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Garlic *Alium sativium* (*L.*) Volatile Oils as Fumigant for the Control of the Red floor *beetle Tribolium castaneum* (adult)

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Abstract: Laboratory experiments were carried out to evaluate the efficacy of the fumigant action of garlic volatile oils on the control of *Tribolium castaneum* (adult) in small plastic cups (200 ml) and jute sacs (100 g) as well as to shed light on suitable fumigation period and oil concentrations. Fumigation was carried out for short exposure period (12- 48 hrs) as well as over medium (3-15 days) and longer exposure period (15-60 days). Recoveries were watched upto seven days. Garlic volatile oils were tested at concentrations ranging between 0.1- 20% V/V for the short and medium periods and 0.001 – 1% V/V for the longer periods. The results of the short exposure period (6-48 hrs) indicated that garlic volatile oil was very effective and caused significant death to the test insects (*T. castaneum* (adult)) in cups at all concentrations (after waiting for recoveries for seven days). Effect was dose dependant. Recovery cases noticed were negatively related to the dose. Garlic oil vapours penetrated through jute sacs and caused significant mortalaties to the test insects inside the sacs at all concentrations (0.1- 20% V/V) in medium exposure period (3-15 days) as compared to control. Effect was dosage related.The efficacy was tested at much lower concentration of garlic oil (0.001 – 1%) and over longer exposure period (15 – 60 days) in sacs. Results revealed that *T. castaneum* (adult) was sensitivite to garlic oil vapor at all concentrations. Effects were also dose related. The performance of garlic fumigation in cups is much similar to that in sacs. Recovery cases were few and negatively related to the dose

Index Terms: *Tribolium castaneum*, garlic volatile oils, fumigation.

INTRODUCTION .I

Sorghum (*Sorghum bicolor* "L." Moench) is of tropical and sub-tropical origin. It is the staple food for many people in Africa, including the Sudan (Shazali and Ahmed, 1998), Asia and is a major animal feed in Argentina, Australia, Mexico, USA and South Africa (Doggett, 1970; de Wet, *et al*., 1976). It is the most important world cereal crop (More, *et al*., 1992) and most popular food grain in the tropics and subtropics (Elhag, 1992; Shazali, *et al*., 1996). In the Sudan, sorghum is widely grown in areas of sufficient rainfall or under irrigation and is the most popular food grain (Elhag, 1992; Shazali, *et al*., 1996) .However sorghum face the problems of insects and diseases. Food losses in the tropics and subtropics are higher than in temperate regions of the world, because tropics and subtropics provide optimum conditions for pest multiplication. Losses tend to be high (Shazali, 1987; Harein and Davis, 1992) during the rainy season (June – October) and during storage which also affects the relative abundance and the succession of insects (Shazali, 1982). Significant crop losses could occur during field and storage. The post-harvest losses, was estimated at 5-10% (Anon, 1993). The majority of these losses are due to insect infestation (Ibrahim, 2001). Shaaya, (1997) and Raja *et al*.,(2001) estimated the global post-harvest grain losses caused by the stored product pests by 10-40%. The damage caused by red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenibrionidae) to various stored food commodities like grain, flour and dried fruits was reported to be 15-20% which worth millions of rupees every year in a developing country like Pakistan (Khattak and Shafique, 1986). This pest produced significantly higher weight losses in wheat than other cereals (Lohar, 1997). A positive correlation was observed between damage and proteins and fats of wheat, whereas, negative correlation was found in carbohydrate (Wakil *et al*., 2003). *Tribolium castaneum* is commonly found in shipments from the tropics. Beetles and larvae feed on a range of grains, seeds, processed products, peanuts, legumes, spices, dried fruits, oil seed cake,… etc. Badly infested flour has a sharp odour, brown in colour, and may be unfit for baking. This pest can also attack undamaged wheat kernels. (Laeota, 2005 and Eisa, 1995). It also feeds on *sorghum bicolor* (L.) Moench], wheat (*Triticum aestivam* L.), rice (*Oryza sativa*

ISSN 2201-2796
L.) and millet [*Pennisetum glaucum* (L.) R. Br.]. The infestation could directly result in weight loss and the beetle indirectly imparts a brownish tinge and pungent smell to infested flour by secretion of benzequinones (Appert 1987; Hodges *et al.* 1996).

Stored products represent a ready form of human and/or animal food, therefore, only safe chemicals are allowed for use in such product. However effective and safe alternatives for control of stored product pests are highly needed. There are many plants organic compounds known to affect pest population in different ways. They are cheap, of low mammalian toxicity, and are environment friendly (Pereira, 1983; Singh, *et al.*, 1978). In Sudan many natural products were reported to have promising potential against stored product pests; they include neem (*Azadirachta indica*), hargal (*Solanastima argel*), usher {*Calotropis procera* (Hit.)}, rehan (*Ocimun basilicum*) and garlic *Alium sativium* (L.). The latter is widely used in human food throughout the world which indicates its high safety to mammals. Garlic (*A. sativum*) products have been reported to have, insecticidal, repellent, antifeedant, acaricidal properties (Stoll, 2000), anti-arsenic and antioxidant activities (Chowdhury, *et al.*, 2008). The latter (antioxidant activities) was due to the biologically active lipophilic sulfur-bearing compounds like; allicin, S-allyl-cysteine, dially-di-sulfide and dially L-sulfide (Kodera, *et al.*, 2002; Okada, *et al.*, 2005, Chung, 2006). The activity of diallyl disulfide as fumigant against many insects and pathogen was reported by Wanyee (1999) and Horbery (1998). Feng-Lian Yang *et al*. (2010) reported that garlic essential oil and diallyl trisulfide had strong fumigant activities against *Tribolium castaneum* (adult). However, the fumigant activity of diallyl trisulfide was more potent than that of garlic essential oil. Number of studies showed that garlic essential oil and its major components methyl allyl disulfide and diallyl trisulfide have high toxicity against both *T.castaniam* and *Sitophilus zeamaiz* (Ho *etal* 1996; Huang *etal* 2000). Previuos studies (Abdalla and Abdelbagi, 2015) indicated the promising potential of the fumigant action of garlic volatile oils against the lessor grain borer under laborotary conditions, Therefore the current study was initiated to cast light on the effacay of the fumigant action of garlic volatile oils against the red flour beetle.

MATERIALS AND METHODS .II

Red flour beetle *T. castaneum* was reared on crushed sorghum grain. The insects were reared in an incubator (a thermostatic control Gallenkamp cooled incubator) adjusted at 28ºC and 75% relative humidity (RH). A dish containing sodium chloride (NaCl) was placed to maintain the (RH) (Shazali, 1982). The medium (crushed sorghum grain) was sterilized by rising up the temperature to 120ºC for fifteen minutes in an oven to destroy any infestation present (Winks 1982).The sterilized media was then taken out and stored at room temperature for cultures or experimental purposes. The moisture content of the media was adjusted to 12% using the following formula

$$x = \frac{B-A}{100-B} \times 100$$

Where:

x = the quantity of water to be added to 100 g culture.

A = initial water content of the culture.

B = desired water content of the culture.

The required quantity of water was added to the media and mixed thoroughly, transferred to small air-tight tins and daily shaken for a week period before use (Shazali, 1982).

3:1 *Tribolium castaneum* stock culture:

Five hundred (500) adults of *T. castaneum* were collected from the basic culture and placed in five liter glass jar half filled with media (crushed sorghum grains). Crumpled papers were placed on the culture surface to increase the available space (Ahmed, 1998). The jars were covered with muslin cloth fitted with rubber band, labeled (with species, strain, source and date) and placed in an incubator at 30ºC and 70% RH. Cultures were sieved, after 28 days, old adult insects were removed and newly emerged adult were collected one week later (0-1 week old) and transferred to fresh equilibrated food for another week before use. Therefore, the test insects were 1-2 week old.

3: 2 Garlic oil extraction:

Two varieties of garlic (Dongola and Berber) were obtained from the local market (Omdurman market). The cloves of garlic were cleaned, sliced into small pieces to allow drying. Sliced garlic was dried under shade in dark room for seven days in the winter and twelve days in the summer. The dried slices were milled manually using pestle and morter. The obtained powder was sieved through 25 mesh screen, stored in tightly closed jars wrapped with Aluminum foil and kept in a refrigerator at 4ºC. Three hundred grams of garlic powder were placed in five liter round bottom flask, then 1.5 liter distilled water was added. The mixture was thoroughly shaken for twenty minutes manually; the content was subjected to steam distillation at 65-70Cº for 3 hrs to obtain the distilled volatiles (clear yellow oil). Sodium sulphate (0.1 g/ml oil) was added to absorb the moisture. The obtained oil was kept in the refrigerator at 4ºC for bioassay.

ISSN 2201-2796

3:3 Fumigation procedures in cups:

Air-tight glass fumigation chambers (16×16×16 cm^3) with sliding doors were prepared. Various concentrations of essential garlic oil (0.1, 1, 10 and 20%) were prepared by serial dilution using 76% ethanol. Two milliliter from each concentration was placed in 5 cm i.d glass Petri-dish, each dish was placed open into the respective glass fumigation box, and the boxes were tightly covered. Twenty-five insects *T. castaneum* (adult)) were counted and each set was placed in muslin cloth covered plastic cup, (capacity 200 ml). The cups were carefully placed in the fumigation chambers and the chambers were quickly and tightly covered. Control sets containing glass dish with two ml of 76% ethanol were included. Fumigation procedure continued for 12, 24and 24 and 48 hours. Experimental units were arranged in Completely Randomized Design (CRD) with four replicates.

At the end of the fumigation period, insects were transferred to Petri-dish containing natural diet (crushed sorghum grain). Final mortality, deformation, were watched for seven days

3:4 Toxicity to test insects (medium exposure periods) in Jute sacs

Small sacs each containing 100 g (crushed sorghum grains) were prepared. Twenty five test insects *(T. castaneum* (adult)) (of similar age and size) were introduced. Infested sacs were introduced into the fumigation chambers. Open Petri-dishes containing two ml of the respective concentration sets (0.1, 1, 10, and 20%) were placed in the respective fumigation chambers and fumigation continued for 3.7, and 15 days. Mortality and deformation were recorded. Experimental units were arranged in Completely Randomized Design (CRD) with four replicates.

3.5 Toxicity to test insects (Long exposure period) in Jute sacs:

Small sacs containing similar weight of crushed sorghum grains and similar level of infestation with test insects were prepared: other further dilutions of garlic oil (0.001, 0.01, 0.1 and 1%) were prepared and 2 ml of each concentration were introduced into the respective chambers as previously mentioned. Fumigation period was extended to 15, 30 and 60 days. The environmental conditions and other arrangements were as previously mentioned were recorded.

RESULTS .III

Mortality data for different exposure period (12-48 hrs) was given in Table 1 and

Results indicated that garlic volatile oil caused significant death to the test insects *(T. castaneum* (adult)) in all exposure periods (12-48) compared to control. Effects increased with the increase in the dose with mortality ranging from 8-20%, 8-80% and 4-96% for the 1st (12 hr), 2nd (24 hr) and the 3rd (48 hr) exposure periods, respectively. The majorty of knock down (Kd) effects occurred in the first day while little mortality with no significant recoveries occurred within the proceeding three days,

Table 1: Mortality* of *T. castaneum* (adult) exposed to fumigant action of garlic oil for 12, 24 and 48 hrs plastic cups

Concentrations (%)	Exposure period (hrs)					
	12		24		48	
20	5.00a	(20)	20.00a	(80)	24.00a	(96)
10	4.00b	(16)	18.00b	(72)	20.00b	(80)
1	3.00c	(12)	11.00c	(44)	18.00c	(72)
0.1	2.00d	(8)	6.00d	(24)	12.00d	(48)
0	2.00d	(8)	2.00e	(8)	1.00e	(4)
SE±		0.32		1.56		1.81

Means with the same letters within each column are not significantly different at (P<0.05) according to DMRT.

Numbers in parenthesis are mortality %.

* data transformed to $\sqrt{\% + 0.5}$

SE±**:** Standard error

Results of jute sac fumigation (table 2) and indicated that exposure to garlic oil vapor caused significant mortality in test insect (*T. castaneum* (adult) in all exposure periods (3-15 days) compared to control. Mortality was dose dependent with

ISSN 2201-2796
mortality ranging from 8-76%, 8-76 % and 8-80 % for the 1st exposure period (3 days), the 2nd exposure period (7days) and the 3rd exposure period (15 days) in sacs respectively.

Table 2: Mortality* of *T. castaneum* (adult) exposed to fumigant action of garlic oil for 3, 7 and 15 days jute sacs

Concentrations (%)	Exposure period (days)					
		3		7		**15**
20	19.00a	(76)	19.00a	(76)	20.00a	(80)
10	17.00b	(68)	17.00b	(68)	20.00a	(80)
1	10.00c	(40)	14.00c	(56)	16.00b	(64)
0.1	5.00d	(20)	5.00d	(20)	5.00c	(20)
0	2.00e	(8)	2.00e	(8)	2.00d	(8)
SE±		1.45		1.55		1.88

Means with the same letters within each column are not significantly different at (P<0.05) according to DMRT.
Numbers in parenthesis are mortality %.
* data transformed to $\sqrt{\% + 0.5}$
SE±: Standard error

The results of previous experiment indicated the need to carry similar experiment with low conc. (0.001, 0.01, 0.1 and 1%) but over prolonged period (15-60 days). Mortality data for the prolonged exposure period (15, 30 and 60 days of prolonged fumigation) caused by the fumigant action of garlic oil is displayed in Table 3. The results revealed that the garlic oil volatiles had ability to penetrate jute sacs and caused significant death to *T. castaneum* (adult) in all exposure periods (15-60 days) as compared to control. Effects were dose related with mortality ranging from 4-28%, 8-56% and 12-64% for the 1st, the 2nd, and the 3rd exposure-period in sacs respectively. The performance of garlic fumigation in jute sacs is much similar to that in cups.

Table 3: Mortality* of *T. castaneum* (adult) exposed to the fumigant action of garlic oil for 15, 30 and 60 days in jute sacs

Concentrations (%)	Exposure period (days)					
		15		**30**		**60**
1.0	7.00a	(28)	14.00a	(56)	15.00b	(60)
0.1	5.00b	(20)	12.00b	(48)	16.00a	(64)
0.01	2.00c	(8)	10.00c	(40)	15.00b	(60)
0.001	2.00c	(8)	7.00d	(28)	11.00c	(44)
0	1.00d	(4)	2.00e	(8)	1.00d	(4)
SE±		0.53		6.92		1.23

Means with the same letters within each column are not significantly different at (P<0.05) according to DMRT.
Numbers in parenthesis are mortality %
* data transformed to $\sqrt{\% + 0.5}$
SE±: Standard error

ISSN 2201-2796

Knock down and recovery of *T. castaneum* (adult) induced by exposure to garlic oil vapor is displayed in Table 4. Results indicated that garlic fumigation caused significant knock-down of the treated insect within the 1st 24 hrs following fumigation. Knock-down effects were dose related and significantly different from the control in most cases. Although few cases of recoveries occurred after the 1st 48 hrs, observations were carried out for seven days (until final fixed numbers were observed).

Table 4: Knock (Kd) and recovery (Rec.) of *T. castaneum* (adult) exposed tofumigant action of garlic oil (for 48 hrs)

Days / Conc. (%)	1st day Kd.	1st day Rec.	2nd day Kd.	2nd day Rec.
12 hrs exposure period				
20	34.45a (32)	-	0.71a	4.53a (20)
10	31.95b (28)	-	0.71a (0)	2.12b (4)
1	26.56c (20)	-	0.71a (0)	3.54a (12)
0.1	16.43d (0)	-	0.71a (0)	0.71c (0)
0	0.00e (0)	-	0.71a (0)	0.71c (0)
SE±	2.95*	-	0.14**	0.41**
24 hrs exposure period				
20	16.00a (64)	-	26.56a (20)	0.71b (0)
10	11.00b (44)	-	20.27ab (12)	0.71b (0)
1	5.00c (20)	-	0.00c (0)	2.12a (4)
0.1	4.00d (16)	-	0.00c (0)	0.71b (0)
0	0.00e (o)	-	11.54b (4)	0.71b (0)
SE±	1.21	-	2.65*	0.24**
48 hrs exposure period				
20	20.00a (80)	-	0.00b (0)	0.71b (0)
10	18.00b (72)	-	0.00b (0)	4.06a (16)
1	15.00c (60)	-	34.45a (32)	0.71b (0)
0.1	8.00d (32)	-	29.33a (24)	0.71b (0)
0	0.00e (0)	-	23.58a (16)	0.71b (0)
SE±	1.40	-	3.09*	0.34**

Means with the same letters within each column are not significantly different at (P<0.05) according to DMRT.

Numbers in parenthesis are mortality %.

Zero recoveries -

SE±: Standard error

DISCUSSION .IV

Synthetic pesticides have caused serious problem in the environment, these problems include contamination of the biosphere, toxicity to man, animals,, beneficial insects and other non-target organisms. These problems had drown the attention of public and policy makers to the need to adopt new pest management strategies (Saxena *et al*., 1981; Parma, 1992; Jaiswal and Srivastava 1993), based on soft insecticidal chemicals of low environmental persistance, highly specific, cheap, available and bio-degradable. Plant materials (alkaloids, terpenoids, phenolic flavonaids, chramenses and other miner chemicals) could be used as safe alternatives to conventional synthetic chemicals.

One of the promising sources of natural products is garlic (*A. sativum*) (Stoll, 2000). Garlic oil was found to have insecticidal activity against insects (Stoll, 2000). In Sudan investigations carried by Ahmed (1998), Abdalla (2003), Khiralla (2007), Taha (2008), Elsonoussy (2009) and (Abdalla and Abdelbagi, 2015) indicated the promising potential of garlic products in the control of stored pests. In the current study, various experiments were carried out under laboratory conditions to investigate the efficacy of the fumigant action of garlic volatile oil against *T. castaneum* (adult). Evaluation was done in cups and in sacs and for various exposure periods with various concentrations.

Results of cup fumigation (< 48hrs with concentrations range from 0.00-20%) indicated that exposure to garlic volatiles vapour was able to cause significant death to test insect. Most tested concentrations were significantly better than the control and

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dose-related. Increasing the exposure period caused significant improvement in efficacy as indicated by the progressive increase in the number of dead insects. These findings agrees with Ahmed (1998), Wanyee *et al* (1999) and (Abdalla and Abdelbagi, 2015) who showed that many stored product pests were sensitive to garlic oil vapour. Generally increasing exposure periods increase the efficiency of the oil vapour. They added that increasing the pre-accumilation period results in better efficacy.

The follow-up of knock-down and recoveries after exposure for seven days indicated that the majority of knock-down effects occurred within the 1st 48hrs following the exposure period. Small increments (< 5%) knock-down occured within the next few days. Knock-down effects follow the trend out-lined earlier for mortality pattern with effects being dose related and significantly different from the control in most cases. Despite this, recovery was watched for seven days.The knock-down and recoveries among garlic fumigated insects were reported by Ahmed (1998). The significance of watching recoveries of poisoned or treated pests is essential for better reading of the end-point mortality as evaluated by Winks (1982); Beard (1949). The end-points mortality was defined by Beard (1949) as observation period where treated individuals either died or recovered (i.e. the poison have exerted its full toxic effect). The ability of garlic volatile oils to cause significant knock-down needs further evaluation as whether it can be utilized in management strategies of storage pest specially when testing lower dosage of pesticides or environmentally soft chemicals which have lower or slow toxic action.

The applicability of garlic fumigation for the control of store pest requires evaluation of the penetration ability of vapor through the packing materials (mostly jute sacs in Sudan). Therefore, further experiments were designed to evaluate the efficacy of garlic fumigation against the test insects in jute sacs. From our previous findings it was noticed that *T. castuneum* (adult) was sensitive to garlic vapours and therefore it was decided to increase the exposure period to 15 days. Results indicated that garlic vapour caused significant mortality to test insects. Effect was dose-related and significantly different from the control in most cases. Increasing the exposure period resulted in progressive increase in mortality. Garlic products were reported to have insecticidal action against many storage pests (Ahmed,1998; Wanyee *et al* 1999; Stoll, 2000; Abdalla ,2003; Khiralla, 2007; Taha, 2008 ;Elsonoussy 2009 and Abdalla and Abdelbagi, 2015).

Garlic oil is made up of sulphar derivatives, mainly; allicin, S-allyl-cysteine, dially-di-sulfide and dially L-sulfide (Kodera, *et al.*, 2002; Okada, *et al.*, 2005, Chung, 2006). The activity of diallyl disulfide as fumigant against many insects and pathogen was reported by Wanyee (1999) and Horbery (1998). *Tribolium castaneum* was highly sensitive to garlic fumigation, 100% mortality was obtainted at the concentration of 8 µl/l with exposure period of only 4 days for diallyl trisulfide, but a longer exposure period (6–7 days) was needed for garlic essential oil (Feng-Lian Yang etal (2010)). The contact toxicity, fumigant toxicity, and antifeedant activity of methyl allyl disulfide and diallyl trisulfide of garlic (*Allium sativum* L.) , were tested against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motschulsky y. The results of fumigant toxicities of diallyl trisulfide were greater than that of methyl allyl disulfide to the adults of both species of insects. However, *T. castaneum* adults found to be more sensitive to the garlic componants than S. *zeamais* adults. *T. castaneum* Older larvae were more sensitive to the contact toxicity, whereas younger larvae were more sensitive to the fumigant. toxicity. Both compounds reduced egg hatching of *T. castaneum* and subsequent emergence of progeny (Yan Huang etal 2000)

From these findings it is very clear that garlic volatile oil had fumigant action which can exert toxicity to test insects and can easily penetrate jute sacs which are normally used for packing of stored grains. The expected low mamalion toxicity of garlic (being a normal additive of human food) adds to its merits.

The efficacy of garlic fumigation against *T. castuneum* (adult) in jute sacs using much lower concentration of the oil (0.001-1%) accompanied with increase in exposure period was also evaluated. The objective of study is to evaluate suitability for longer storage period. The results obtained indicated the efficacy of garlic oil fumigation even at this extended period and much lower concentration. Increase in population of test insects is expected to occur in prolonged storage, however it was not possible to evaluate this parameter in this experiment because proper sexing of test insects was not done and therefore any evaluation of multiplication of test insects could give false results. The mortality of long storage was calculated based upon the introduced insects which were quite distinguishable from newly emerged adults or larvae. Natural mortality as indicated by longevity of test insects under Sudanese environment normally exceeds the preliminary observation of this study. It indicated the progressive reduction in the rate of multiplication of treated insects. Effects appear related to dose and exposure period and different from the control. Essential oil from fresh garlic cloves (obtained by steam distillation) can cause dose-related mortality of egg, larvae and adult of *T. castaneum* with eggs being more susceptible than adult and larvae (Ho-SH *et al.*, 1997). Further garlic products were reported to have antifeedant and repellent activities against storage and field crop pests (Ho-SH, 1997; Wanyee *et al.*, 1999 and Stoll, 2000). Evaluation of the prolonged fumigation of stored sorghum indicated that such treatment could give significant protection of stored commodities. The level of protection is negatively related to the dose and range from two folds to ten fold in weight loss depending upon exposure period.

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