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FUMIGANT AND CONTACT TOXICITY OF GARLIC OIL ON *Callosobruchus chinensis* (L.), *Callosobruchus maculatus* (F.), *Tribolium castaneum* (HBST.) AND *Sitophilus oryzae* (L.)

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ABSTRACT

Toxicity of garlic oil was tested against adults of *Callosobruchus chinensis* (L.), *C. maculatus* (F.), *Tribolium castaneum*(Hbst.) and *Sitophilus oryzae*(L.) using air tight jars for the fumigant toxicity and topical application for the contact toxicity. The study revealed that adults of *C. chinensis* were more susceptible to the fumigant toxicity of garlic oil than *C. maculatus* and *T.castaneum,* while *S. oryzae* adults were the least susceptible one. The LC_{50} values were 0.081, 0.21, 0.22 and 1.77µ / L air for the above-mentioned insects, respectively. In the contact bioassay, *C. chinensis* , *C. maculatus* and *T.castaneum* were more susceptible to garlic oil than *S oryzae*. The LD_{50} values were 10.3, 10.4 , 11.6 and 50.3 ppm/insect for the four tested adult insects, respectively.

Keywords: Stored grain insects, garlic oil, fumigant toxicity, contact toxicity.

INTRODUCTION

There is an increasing interest in plant and microbial products as sources of insecticides, due to the environmental hazards caused by synthetic insecticides (Arnason *et al.*, 1989 and Jacobson, 1989).

Many developing countries cannot import the newer, more expensive and sometimes less environmentally damaging pesticides (Don-Pedro, 1996). It is worth while therefore to look for alternative sources of pesticides in the regions concerned. Among these sources are various spices traditionally used for protecting food stuffs against inects (Golob *et al.*, 1999).The popular household use of these spices as insect repellents for preserving food grains had led to experimental evaluation of them for possible use as pesticides (Arnason *et al.*, 1989).

One of such spice is garlic, *Allium sativum* L. (Family Liliaceae) which has been used worldwide as a food, spice, and medicine by various populations including the ancient peoples of Egypt, Greece, Rome, and India (Stoll and Seebeck, 1951). The medicinal uses of garlic are diverse. Garlic possesses antibacterial, antifungal and anti-oxidant properties (Sallam *et al.*, 2004; Gowda *et al.*, 2004; and Bakri and Douglas, 2005). Cavallito and Bailey (1944) isolated its active principle, allicin, and showed its broad spectrum activity against various human pathogenes. Sundaram and Milner (1996) demonstrated the growth-inhibiting action of garlic on various human tumors. Garlic extracts contain the compound ajoene, which is a potent inhibitor of platelet aggregation (Block *et al.*, 1986) as well as anti-leukemia agent (Hassan, 2004).

The effect of garlic cloves and garlic oil on insects had been investigated by Amonkar and Reeves (1970), they found that methanolic extract of garlic cloves and its oil had high larvicidial effect on 3rd and 4th stage larvae of *Culex* and *Aedes*. They added that both the extract and the oil had highly larvicidal effect on larvae of highly insecticide-resistant strains of *A. nigromaculis* (Ludlow).

Pandey *et al.*, (1976) found that petroleum ether extract of garlic repels *Callosobruchus chinensis* adults. On the other hand, garlic oil possesses insecticidal activity against the Khapra beetle, *Trogoderma granarium* Everts (Bhatnagar-Thomas and Pal, 1974). Ho *et al.*, (1996) revealed that garlic oil had toxic effect against eggs, larvae and adults of *Tribolium castaneum* and adults of *Sitophilus zeamais*. They added that garlic oil is effective in reducing F1 progeny production in both *T. castaneum* and *S. zeamais*. Kim *et al.*, (2003 a and b) found that garlic oil had high fumigant toxic effect towards *C. chinensis* and moderate one against *Lasioderma serricorne* and *S. oryzae*. Swidan (2005) mentioned that chopped garlic bulbs exhibited high fumigant toxic effect towards *C. chinensis, Rhizopertha dominica* and *T. castaneum*, while it shows low toxicity towards *S. oryzae* adults. On the other hand ,Regnault-Roger and Hamraoui (1993) mentioned that fresh cut cloves of *A. sativum* had no toxic effect against adults of *Acanthoscelides obtectus*.

On the basis of those findings, the laboratory study described herein examined the fumigant and contact toxicity of garlic oil against adults of four main species of stored grain beetles in Egypt. The insects tested were

Callosobruchus chinensis, C. maculates, Tribolium castaneum and *Sitophilus oryzae*.

MATERIALS AND METHODS

A: Insects:

Stock culture of the tested insects species was maintained at the laboratories of Faculty of Education, Alexandria University since 1997. *C. chinensis* and *C. maculatus* were reared using the procedure previously described by Strong *et al.* (1968). *S. oryzae* and *T. castaneum* were reared according to the FAO method (1974).

***B*: Oil preparation:**

garlic oil was extracted from garlic cloves purchased from local market by water steam distillation (Furniss *et al.* 1984) after extraction; the oil was dried over anhydrous sodium sulfate and stored in a refrigerator at 5° C.

C: Fumigant toxicity:

In order to test the fumigant toxicity of garlic oil on the tested insects, gastight glass jars of 500 ml volume with screwed metallic caps were used as exposure chambers.A Watman No. 1 filter paper (2.0 cm diameter) was glued on the underside of the cap and impregnated with aliquots of 25 µl of an appropriate five different concentrations of garlic oil in acetone; 25 µl of acetone alone was applied to controls. The solvent was allowed to evaporate for 2 minutes and the cap containing the treated filter paper was screwed tightly onto the glass jars containing 40 adults of each tested insect species(12-24 hour old adults *C.chinensis*, *C. maculatus* and 7-days old adults *T. castaneum* and *S. oryzae*). Three replicates were set up for each of the tested five concentrations of garlic oil and the control. Glass jars were then kept in an incubator at 30 ± 1 °C and 45-55% RH. Mortalities were recorded 24, 48, 72 and 96 hours after treatment. Mortality was assessed by immobility of the insects and habits characteristic of death. Immobile insects were exposed to the influence of gentle heat from a 20W halogen lamp to further assess mortality.

D: Contact toxicity:

A series of dilutions of garlic oil was prepared using analytical reagent acetone as a solvent. Adults of *C. chinensis, C. maculatus* ***(12-24 hour old)***, *T. castaneum* and *S. Oryzae(7-10 days old)* were anthesized on crushed ice, then they were treated topically with 1 µl of an acetonic solution of garlic oil on the thoracic dorsum of the tested insects using microapplicator. The solvent was allowed to evaporate for 2 minutes. Five concentrations of garlic oil were were tested. Control insects were treated with acetone alone. After treatment, insects were transferred to 5cm Petri dishes and kept in incubator at 30 ± 1 °C and 45-55% RH. Twenty insects were used for each concentration of garlic oil as well as control respectively. Each concentration was repeated six times. Mortality was recorded 24, 48, 72 and 96 hours after treatment. Mortality was assessed by immobility of the insects and habits characteristic of death. Immobile insects were exposed to the influence of gentle heat from a 20W halogen lamp to further assess mortality.

E: Statistical analysis:

The percent mortality was transformed to the angular scale for analysis of variance (ANOVA) as given by Steel and Torrie (1980). The statistical analysis was done using SAS program (SAS, 1985).Treatment means were compared using FLSD 0.5 (Fisher's least significant difference). Data obtained from the various dose – response bioassays were subjected to probit analysis (Finny, 1971) to estimate LD_{50}, LD_{90}, LC_{50} and LD_{90} of garlic oil.

RESULTS

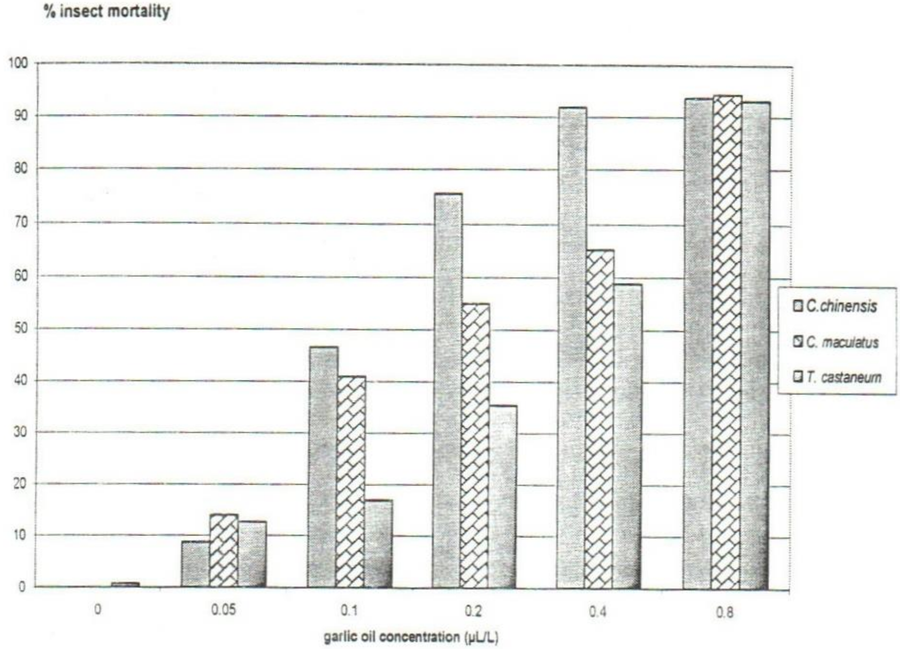
Results of fumigant and contact toxicities of garlic oil against *C. chinensis, C. maculatus, T. castaneum* and *S. oryzae* were represented in tables 1-3, and figures 1 (a.b) and figure 2.

Table 1: Mean percent of dead *C. chinensis, C. maculatus, T. castaneum* and *S. oryzae* after fumigation with different concentrations of garlic oil.

Concentra-tions µL/L air	Mean percent of dead insects					
	C. chinensis	Concentra-ions µL/L air	*C. maculatus*	*T. castaneum*	Concentraions µL/L air	*S. oryzae*
Control	0.0^{e}	Control	0.0^{e}	0.8^{e}	Control	1.3^{d}
0.05	8.8^{d}	0.1	14.0^{d}	12.7^{d}	1	19.4^{c}
0.1	46.7^{c}	0.2	41.0^{c}	16.9^{d}	2	49.8^{b}
0.2	75.6^{d}	0.4	55.0^{bc}	35.4^{c}	4	51.9^{b}
0.4	91.9^{a}	0.8	65.2^{b}	58.8^{b}	8	66.0^{a}
0.8	93.8^{a}	1.6	94.4^{a}	93.1^{a}	16	71.9^{a}

Means having the same letter are not significantly different according to FLSD .05 performed on the angular scale.

A



B

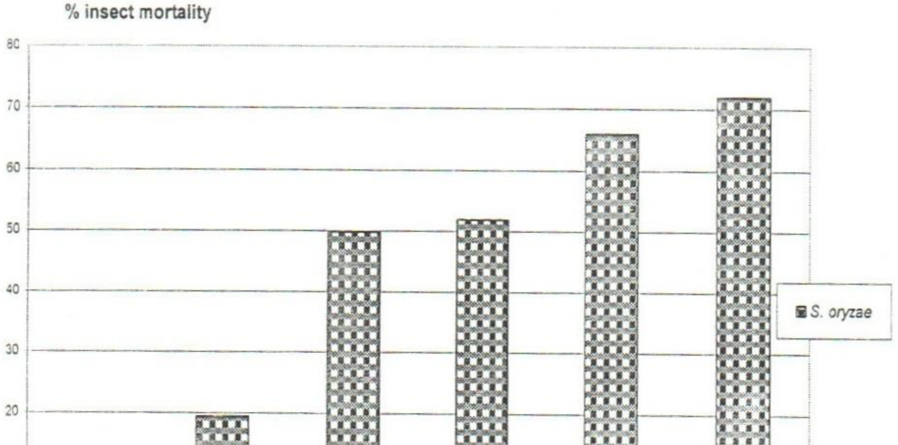


Figure 1: Effect of fumigant toxicity of garlic oil on (A) *C. chinensis* , *C. maculatus* , *I. castaneum* and (B) on *S.aryzae* .

Table 2: Mean percent of dead *C. chinensis*, *C. maculatus*, *T. castaneum* and *S. oryzae* after topical application with different concentrations of garlic oil.

Concentrations (ppm/insect)	Mean percent of dead insects				
	C. chinensis	*C. maculatus*	*T. castaneum*	Concentrations (ppm/insect)	*S. oryzae*
Control	2.7^{d}	4.2^{c}	2.5^{d}	Control	3.5^{d}
5	3.3^{d}	14.2^{b}	3.3^{d}	20	1.5^{d}
10	30.0^{c}	19.2^{b}	20.4^{c}	40	21.5^{c}
20	67.1^{b}	27.9^{b}	39.6^{b}	60	33.5^{c}
30	84.2^{a}	89.2^{a}	78.3^{a}	80	68.0^{b}
40	93.8^{a}	94.6^{a}	87.5^{a}	100	89.8^{a}

Means having the same letter are not significantly different according to FLSD .05 performed on the angular scale.

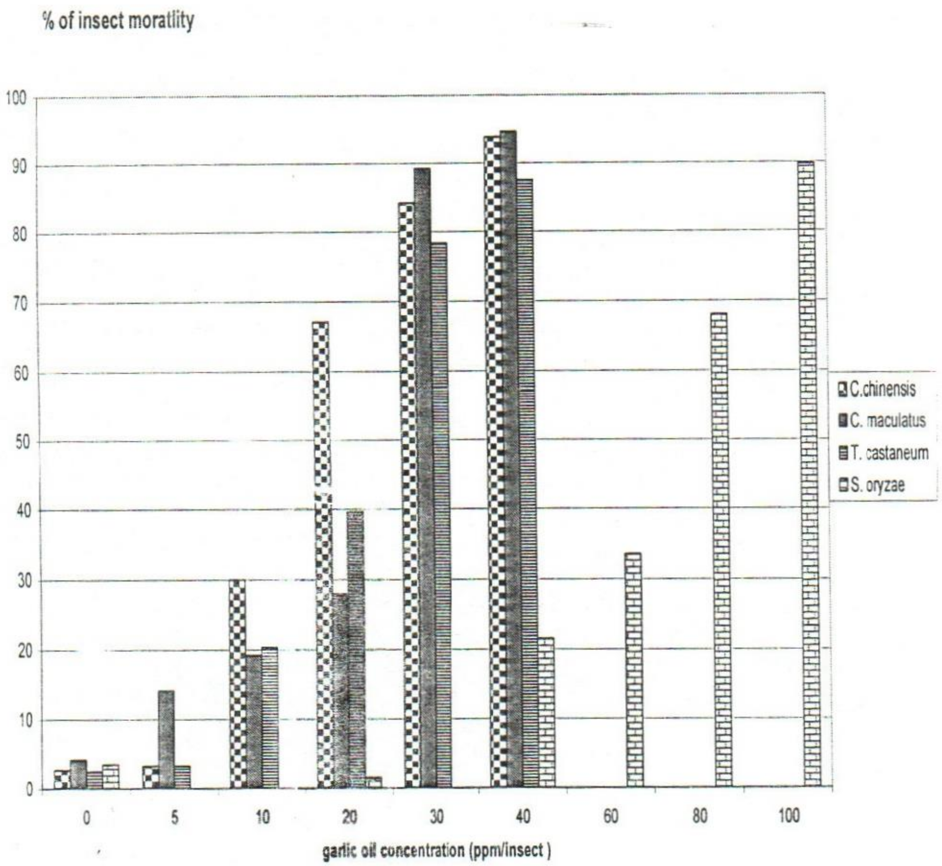


Figure 2: Effect of topical application of garlic oil on *C. chinensis* , *C. maculatus* , *T. castaneum* and on *S.oryzae* .

Table 1 and figure 1 (a, b) shows the fumigant toxic effect of garlic oil against the tested insects. As seen from table a 93.8% mortality was achived in *C. chinensis* adults after fumigation with 0.8µl/L of garlic oil. Only 65.2% and58.8 % mortality was achieved in *C. maculatus* and *T. castaneum* after fumigation with the same concentration of garlic oil (figure 1a).

When *C. chinensis, C. maculatus and T. castaneum* adults were fumigated with 0.4 µl/L of garlic oil, the mean percent of mortality were 91.9, 55.0 and 35.4 respectively. At concentration of 0.2 µl/L of garlic oil the mean perent of mortality was only 41.0 and 16.9 in both *C. maculatus* and *T. castaneum* respectively. Table 1 also shows that when *C. chinesis, C. maculatus and T. castaneum* adults were fumigated with 0.1 µl/L of garlic oil, the mean percent of mortality was 46.7, 14.0 and 12.7, respectively.

Table 1 and Figure 1b also show the fumigant toxic effect of garlic oil towords *S. oryzae* adults .As seen from the table , no significant difference was observed in the mean percent of dead insects after fumigation with 16 and 8µl/ L and concentrations of 4 and 2 µl/L of garlic oil , respectively . A significant difference in the mean percent of dead weevils was observed between concentration of 1 µl/L of garlic oil and the control group.

Table 2 and figure 2 show the mean percent of dead *C. chinensis, C. maculatus, T. castaneum* and *S oryzae* after topical application with different concentrations of garlic oil. The table shows that there is no significant defference between the mean percent of dead *C. chinensis, C. maculatus* and *T. castaneum* when they treated topically with 40 and 30 ppm/ insect of garlic oil .

A Significant difference in the mean percent of dead *S. oryzae* was observed after treatment with concentrations of 100 and 80 ppm/insect of garlic oil. Table 2 also shows a significant difference between the mean percent of dead *C. chinensis* and *T. castaneum* when they topically treated with 20 and 10 ppm/insect with garlic oil. On the other hand, no significant difference was observed in the mean percent of dead *C. maculatus* at the aformentioned concentrations. As for *S. oryzae* no significant difference was observed in the mean percent of dead insects after treatment with 60, 40 ppm/insect and the control group respectively. Table 2 also shows a significant difference in the mean percent of dead *C. maculatus* after treatment with 5 ppm/insect garlic oil and the control group. No significant difference was observed in the mean percent of dead *C. chinensis, C. maculatus* at the aformentioned concentration and the control group.

Table 3 Shows the LC50, 90 values of of fumigant toxicity and LD50, 90 values of contact toxicity of garlic oil for the tested insects. It is clearly seen from the table that *C.chinensis* adults is more susceptible to the effect of garlic oil followed by *C. maculatus and T. castaneum* adults *S. oryzae* adult is in the most tolerant insect to the effect of the oil .

Table 3: $LC_{50, 90}$ values of fumigant toxicity and $LD_{50, 90}$ values of topical application of garlic oil for *C. chinensis*, *C. maculatus*, *T. castaneum* and *S. oryzae*.

Tested insect	Fumigant toxicity (µL/L)		Contact toxicity (ppm/insect)	
	LC_{50}	LC_{90}	LD_{50}	LD_{90}
C. chinensis	0.081	0.14	10.3	17.3
C. maculatus	0.21	0.5	10.4	17.11
T. castaneum	0.22	0.51	11.6	17.8
S. oryzae	1.77	7.66	50.3	91.6

DISCUSSION

Results of the present investigation showed that all the tested insects responded, with different degrees, to the fumigant and topical application of garlic oil. The advantage of the topical application technique is that the actual dose causing mortality to the tested insects can be determined exactly.

On the basis of LC 50s values *C. chinensis* adults were more sensitive to the fumigant effect of garlic oil than the other tested insects. These values are 0.081 µl/L air for *C. chinensis*, 0.21 and 0.22 µl/L air for C. *maculatus* and *T. castaneum* respectively. The LC50 value of garlic oil for *S. oryzae* was found to be 1.77µl/L air.

The differences in responses of the different insect species could be attributed to the morphological and behavioral differences between the insects. Sarc and Tunc (1995) tested the mortality effect of essential oils extracted from different plant species on adults of *T. castaneum, S. oryzae* and *Ephestia kuehniella*. They found that the tested insects responded differently when exposed to the same essential oil at the same dose and over the same period of time. Ho *et al.* (1996) tested the effect of garlic oil on adults of *T. castaneum* and *S. zeamais* using filter paper impregnation technique. They found that *T. castaneum* adults were more susceptible to garlic oil than *S. zeamais*. Kim *et al.* (2003 a and b) found that garlic oil had high fumigant toxic effect towords *C. chinenisis* and moderate one against *L. serricorne* and *S. oryzae* respectively . Swidan (2005) found that chopped garlic bulbs exhibited high fumigant toxic effect towords *C. chinensis* , *R. dominica and T. castaneum* , while they showed low toxicity towords *S. oryzae* adults.

Results of the present investigation showed that the tested adult insects respond to the two type of bioassay, i. e. fumigant and topical application. Don-Pedro (1996) mentioned that topical application of citrus oils to *C. maculatus* , *S. zeamais and Dermestes maculates* had little or no effect to the tested insects on the contrary of fumigant toxicity. He added that the toxic volatile components of the oil apparently evaporated into the surrounding air space faster than they penetrated the insect cuticle on contact. The differences in the results presented herein and those of Don-Pedro (1996) could be attributed to the differences in the chemical composition of the tested oils.

Results of present investigation showed that garlic oil could be useful for managing adult coleopterous insects such as *C.chinensis*, *C.maculatus,*, *T.castaneum* and *S.oryzae*. The fact that these insects responds to the fumigant, as well as contact action of garlic oil, provide an adequate way of the control of stored product insects. Works are in progress for the investigation of insecticidal constituents against coleopterous stored product insects from plant extracts and essential oils, insecticidal mode of action of the constituer ts and appropriate formulation types, for their utilization in grain stores or enclosed spaces .

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التأثير السام بالملامـــسة و التبخيرلزيـــت الثـــوم علـــى *Callosobruchus chinensis* (L.), *Callosobruchus maculatus* (F.), *Tribolium castaneum* (Hbst.) and *Sitophilus oryzae* (L.)

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تم دراسة التأثير السام لزيت الثوم على الحشرات البالغة لكل من :
Callosobruchus chinensis, Callosobruchus maculatus, Tribolium castaneum and *Sitophilus oryzae.*

باستخدام كل من برطمان زجاجي محكم الغلق في حالة دراسة التأثير الـــسام المتطـــاير و الملامس لزيت الثوم .

كانت الحشرات البالغة لـــ *C. chinensis* أكثرحساسية قابلية للأبخرة السامة لزيـــت الثوم ، عن *C. maculatus & T. castaneum* في حين وجد أن *S. oryzae* كانت أكثر مقاومة لسمية زيت الثوم .

و لقد وجد أن قيمة LC_{50} هي (٠,٠٨١ ، ٠,٢١ ، ٠,٢٢ ، ١,٧٧ ميكروليتر / ليتـــر هواء) لكل من *C. chinensis , C. maculatus, T. castaneum & S. oryzae*

في حالة التأثير بالملامسة وجد أن كل من *C. chinensis & C. maculatus* أكثر تأثراً لسمية لزيت الثوم من *T. castaneum* .

أما قيمة (LD_{50}) فقد وجد أنها (١٠,٣ ، ١٠,٤ ، ١١,٦ جزء مـــن المليـــون) للـــثلاث الحشرات التي أجري عليها الاختبار بنفس التتابع .

من جهة أخرى وجد أن *S. oryzae* أكثر حشرة مقاومة لسمية تلامس زيـــت الثـــوم (قيمة LD_{50} هي ٥٠,٣ جزء من المليون) .