Garlic Volatile Oil as Promising Fumigan for the Control of the Lesser Grain borer *Rhyzopertha dominica* (adult)

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***Abstract*- Laboratory experiments were carried out to investigate the efficacy of the fumigant action of garlic volatile oil on *Rhyzopertha dominica* (adult) in small cups (200 g) and jute sacs (100 g) as well as to shed light on suitable fumigation period and concentration. Garlic volatile oils were tested at concentrations ranging between 0.1 and 20% v/v for short exposure (6-48 hrs) and medium exposure periods (3-15 days), however for longer exposure period (15-60 days) the concentrations were between 0.001-1.0 v/v. The results of the short periods indicated that garlic volatile oils are very effective and caused significant mortalities to the test insects in cups at all concentrations tested (after waiting recoveries for seven days) compared to control. Effect was dosage related. and the insect was sensitive to garlic fumigation. The results of medium (3-15 days) and longer (15-60 days) exposure periods indicated that garlic oil was able to penetrate through sacs and cause significant death to the test insect (*R. dominica* (adult)) compared to control. Effect was dose dependant. The performance of garlic fumigation in cups is much similar to that in sacs. Recovery cases noticed were few and negatively related to the dose.**

***Index Terms*— Rhyzopertha dominica garlic control**

I. Introduction

Sorghum is a major crop grown in the tropics and subtropics (Hall, 1970), and it is the staple food in many countries including the Sudan (Shazali and Ahmed, 1998). Wheras sorghum is widely grown in areas of sufficient rainfall and/or under irrigation, is the most popular food grain (Elhag, 1992; Shazali, *et al*., 1996). However sorghum production is faced problems of insects and diseases. Significant crop losses could occur during field and storage. . Among 13 species of insect pests of stored grains in Renk,Sudan; The two species *Trogoderma granarium* (Khapra beetle) and *Rhyzopertha dominica* cause most of the damage to stored grains, however , Lesser grain borer caused more damage to the sorghum local cultivars (Fetterita and Gadam Alhamam) than the *Trogoderma granarium* (Doka, 2011)

The most important insects of stored maize and sorghum were the *Sitophilus zeamaiz* (Mostchulsky) and *Rhyzopertha.dominica* (F.) (Hoppe, 1986). The later is the one of the most important primary storage pest of grain cereals in central and northern Sudan specially Gezira, Khartoum, River Nile and Northern States (Abdalla, 1986).However *Rhyzopertha dominica* is a major pest of wheat (Flinn *et a*l., 2004), rice (Chanbang *et al*., 2008), maize and sorghum (Jood et al., 1992 and More, *et al*., 1992) around the world. Both larvae and adult cause loss in the quantity (Emery and Nayak, 2007 and Sanchez-Marinez et al., 1997), quality of stored seeds (Sanchez-Mariñez *et al*., 1997) in addition to the cost of the prevention and control (Cuperus *et al*., 1990; Anonymous, 1998). Weight losses of *R. dominica* was estimated as 40%, compared to 19%, 14% and 10% for *Sytophilus oryzae*, *Triboliumcastaneum* and *Ephestia cautella*, respectively (Sittusuang and,Imura,1987). Nevertheless the beettle feeds on seed germs reducing germination ability and vigour of the grains (Bashir, 2002). Jood *et al*., (1992) reported that *R. dominica* infestation affect the nutritional value of wheat, maize and sorghum grains changing the calcium, phosphorus, zinc, iron, copper and manganese contents. Jood and Kapoor (1992) also observed a reduction in the starch digestibility of maize, rice and sorghum.

Store products represent a ready form of human and/or animal food, therefore, only safe chemicals are allowed for use in such products. Therefore, effective and safe alternatives for the 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 store pests; they include *Trigonella foenum graecum* (L.) (Fenugreek), *Azadirachta indica* (A. Juss) (Neem), *Nicotiana* spp (nicotine), *Calotropics procera* (Sadon apple, Usher), and garlic *Alium sativium* (L.). The latter is widely used as a major component of many Asian diets and as spices for human food throughout the world. This wide use in human food indicates its high safety to mammals. Recently garlic has been reported to have antioxidant activities, anti-arsenic activities (Chowdhury, *et al*., 2008), insecticidal, repellent, antifeedant, and acarocidal properties (Stoll, 2000). It was found effective against cut worms, Aphids, bollworm, pod borer of pigeon pea, jassid,

cabbage,white fly, caterpillars, Colorado beetle larvae , and butter flies (Stoll, 2000 and Greenstock, (1970)

Ahmed (1998) reported that the increase in the accumulation period of garlic oil result in faster kills. However hundred percent mortality in *T. castaneum* and *R. dominica* occurred in 75 and 60 minutes respectively. The current study aimed at evaluating the effacay of garlic volatile oils against the adult stage of *R. dominica* in plastic cups and jute sacs.

II. MATERIALS AND METHODS

Lesser grain borer *R. dominica* was cultured on sound sorghum grain (Fetarita). The insect was 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 media (sound sorghum grains) was sterilized by rising the temperature to 120ºC for fifteen minutes in an oven to destroy any infestation presence (Winks 1982).The sterilized media was then taken out and stored at room temperature for culture 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 for the culture.

B = desired water content for 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 used (Shazali, 1982).

3: 1 *R. dominica* Stock culture:

Three hundred adults of *R.dominica* were collected from the media and placed in glass jars. Jars were closed with muslin cloth tightly fitted with rubber bands. Cultured insect were labeled (with species, strain, source and date) and placed in an incubator at 30ºC and 70% RH. Culture were sieved after 28 days, old adults were removed and newly emerged adults were collected one week later and placed in fresh equilibrated food for another week (Ahmed, 1998). 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 were dried under shade in dark room for seven days in winter and twelve days in 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 subject to steam distillation at 65-70ºC 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.

3:3 Fumigation procedure in plastic cups (short exposure period):

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 (*R. dominica*, 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, 24 and 48 hours. Experimental units were arranged in Complete Randomized Design (CRD) with four replicates.

At the end of the fumigation period, insects were transferred to Petri-dishes containing natural diet (sound sorghum). Final mortality, recoveries, deformation were watched after seven days (White, 1982).

3.4. Fumigation procedure in Jute sacs (medium exposure periods):

Small sacs each containing 100 g (sound sorghum grains) were prepared. Twenty five of the test insect (*R. dominca* (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 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 Fumigation procedure in Jute sacs (Long exposure period):s:

Small sacs containing similar weight of sound 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, other arrangements and observations were as previously mentioned.

RESULTS AND DISCUSSION

The use of 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 which include secondary compounds (alkaloids, terpenoids, phenolic flavonaids, chramenses and other miner chemicals) could be used as safe alternatives to conventional synthetic chemicals in IPM strategies. These compounds affect insects in various ways; they can disrupt major metabolic pathways leading to rapid death, may act as attractant, deterrents, phago-stimulants, antifeedants, modifier of ovipostion, may retard or accelerate

development or interfere with insect's life cycle in other way (Bell *et al*., 1990).

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 the preliminary investigations carried by Ahmed (1998) indicated the promising potential of garlic products in the control of stored product 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 leaser grain borer *R. dominica* (adult); Evaluation was done in cups and in sacs and for various exposure periods with various concentrations (Table 1, 2, 3, and 4).

The final mortality (after waiting for recoveries for four days for cup experiment and seven days for sac experiments) caused by the fumigant action of garlic oil is displayed in Table1. Significant recoveries occurred within the first 48 hrs although insect were watched for seven days post-exposure periods. Mortality data for different exposure period (6-48 hrs) was given in Table **I** . Results of cup fumigation (< 48 hrs with concentrations range from 0.00-20%) indicated that exposure to garlic volatiles was able to cause significant mortalities to test insect. Most tested concentrations were significantly better than the control and effects were dose-related i.e. increasing the exposure period from 6 hrs to 48 hrs, caused significant improvement in efficiency as indicated by the progressive increase in the number of dead insects.

Table1. Final mortality* of *R.dominica* (adult) exposed to garlic oil vapors in plastic cups

Concentrations (% V/V)	**exposure period (hrs)**			
	6	**12**	**24**	**48**
20	10.00a (40)	23.00a (92)	25.00a (100)	25.00a (100)
10	7.00b (28)	18.00b (72)	25.00a (100)	25.00a (100)
1	7.00b (28)	10.00c (40)	23.00b (92)	22.00b (88)
0.1	5.00c (20)	6.00d (24)	11.00c (44)	12.00c (48)
0	1.00d (4)	4.00e (16)	5.00c (20)	3.00d (12)
SE±	0.78	1.23	1.90	2.01

Means with the same letter within each column are not significantly different at P ≤ 0.05, according to Duncan Multiple Range test.

Numbers in parenthesis are mortality percentage.

SE: Standard error

*****Mortality was reported after watching recoveries for four days in normal diets.

The follow-up of knock-down and recoveries after exposure in normal diet (fatarita) for seven days (Table **IV)** indicated that the majority of knock-down effects occurred within the 1st 48 hrs following the exposure period. Small increasments (< 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 differ from the control in most cases.

The applicability of garlic fumigation for the control of storage pests requires evaluation of the penetration ability of the vapor through the packing materials (mostly jute sacs in Sudan). Therefore, further experiments were designed to evaluate the efficacy of garlic fumigation against test insects in jute sacs. From the previous findings it was noticed that *R. dominica* adults were sensitive to garlic vapours and therefore it was decided to to increase the exposure period to 15 days . The results (Table **II**) . The 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.

Table II.. Mortality of *R.dominica* (adult) exposed to garlic oil vapors in sacs for 3, 7 and 15 days

Concentrations (% V/V)	**exposure period** (days)		
	3	**7**	**15**
20	20.00a (80)	24.00a (96)	25.00a (100)
10	20.00a (80)	21.00b (84)	23.00b (92)
1	18.00b (72)	17.00d (68)	19.00c (76)
0.1	17.00c (68)	18.00c (72)	17.00d (68)
0	2.00d (8)	2.00e (8)	3.00e (12)
SE±	1.90	0.96	1.86

Means with the same letter within each column are not significantly different at P ≤ 0.05, according to Duncan Multiple Range test.

Numbers in parenthesis are mortality percentage.

SE: Standard error.

The efficacy of the fumigant action of garlic oil against storage insects were reported earlier by Ahmed (1998) and Wanyee *et al* (1999). Garlic products were reported to have insecticidal action against many storage and vegetable pests (Amonkar and Reeves, 1970; Bhatnagar and Pal ., 1973 and Stoll, 2000).

Garlic oil is made up of sulphur derivatives, mainly diallyl disulfide, allyl propyll disulfide, Allyl sufoxide, alicin and

other miner compounds (Fenaroli, 1975 and Tlal, 1994). Garlic has antifungal, and other medical properties (Hayashi and Sano, 1993). The activity of diallyl disulfide as fumigant against many insects and pathogen was reported by Wanyee (1999) and Horbery (1998). 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 results clearly marked sensitivity of *R. dominica*, adult in jute containers. The fact that this pest is a major pest of stored cereals in Sudan and the cereals are the main food and usually consumed by human and an animal add more merits to its applicability in the control of store product pests. The expected low mamalion toxicity of garlic (being a normal additive of human food) further adds to its merits.

The efficacy of garlic fumigation against *R. dominica* (adult) in jute sacs using much lower concentration of the oil (0.001-1%) accompanied with increase in exposure period was also evaluated Table **III**. 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 insectsis expected to occur in prolonged storage, however it was not possible to evaluate this parameter in the experiments done because proper sexing of test insects was not done and therefore any evaluation of multiplication of test insects could give false results. The mortality reported 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 as indicated by the progressive reduction in the rate of multiplication of treated insects. Effects appear related to dose and exposure period and differ from control.

Table III. Mortality* of *R.dominica* (adult) exposed to garlic oil vapors in sacs for 15, 30 and 60 days

Concentrations (% V/V)	**exposure period** (days)		
	15	**30**	**60**
1	12.00a (48)	16.00a (64)	19.00a (76)
0,1	11.00a (44)	12.00c (48)	18.00b (72)
0.01	6.00b (24)	13.00b (52)	15.00c (60)
0.001	3.00c (12)	12.00c (48)	13.00d (52)
0	1.00d (4)	2.00d (8)	3.00e (12)
SE±	1.00	6.92	1.37

Means with the same letter within each column are not significantly different at $P \leq 0.05$, according to Duncan Multiple Range test.

Numbers in parenthesis are mortality percentage.

SE: Standard error.

The ability of allyl disulfide (active ingredient of garlic) to decrease the fecundity and adult emergence of *R. dominica* was reported by Singh *et al*. (1996). 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 (Green stock, 1970; 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. Garlic extract was reported as the best natural protectant of stored grain in India against *R. dominica* and *S. oryzae* (procash *et al*., 1982). The antifeedant activity of garlic products were reported by Green stock (1970) and Wanyee (1999) as mentioned earlier.

Table IV. Knock (Kd) and recovery (Rec.) of *R. dominica* (adult) exposed to garlic oil vapors in plastic cups (for 48 hrs)

a-6 hrs exposure period				
Days / **Conc.**	**1st day**		**2nd day**	
	Kd.	**Rec.**	**Kd.**	**Rec.**
20	**12.00a (48)**	-	0.71a	29.33b (24)
10	8.00c (32)	-	0.71a (0)	10.43b (8)
1	10.00b (40)	-	0.71a (0)	26.55ab (20)
0.1	4.00d (16)	-	0.71a (0)	11.54c (4)
0	1.00e (4)	-	0.71a (0)	0.00d **(0)**
SE±	0.91	-	0.10	2.62
b-12 hrs exposure period				
20	13.00bb (52)	-	0.71a	29.33a (24)
10	15.00a (60)	-	0.71a (0)	16.43b (8)
1	11.00c (44)	-	0.71a (0)	26.56ab (20)
0.1	5.00d (20)	-	0.71a (0)	11.54c (4)
0	0.00c (0)	-	0.71a (0)	0.00d (0)
SE±	1.17	-	0.07	3.08
c-24 hrs exposure period				
20	25.00a (100)	-	0.71b (0)	0.71b (0)
10	19,008 (76)	-	2.12a (4)	0.71b (0)
1	10.00c (40)	-	0.71b (0)	2.12a (4)
0.1	8.00d (32)	-	0.71b (0)	2.92a (8)
0	0.00c (0)	-	2.92a (8)	0.71b (0)

SE±	1.96	-	0.23	0.35
d-48 hrs exposure period				
20	24.00a (65)	-	0.71b (0)	0.71c (0)
10	18.00b (72)	-	0.71b (0)	3.54a (12)
1	10.00c (40)	-	0.71b (0)	3.54a (12)
0.1	0.00d (0)	-	2.92a (8)	0.71c (0)
0	1.00d (4)	-	0.71b (0)	2.12b (4)
SE±	1.99	-	0.17	0.35

Means with the same letter within each column are not significantly different at $P \leq 0.05$, according to Duncan Multiple Range test.

Numbers in parenthesis are knock down or recoveries (%).

SE: Standard error

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