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EFFECT OF ALLYL DISULFIDE FROM ESSENTIAL OIL OF GARLIC ON ADULTS OF FOUR SPECIES OF STORED GRAIN INSECTS, *Callosobruchus chinensis* (L.), *Callosobruchus maculatus* (F.), *Tribolium castaneum* (HBST.) AND *Sitophilus oryzae (L.)* .

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ABSTRACT

Allyl disulfide, a major constituent of the essential oil garlic, *Allium sativum,* was tested against *Callosobruchus chinensis* (L.), *C. maculatus* (F.), *Tribolium castaneum* (Hbst.) and *Sitophilus oryzae* (L.) for fumigant and contact toxicity . The study revealed that allyl disulfide was more toxic to *C. chinesis* than the other insects in both fumigant and contact toxicity. The LC50 values of fumigant toxicity were 0.64, 0.63, 0.46 and 3.75µL/Lair for *C. chinensis*, *T.castaneum* and *S. oryzae*, respectively. As for the contact toxicity, which was performed using topical application, the LD50 values were 22.1, 22.8, 24.2 and 94.0 ppm /insect for *C. chinensis, C. maculatus, T.castaneum* and *S. oryzae,* respectively.
Keywords: allyl disulfide, fumigant toxicity, contact toxicity. stored grain insects.

INTRODUCTION

Since antiquity, plants and plant products have been shown to display not only their pharmacological benefits but other biological properties including pesticidal activities. Among these interesting plants *Alliums* pp show pesticidal effects (Golob *et al.*1999) most often linked to volatile substances derived from sulfur amino acids.

When cell membranes are ruptured the amino acids, S-alk(en)y-cysteine sulfoxides , come into contact with alliinase enzymes present in unstable (Lancaster *et al.*1981). S-alk(en)yl-cysteine sulfoxide are then cleaved to produce alk(en)yl sulphenic acids that rearrange to from vestibule thiosulfinates in all *Allium* spp. (Block *et al.*1992).

Depending on break down conditions diallyl thiosulfinate, the main thiosulfinate from garlic is transformed into 66% disulfide, 14% monosulfide and 9% trisulfide along with various proportions of thiosulfonate, vinyldithiines and ajoene (Block *et al.*1984).

The secondary substances of *Allium sativum* have been studied for thier pesticidal effects. Sulfur compounds in *Allium* have been shown to have not only insecticidal, acaricidal, nematicidal, herbicidal, fungicidal and bactericidal effects, but also repellents against arthropods (Auger *et al.* 2004).

Few studies had been done on the toxic effect of the constituents of garlic on stored grain insects. Chiam *et al.* (1999) tested the effect of allyl disulfide on adults and larvae of *T. castaneum* and adults of *S. zeamais* . They found that allyl disulfide had adultcide, ovicide and larvicide effect towards *T. castaneum* and *S. zeamaiz.* Huang *et al.* (2000) found that methyl allyl disulfide and diallyl trisulfide has toxic effect towards *S. zeamais* and *T.*

castaneum. Koul (2004) found that sulfur compounds had toxic and deterrent effect towards adults of *S. oryzae* and *T. castaneum*.

Based on the results of the various workers on the bioactivity of garlic constituents on storedgrain insects, the present study was conducted to evaluate the contact and fumigant toxicity of allyl disulfide, a major volatile component from garlic essential oil on adults of *Callosobruchus chinensis, C. maculatus, T. castaneum* and *S. oryzae*.

MATERIALS AND METHODS

A: Insects:

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

B: Chemical:

Allyl disulfide (80% purity) was purchased from Sigma-Aldrich-Inc., U.S.A. The chemical was diluted with analytical reagent acetone to prepare the desired concentrations.

C: Bioassay:

(I) Fumigant toxicity:

In order to test the fumigant toxicity of allyl disulfide 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 allyl disulfide in acetone; 25 µl of acetone alone was applied to controls. The solvent was allowed to evaporate for 2 minuets and the cap containig the treated filter paper was screwed tightly onto the glass jars containing 40 adult of each tested insects(12-24 hour old for *C. chinensis,C.maculatus* and 7-10 days old for *T. castaneum* and *S. oryzae*) . Three replicates were setup for each of the tested five concentrations of allyl disulfide and the control. Glass jars were then kept in incubator at 30 ± l°C and 45-55% R H. Mortality was assessed by immobility of the insects and habits charecteristic of death. Immobile insects were exposed to the influence of gentle heat from a 20 W halogen lamb to further assess mortality.

(II) Contact toxicity

A series of dilutions of allyl disulfide was prepared using analytical reagent acetone as a solvent. Adults of the tested insects (12-24 hours old for *C. chinensis* and *C. maculatus* and 7-10 days old for *T. castaneum* and *S. oryzae*) were anthesized on crushed ice, then they were treated topically with 1 µl of an acetonic solution of allyl disulfide on the thoracic dorsum of the tested insects using micro applicator. Solvent was allowed to evaporate for 2 minuts. Five concentrations of allyl disulfide were tested. Control insects were treated with acetone alone. After treatment, insects were transferred to 5 cm Petri dishes and kept in incubator at 30 ± l°C and 45- 55% RH. Twenty insects were used for each concentration of allyl disulfide and the control group, 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 20 W halogen lamp to futher assess mortality.

D: Statistical analysis:

Percent mortality was transformed to the angular scale for analysis of variance (ANOVA) as given by Steel and Torrie (1980), and the analysis was done using SAS program (SAS,1985).Treatment means were compared using FLSD 0.5 (Fishers least significant difference). Data obtained from the various dose- response bioassays were subjected to probit analysis to estimate LD50, LC90, LC50, and LD90 of allyl disulfide, (Finny, 1971).

RESULTS

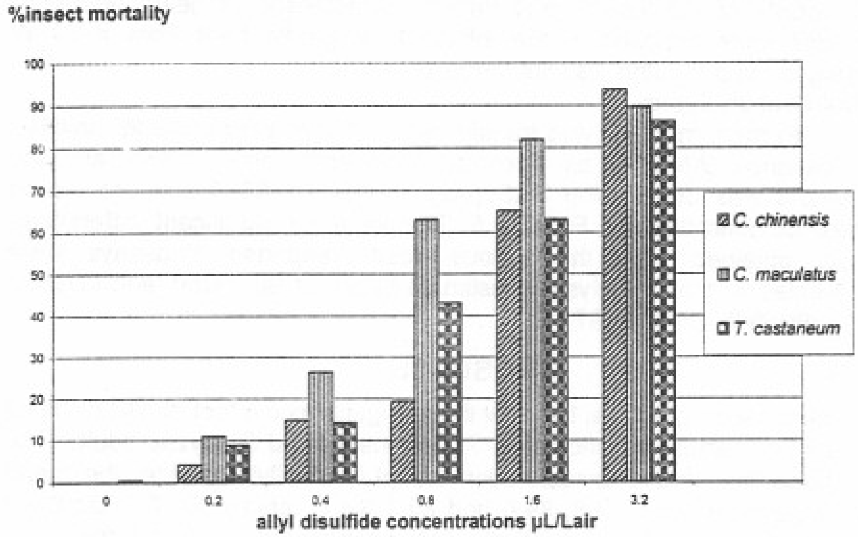
Table 1and figure 1(a, b) show the fumigant toxic effect of allyl disulfide towords *C. chinensis*, *C. maculatus*, *T. castaneum* and *S. oryzae* adults. The table shows that at the concentration 3.2 µL/L of allyl disulfide, the mean mortality percents were 93.8, 89.6 and 86.3 for *C. chinensis*, *C. maculatus* and *T. castaneum* adults, respectively. At the concentration 30 µL/L the mean mortality percent for *S. oryzae* was 88.3. Table 1 also shows that at concentratio of 1.6 µL/L of allyl disulfide, the mean percents of mortality were 65.2, 81.9 and 63.1 for *C. chinensis*, *C. maculatus* and *T. castaneum* , respectively. At concentration 20 µL/L air the mean mortality percent was 88.1 for *S. oryzae*. At concentration 0.8 µL/L of allyl disulfide, the mean mortality percents were 19.4 for *C. chinensis* and 63.1 and 43.1 for *C. maculatus* and *T. castaneum*, respectively. 62.7 % mortality was achived when *S. oryzae* was fumigated with 10 µL/L air of allay1 disulfide. Table 1 also shows that the mean percent of mortality was gradually declained when the tested insects were fumigated with 0.4, 0.2, 5 and 2 µL/L of allay1 disulfide, respectively.

Table 1: Mean mortality percentage for *C. chinensis*, *C. maculatus*, *T. castaneum* and *S. oryzae* after fumigation with different concentrations of allyl disulfide.

Concentrations µL/L air	Mean percent of insect mortality				
	C. chinensis	*C. maculatus*	*T. castaneum*	Concentrations µL/L air	*S. oryzae*
Control	0.0^{e}	0.0^{e}	0.4^{e}	Control	0.0^{e}
0.2	4.2^{e}	11.0^{d}	8.8^{c}	2	21.3^{d}
0.4	14.8^{cd}	26.4^{c}	14.2^{c}	4	41.7^{c}
0.8	19.4^{c}	63.1^{b}	43.1^{c}	10	62.7^{b}
1.6	65.2^{b}	81.9^{a}	63.1^{b}	20	88.1^{a}
3.2	93.8^{a}	89.6^{a}	86.3^{a}	30	88.3^{a}

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

A



B

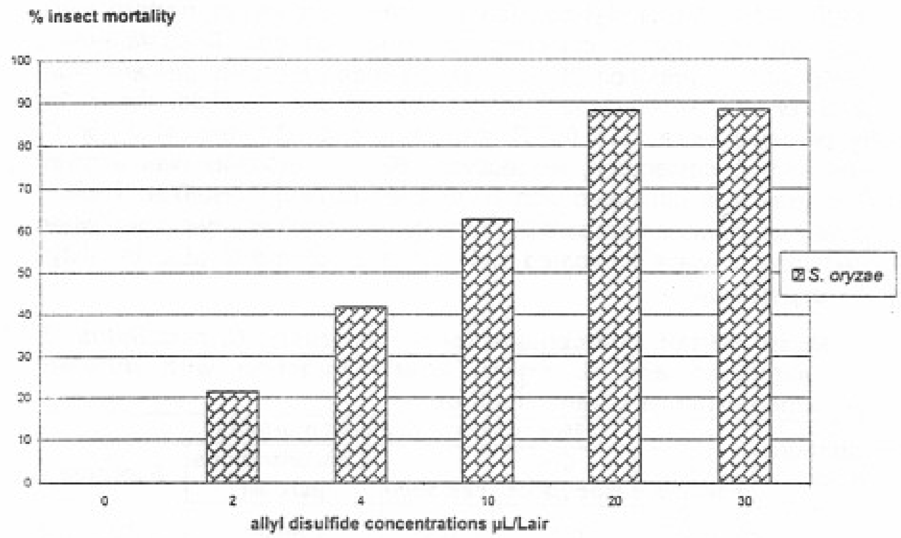


Figure 1a: Effect of fumigant toxicity of allyl disulfide on (A) *C. chinensis, C. maculates* , *T. castaneum* and (B) on *S. oryzae*

Table 2 and figure2 (a, b) shows the mean mortality percents for *C. chinensis*, *C. maculatus, T. castaeum* and *S. oryzae* after topical application with different concentrations of allay disulfide. It is clearly seen from the table that there is significant differences in the mean mortality percent for *C. chinensis* due to treatment with 50 and 40 ppm/insect of ally1 disulfide, respectively. No significant difference is observed in the mean mortality percent for *C. maculates* and *T. castaneum* after treatment with the same concentration. The table also shows that there is no significant difference in the mean mortality percent for *C. chinensis*, *C.maculatus* and *T.castaneum* after topical application with 30 and 20 ppm/insect of allyl disulfide. Their is a significant difference in the mean mortality percent for *C. chinensis* and *C. maculatus* after topical application with 10 ppm/insect of allyl disulfide and the control group. No significant difference is observed in the mean mortality percent for *C. castaneum* after treatment with the aforementioned concentration and the control group.

Table 2: Mean mortality percentage for *C. chinensis*, *C. maculatus*, *T. castaneum* and *S. oryzae* after topical application with different concentrations of allyl disulfide.

Concentrations (ppm/insect)	Mean percent of insect mortality				
	C. chinensis	*C. maculatus*	*T. castaneum*	Concentrations (ppm/insect)	*S. oryzae*
Control	0.8^d	1.3^e	1.7^d	Control	0.5^e
10	12.9^c	7.9^d	2.1^d	50	15.3^d
20	17.9^c	22.1^c	16.7^c	100	33.0^c
30	33.8^b	36.7^b	42.5^b	200	58.8^b
40	42.5^b	71.3^a	76.5^a	300	88.3^b
50	81.7^a	82.9^a	81.7^a	400	88.8^a

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

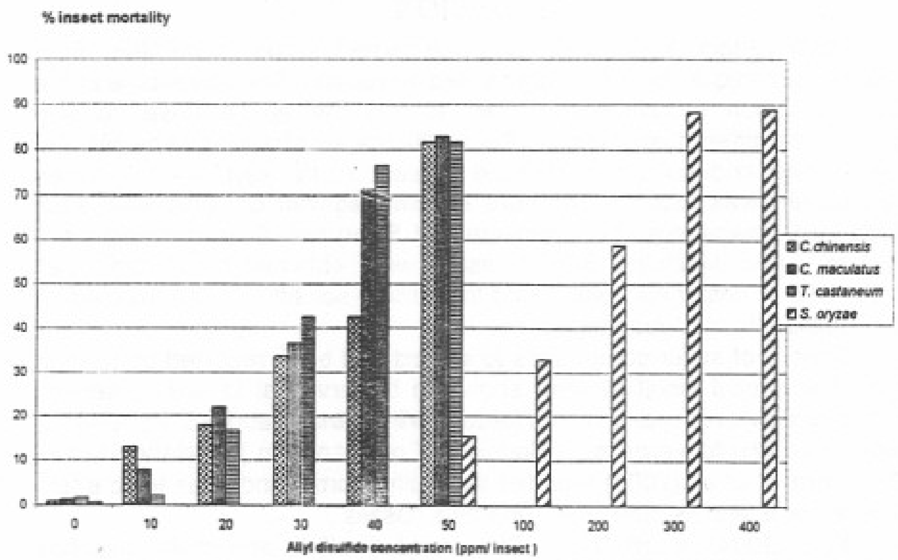


Figure 2: effect of topical application of allyl disulfid on *C. chinensis* , *C. maculates* *T. castaneum* and *S.aryzae* .

Effect of topical application of allyl disulfide on *S. oryzae* adult is also represented in table 2. As seen from the table, no significant difference is observed in the mean mortality percent for *S.oryzae* after treatment with 400 and 300 ppm/insect of allyl disulfide, respectively. Significant differences were observed in the mean mortality percent for *S. oryzae* between 200 and 100 ppm/insect of allyl disulfide and between 50 ppm/insect and the control group.

Tεble 3 shows the LC50, LC 90 values of fumigant toxicity and LD 50,LD 90 values of contact toxicity of allyl disulfide for *C.chinensis, C. maculatus*, *T. castaneum* and *S.oryzae* adults. It is seen that *C.chinensis* adults are more sensitive to the fumigant and contact toxicity of allyl disulfide exhibiting LC50 and LD50 values of 0.46 µL/Lair-and 22.1 ppm, respectively. *C.maulatus* and *T. castanum* adults responded, more or less, equally to the fumigant and contact toxicity of allyl disulfide. *S. oryzae* was the most tolerant insect towards the tested compound.

Table 3: LC_{50}, and LC_{90} values of fumigant toxicity and LD_{50} , LD_{90} values of contact toxicity of allyl disulfide for *C. chinensis*, *C. maculatus*, *T. castaneum* and *S. oryzae*. adults.

Tested insect	Fumigant toxicity (µL/Lair)		Contact toxicity (ppm/insect)	
	LC_{50}	LC_{90}	LD_{50}	LD_{90}
C. chinensis	0.46	1.31	22.1	38.8
C. maculatus	0.63	1.14	22.8	41.3
T. castaneum	0.64	1.94	24.2	48.1
S. oryzae	3.75	12.65	94.0	244

DISCUSSION

Most contact toxicity tests have bee carried out using the filter paper impregation technique, which simulates filed conditions. The advantage of the topical application technique, however, is that the ac ual dose cousing mortality in the different species or different life stages of a single species can be determined and compared. In The present study, allyl disulfide when applied topially was found to be more effective against *C. chinensis* adult followed by *C. maculatus, T. castaneum* and *S. oryzae* .*S oryzae* was more tolerent to allyl disulfide . Similar results were obtained by Chiam *et al.* (1999) in whit *T. castaneum* was found to be more sensitive to the toxic effect of allyl disulfide than *S. zeamais* adults.

Toxicity of sulfur compounds to insects had been reported by several authors. Di-n-propyl disulfide were shown to be larvicidal to *Aedes aegypti* Linnaeus (yellowfever mosquito), *Heliothis Virescens* (Fabricius) (tobacco budworm) and *Helicoverpa zea* (Boddie) (Corn eεrworm) (Balandrin *et al.* 1988). Dugravot *et al.* (2003) reported that sulfur compounds has toxic effect on *C. maculatus* and its parasitoid *Dinarmus basalis* .

Koul (2004) reported that Di-n-propyl disulfide and diallyl disulfide have toxic and deterrents effect on *S.oryzae* and *T. castaneum* adults. He added that both compounds were more toxic to *T. castaneum* when compared with *S.oryzae.*

Huang et. al. (2000) tested the contact and fumigant toxicity of methyl allyl disulfide and diallyl trisulfide (major constituents of essential oil of garlic) on *S. zeamaiz* and *T.castaneum.* They found That the tested compounds were more toxic to *T. castaneum* adults than to *S. zeamais* adults . Auger *et al.* (2004) tested the toxicity of 3 sulfur compounds from garlic oil on the termites *Reticulitermes santonensis* and *R. grassei.* Tested compounds were dimethyl disulfide, dipropyl disulfide and diallyl disulfide. They found that *R. grassei* was more sevsitive to the effect of each disulfide than *R. santonensis.*

The insecticidal effect of sulfur compounds requires knowledge of their toxic mode of action. Dugravot et. al. (2003) suggested that sulfur compounds could inhibit the mitochondrial respiratory chain with the target being the cytochrome oxidase .

In conclusion, sulfur compounds from plants exhibited great insecticidal activities with potential application in crop protection. They are of interest because they could become an alternative of natural origin, rather than conventional synthetic compounds and even a replacement of methyl bromide. To complete the knowledge of insecticidal effects of sulfur compounds, futher studies on stored product insects have to be performed.

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تأثير ثنائي كبريتيد الأليل – من الزيت الطيار للثوم – علـــي بعـــض أنـــواع مـــن حشرات الحبـــوب المخزونـــة وهـــي ***Callosobruchus chinensis* (L.), *Callosobruchus maculatus* (F.), *Tribolium castaneum* (Hbst.) and *Sitophilus oryzae* (L.) .**

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يعتبر ثنائي كبيريتيد الاليل مكون رئيسي من مكونات زيت الثوم الطيار . تم أختبار هذه المادة علـــي اربعة من حشرات الحبوب المخزونة وهي :–

Callosobruchus chinensis, Callosobruchus maculatus, Tribolium castaneum and *Sitophilus oryzae.*

وذلك باستخدام برطمان زجاجي محكم الأغلاق لدراسة التأثير السام لأبخرة تلك المادة بالأضـــافة إلي المعاملة بالملامسة لبيان تأثير السميةبالملامسة لمادة *ثنائي كبيريتيد الاليل*.

وجد أن الطور البالغ لحشرة *C. chinensis* هو أكثر الاطوار البالغة تأثرا لأبخرة تلك المادة وكانت قيمة LC_{50} لها هي 0.46 µL/Lair

أما حشرتي *C. maculatus,* و *T. castaneum* فكانت قيمة LC_{50} ليما هي µL/Lair 0.46 , 0.63 . بين البحث أن حشرة *S. oryzae* هي أقل الحشرات المختبرة تأثرا بـــابخرة *ثنـــائي كبيريتيد الاليل*وكانت قيمة LD_{50} لها 3.75 µL/Lair

أما في حالة التأثيربالملامسة لمادة ثنائي كبريتيد الأليل علي الحشرات تحت الأختبار فكانت قيمة LD_{50} لحشرة *C. chinensis* هي ٢٢,١ *جزء من المليون وقيمة* LD_{50} لحشرة *C. maculatus* هي ٢٢,٨ جزء من المليون وقيمة LD_{50} لحشرة *T. castaneum* هي ٢٤,٢ جزء من المليون . أما تلك القيمة للحشرة S. oryzae فكانت ٩٤,٠ جز من المليون لكل حشرة .