filled with sound grain of cowpea, local varieties Black eye, then covered with a muslin cloth, fixed with rubber

bands and kept in the Laboratory at room temperature for months. Newly emerged (24 hours) insects were used. Female were kept for 10-15 minutes with a surplus of newly emerged males, to allow mating.

Garlic Preparation

The cloves of the Sudanese and Chinese garlic were obtained from Northern state Dongla El- Seleem and from Omdurman (Soug El-mahaseel) respectively. The collected samples were cleaned and cut into small pieces and left to dry (for 30 to 40 days for Sudanese and two weeks for Chinese garlic). The dried slices were manually milled into a fine powder using pestle and Mortar.

Volatile Oils

Volatile oils were obtained by Soxhlet and steam distillation for Sudanese and Chinese cultivars respectively. Egyptian oil was obtained as ready-made oil from Egyptian Company (El-captain Company for Extraction of Natural oils, CAP PHARM, Cairo, Egypt).

Soxhlet Extraction of Sudanese Garlic Oil

Five hundred grams of Sudanese garlic powder were placed in five liters round bottom flask, two liters of ethanol (96%) were added. The mixture was subjected to Soxhlet extraction at 60 to70 ºC for six hrs. The oil obtained was kept in a refrigerator at 4 ºC.

Steam Distillation of Chinese Garlic

Steam distillation was done according to the method adopted by Abdalla [9-10]. Three hundred grams of Chinese garlic powder were placed in five liters round bottom flask and 1.5 liters of distilled water were added. The mixture was thoroughly shaken for 20 minutes. The contents were subjected to steam distillation at 65 - 70 ºC. For three hours. Sodium sulfate at 0.1g/ml oil was added to absorb the moisture. Oil was collected in glass containers and kept in a refrigerator at 4 ºC.

Assessment of Ovicidal Effects

Laboratory experiments were carried out to study the ovicidal effects of Sudanese, Chinese and Egyptian garlic volatile oils. Ovicidal test of the volatile oils was done by fumigating eggs to different concentrations of oils. Adults of *C. maculatus* L., 24 hrs old, were allowed to lay their eggs on sound grains of *Vigna unguculata* local variety for 24 to 72 hrs. Seeds bearing about 10 eggs were selected from treatments culture using a hand lens. A total of 100 eggs were tested per replicate. Selected seeds with their eggs were introduced into opened glass jars. Series of dilution (0.01%, 0.1%, 1%, 5% and 10%) of each of garlic oils were prepared using ethanol 5 %. About 2.5 ml of each concentration were placed in Petri-dishes and placed open into fumigation chambers for 3 days. The treated eggs were transferred to open glass jars covered with muslin cloth fixed with a rubber band and kept in an incubator at 28 ºC and 60 - 80% RH. The emerged insects from the test eggs were counted every 24 hrs for 7 days. The eggs were considered dead if not hatched after 18 days. The treatments were arranged in a completely randomized design with four replicates. Sets containing ethanol 5% and distilled water were used as a control. An additional untreated control was included.

Statistical Analysis

Data were subjected to analysis of variance and means were separated by least significant difference (LSD).

3. Results

Ovicidal Effects of Garlic Oils

The results showed that fewer eggs were laid by the females after exposure to Sudanese, Chinese and Egyptian garlic volatile oils. The respective average number of eggs laid after exposure to different concentrations of Sudanese, Chinese, and Egyptian garlic oils were; 5.4, 4.0, 4.1 after 24 hrs; 0.7, 0.1, 0.1 after 48 hrs 0.2, 0.5, 0.5 after 72 hrs. There were significant differences between all treatments of garlic oils. The respective average reduction of a number of eggs laid after exposure to Sudanese, Chinese and Egyptian oils were 80-99, 91-99 and 88-99.7%. However, the numbers of laid eggs were significantly lower after 24 hrs of exposure to the concentrations 5 and 10% (Figures 1-3).

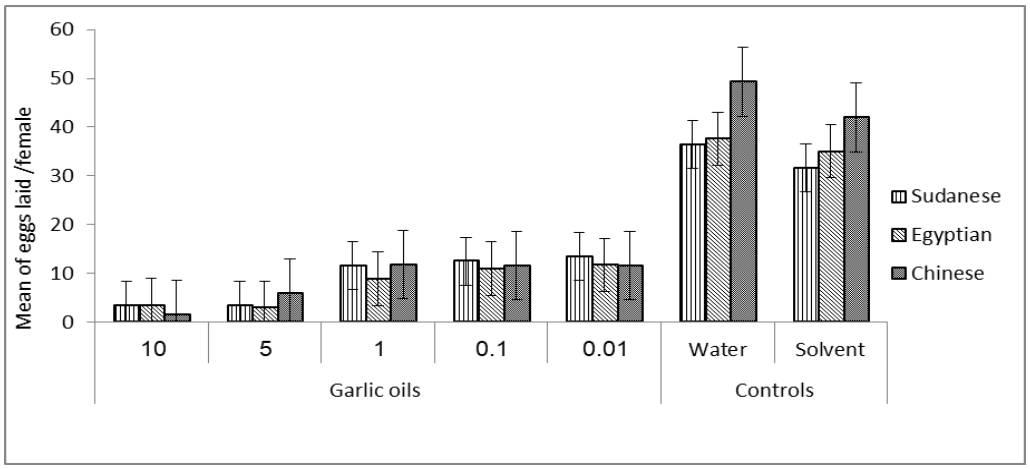

Figure 1. Mean number of eggs Laid by *C. maculatus* female fumigated with garlic oil for 24 hrs, vertical bars represent the SE ±.

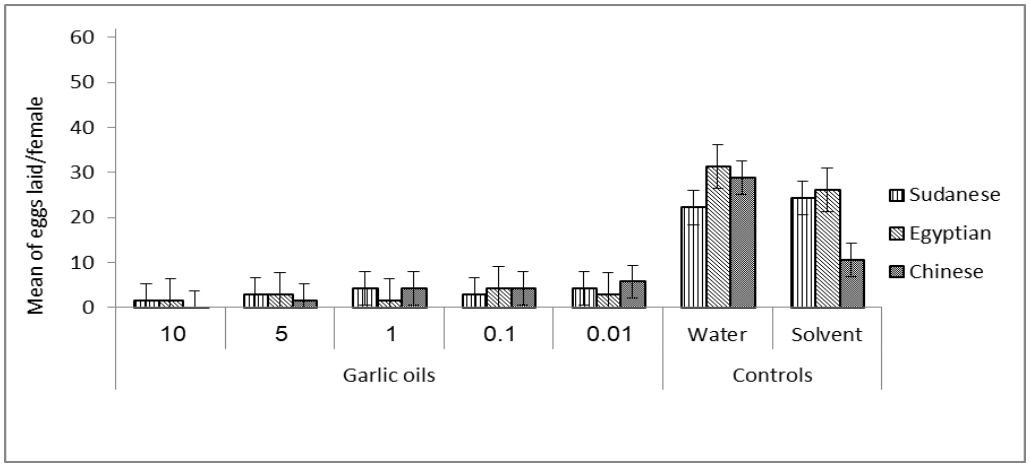

Figure 2. Mean number of eggs Laid by *C. maculatus* female fumigated with garlic oil for 48 hrs, vertical bars represent the SE ±.

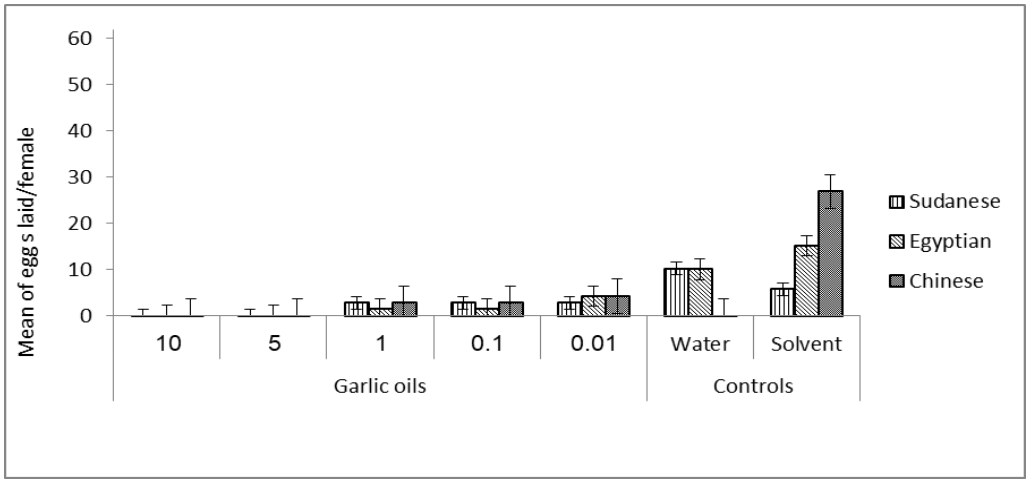

Figure 3. Mean number of eggs Laid by *C. maculatus* female fumigated with garlic oil for 72 hrs, vertical bars represent the SE ±.

Hatchability Effect of Garlic Oils

Hatchability's were watched for seven days post-exposure period. Volatile oil of garlic cultivars manifested variable ovicidal effects on eggs of the cowpea beetle. Average number of eggs hatched after exposure to the lowest concentration (0.01%) of Sudanese, Chinese and Egyptian oils were; 0.25 after one day and 1.00 after seven days, 0.00 after one day and 0.75 after seven days and 0.00 after one day and 0.75 after seven days. The upper dose (10%) of the three garlic oils caused complete inhibition of eggs hatchability compared to the others concentrations (Tables 1-3).

Table 1. Percentage hatchability of *C. maculatus* eggs fumigated with Sudanese garlic oil in glass jars for a week

Exposure time (days)	Untreated control	Treated with distilled water only	Treated with solvent only	Garlic oil Concentration (%)					LSD	SE±	C.V%
				0.01	0.1	1	5	10			
1	1.00^{B}	3.25^{A}	2.75^{A}	0.25^{BC}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.86	0.58	64.69
2	4.75^{A}	4.75^{A}	5.25^{A}	0.50^{B}	0.25^{B}	0.25^{B}	0.00^{B}	0.00^{B}	0.98	0.67	33.99
3	6.25^{AB}	6.00^{AB}	7.25^{A}	0.75^{C}	0.50^{C}	0.00^{C}	0.00^{C}	0.00^{C}	1.02	0.71	26.93
4	8.00^{B}	9.00^{AB}	9.50^{A}	1.00^{C}	0.50^{C}	0.25^{C}	0.00^{C}	0.00^{C}	1.29	0.88	25.03
5	10.50^{A}	11.00^{A}	10.25^{A}	1.00^{B}	0.75^{B}	0.25^{B}	0.00^{B}	0.00^{B}	1.44	0.98	23.33
6	11.75^{A}	12.00^{A}	11.25^{A}	1.00^{B}	0.75^{B}	0.50^{B}	0.00^{B}	0.00^{B}	1.50	1.03	22.03
7	11.75^{A}	12.25^{A}	11.25^{A}	1.00^{B}	0.75^{B}	0.50^{B}	0.00^{B}	0.00^{B}	1.46	1.00	21.33

Means followed with the same letter (s) in the same row are not significantly different at P = 0.05 according to LSD.

Table 2. Percentage hatchability of *C. maculatus* eggs fumigated with Chinese garlic oil in glass jars for a week

Exposure time (days)	Untreated control	Treated with distilled water only	Treated with solvent only	Garlic oil Concentration (%)					LSD	SE±	C.V%
				0.01	0.1	1	5	10			
1	1.00^{B}	3.25^{A}	2.75^{A}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.82	0.56	63.89
2	4.75^{A}	4.75^{A}	5.25^{A}	0.25^{B}	0.25^{B}	0.25^{B}	0.00^{B}	0.00^{B}	0.89	0.61	32.66
3	6.25^{B}	6.00^{B}	7.25^{A}	0.25^{C}	0.00^{C}	0.25^{C}	0.00^{C}	0.00^{C}	0.99	0.68	27.08
4	8.00^{B}	9.00^{AB}	9.50^{A}	0.50^{C}	0.00^{C}	0.25^{C}	0.00^{C}	0.00^{C}	1.29	0.88	25.95
5	10.50^{A}	11.00^{A}	10.25^{A}	0.75^{B}	0.25^{B}	0.25^{B}	0.00^{B}	0.00^{B}	1.52	1.04	25.23
6	11.75^{A}	12.00^{A}	11.25^{A}	0.75^{B}	0.25^{B}	0.50^{B}	0.00^{B}	0.00^{B}	1.58	1.08	23.67
7	11.75^{A}	12.00^{A}	11.25^{A}	0.75^{B}	0.75^{B}	0.50^{B}	0.00^{B}	0.00^{B}	1.58	1.08	23.67

Means followed with the same letter (s) in the same row are not significantly different at P = 0.05 according to LSD.

Table 3. Percentage hatchability of *C. maculatus* eggs fumigated with Egyptian garlic oil in glass jars for a week

Exposure time (days)	Untreated control	Treated with distilled water only	Treated with solvent only	Garlic oil Concentration (%)					LSD	SE±	C.V%
				0.01	0.1	1	5	10			
1	1.00^{B}	3.25^{A}	2.75^{A}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.00^{C}	0.82	0.56	63.89
2	4.75^{A}	4.75^{A}	5.25^{A}	0.00^{B}	0.25^{B}	0.00^{B}	0.00^{B}	0.00^{B}	0.89	0.61	32.66
3	6.25^{AB}	6.00^{B}	7.25^{A}	0.50^{C}	0.25^{C}	0.25^{C}	0.00^{C}	0.00^{C}	1.03	0.71	27.59
4	8.00^{B}	9.00^{BA}	9.50^{A}	0.75^{C}	0.25^{C}	0.25^{C}	0.00^{C}	0.00^{C}	1.37	0.94	27.13
5	10.50^{A}	11.00^{A}	10.25^{A}	0.75^{B}	0.50^{B}	0.25^{B}	0.00^{B}	0.00^{B}	1.53	1.05	25.16
6	11.75^{A}	12.00^{A}	11.25^{A}	0.75^{B}	0.50^{B}	0.50^{B}	0.00^{B}	0.00^{B}	1.58	1.08	23.62
7	11.75^{A}	12.00^{A}	11.25^{A}	0.75^{B}	0.75^{B}	0.50^{B}	0.00^{B}	0.00^{B}	1.58	1.08	23.35

Means followed with the same letter (s) in the same row are not significantly different at P = 0.05 according to LSD.

4. Discussion

Botanical treatments are particularly relevant for small-scale subsistence farmers during post-harvest storage of their commodities [14-15]. Therefore, today, researchers are seeking new classes of naturally occurring pesticides that might be compatible with newer pest control approaches [16-18].

In the Sudan preliminary investigations done by Ahmed [8]; Abdalla [9-10], Khiralla [11], Taha [12], El-sonoussy [13], Ahmed and Abdelbagi [7] indicated the great potential of garlic products in the control of stored insect pests. These results indicated the superior sensitivity of Bruchids to various types of garlic essential oils. Garlic fumigation caused significant reduction of store pests and improvement of stored grain quality [8].

In the current study, laboratory experiments were conducted to investigate the efficacy of the fumigant action of three of garlic oils (Sudanese, Chinese and Egyptian) against the eggs of cowpea beetle *C. maculatus*.

The results showed that fewer eggs were laid after three days of exposure to all concentrations of garlic volatile oils. There were significant differences between treatments and the controls. This result obtained agree with those reported by El-sonoussy [13] who stated that garlic volatile oils from all three cultivars (Sudanese, Chinese and Egyptian) showed a typical ovicidal effect against test eggs of *B. incarnatus*. He also added that the test eggs were more

sensitive than the adult stage of the test insect (*B. incarnatus*) and this may be due to easy penetration into the eggs. The result also are in agreement with result obtained by Neveu et al. [19] who reported that garlic compound may toughen the structure of egg, preventing hatching in a way similar to that of dehydration.

The ovicidal effects of garlic volatiles to eggs of four cotton insect pests, *Earias vittela* (F.), *Dysdercus koenigii* (F.), *S. litura*, and *Helicoverpa armigera* (Huubert), have been reported by Gurusubramanian and Krishna [20]. HO et al. [21] reported that volatile oil from fresh garlic cloves (obtained by steam distillation) can cause dose related mortality against eggs, larvae and adult of *Tribolium castneum* with eggs being more susceptible than adult and larvae.

The results have indicated the great potential of the three types of garlic oils for control of this important store pest of cowpea and other legumes. The tested garlic oils resulted in significant reduction in eggs lying and hatchability of the *C. maculatus*. Further investigations are needed to isolate and identify active substances in the oil, improve extraction procedure and validate the obtained results under typical storage conditions.

5. Conclusions

The results of the current study indicated that volatile oils from the three garlic cultivars could reduce the number of eggs laid by *C. maculatus* and cause significant inhibition of eggs hatchability. Effects were dose and time-related. The three types of oils exerted similar effects in the reduction of number of eggs laid and hatchability.

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Conflict of Interest

The authors have declared no conflict of interest.

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