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Evaluation of the Fumigant Action of Garlic (*Allium sativum*) Aqueous Extract Against the Cowpea Seed Weevil *Callosobruchus maculatus*, (F.)

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Abstract The efficacy of the fumigant action of garlic aqueous ethyl acetate extract (95:5 v/v) from the Chinese garlic was tested against the adult stage of the cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), under laboratory conditions at the Department of Crop Protection, Faculty of Agriculture, University of Khartoum, Shambat, Sudan. Garlic extracts were prepared by 24 hrs soaking of garlic powder in water: ethyl acetate solution (95:5% v/v). The tested concentrations were 0.1%, 1%, 5% and 10%. The bioassay was done in plastic cups placed in glass chambers with sliding doors. Result indicated that water: ethyl acetate extract was effective against the test insect. The median lethal doses (LD_{50}) for the extracts after 6, 12, 24, 48 and 72 hours were 0.351%, 1.14%, 0.26%, 0.088% and 0.059% while the corresponding LD_{90} values were 14.92%, 7.41%, 1.66%, 2.99% and 1.20%. The respective median lethal times (LT_{50}) values at the concentrations 10%, 5%, 1% and 0.1% were 3.56 hrs, 4.35 hrs, 9.87 hrs and 50.63 hrs with a corresponding LT_{90} of 16.32 hrs, 19.09 hrs, 63.86 hrs and 112.39 hrs. Mortality was found to increase with increase in exposure period and concentration. The responses of test insects were homogenous as indicated by the steep slope of probit lines. Based on the current results 90% mortality can be achieved at concentration 5% within 24 hrs exposure period. No significant recoveries were observed after 4 days following exposure and few non-significant cases of malformation were noticed. Further lines of research were suggested.

Keywords Garlic, Fumigation, *Callosobruchus maculatus* (F.), Sudan

1. Introduction

Cowpeas (black eye peas, or simply beans in many parts of Africa), *Vigna unguilata,* are widely grown in the tropics and sub tropics for human and animal food. Nigeria, Brazil, and Niger are among the major producers and account for over 70% of the world production [1]. Cowpeas constitute the cheapest source of dietary protein and energy for most poor people in the tropical world [2]. The production of pulses and grain in the Sudan was faced by two problems; insect and disease. Significant crop losses could occur in field and storage. The post-harvest losses were estimated at 5-10 % [3]. These losses vary according to the geographical location and climatic condition, from region to another within the country, where it varies from 5% in the north to 20% in the south [4]. The majority of these losses are due to insect infestation [5]. The losses can be direct, loss in weight, or indirect such as reduction in quality of stored product. In contrast to field crops, stored products cannot compensate losses, further the treatment of storage pests with chemical pesticides deserved special consideration and precautionary measures should be taken to protect consumer safety. Store products represent a ready form of human and/or animal food, therefore, only safe chemicals are allowed for use in such product. Biological control, even if effective, is not practiced because of consumer rejection to the presence of insects or pathogens even though they are useful. The above mentioned problems call for urgent, effective and safe alternatives for control of stored product pests. There are many organic compounds of plant origin that has been identified to affect pest population in different ways. They are cheap, of low mammalian toxicity, and are environment friendly. Generally a number of plants product, e.g. oils, powders, ashes, and others, are commonly used by traditional farmers in villages to protect cowpeas from damage in storage [6]. They include; garlic oils, black pepper, lemon oil, palm oil, soybean oil, citrus peels, and activated kaolin. Neem extract was reported effective against beetles [7,8,9]. In Sudan many natural products were reported to have promising potential against store pests; they include Neem, hargal, usher, rehan 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. Garlic was reported to have antioxidant activities. Its activity is due to the biologically active lipophilic sulfur-bearing compounds like; allicin, S-allyl-cysteine (SAC), dially-di-sulfide (DADS) and dially L-sulfide (DAS) [10,11]. Garlic have anti-arsenic activities [12], anti-bacterial activity, anti-cancer, anti-helminthic, anti-protozoan properties [13], hypoglycemic action [14], and hypocholesterolemic effect [15]. Its activity as a pest control agent was reported against mosquito larvae [16], whiteflies, butter flies, cabbage aphids, Colorado beetle [17], lesser grain borer [18], red flour beetle [19] and *Callosobruchus maculatus* on eggs[20]. In the Sudan previous studies [21,9,22,23,24], indicated the promising potential of volatile garlic oils against Sudanese species of *Rhyzopertha dominica*, *Tribolium castaneum*, *C. maculatus* and *B. incarnates*. These promising results, initiated our interest to further develop the method of extraction and bioassay using simple extraction procedure with the cheapest solvent system (water) together more detailed evaluation of the effect of the exposure period on the efficacy of the fumigation. *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) was used for bioassay.

2. Materials and Methods

2.1. Rearing of Test Insects

The test insect *Callosobruchus maculatus* (adult) was collected from local market (Omdurman crops market). The collected insects were placed in three glass jars (capacity 3 kg), half filled with the media (cowpea seeds) to allow flight space for adults. Jars were covered with muslin cloth fitted with rubber bands [25]. Four weeks later the culture was sieved. Sieving was done 24 hrs prior to the test. The old adults were removed and newly emerged adult (0-24 hrs old) were collected and used for the bioassay.

2.2. Media Preparation

The cowpea seeds, variety black eye, were sterilized at 120ºC for fifteen minutes in an oven to eradicate any infestation present [26]. The sterilized cowpea seeds were kept at room temperature for bioassay.

2.3. Garlic Preparation

Garlic (Chinese cultivar) was purchased from the local market. The cloves were peeled, sliced into small pieces (to increase the surface area for drying), placed spread under shade in dark room for 21days in the laboratory, during the period from November to December 2008. The dried slices were milled manually using pestle and mortar. The obtained powder was sieved through 25 mesh screen, stored in tightly closed jars wrapped with Aluminum foil and kept in the lab at room temperature.

Four hundred grams of garlic powder were placed in one liter conical flask, and then 400 ml of ethyl acetate water solution (5:95, v/v) were added. The content was thoroughly shaken manually 4 times, 5 minute each, and then filtered through fine muslin cloth. The obtained extract was kept at room temperature for bioassay.

2.4. Bioassay

Fumigation chambers (16× 16× 16 cm) with sliding doors [9] were used. The effect of various exposure periods; 6, 12, 24, 48, 72 and 96 hrs were evaluated in plastic cups using four concentration of garlic aqueous extract (0.1%, 1%, 5% and 10%; water ethyl acetate solution 95:5, v/v). The experiment was terminated by the fourth day because previous results [9] indicated that test insect can reach 100% mortality within this period. Ten milliliter from each concentration were placed in Petri-dishes. Dishes were then placed open into the fumigation chamber and the chambers were firmly closed. Sets of ten insect of *C. maculates* (adults) were collected and each was placed in muslin cloth covered plastic cup (capacity 200 ml), each containing 30 gram of cowpea seeds. Infested cups were introduced into the fumigation chamber and the chambers were quickly closed with sliding doors. Three control chambers containing dishes with either 10 ml ethyl acetate: distilled water (5:95 v/v), distilled water only or nothing was included. The fumigation chambers were left in laboratory at room temperature, and the procedure continued up to 96 hrs. Observations of mortality, malformation were recorded every 6, 12, 24, 48, 72, and 96 hrs. Recoveries were watched for four days on natural diet. The experimental units were arranged in factorial design and each treatment was replicated three times. The collected data were subjected to Arc sine transformation prior to the analysis. The data was analyzed by factorial Analysis of variance described by [27]. Means were separated using least significant difference (LSD). Probit analysis [28] was done using MINITAB software version 13.20. Control mortality was corrected by Abbott's correction formula [29] as follows:

Pt= (Po-Pc/100-Pc) ×100 where

Pt= percentage of control mortality

Po= observed mortality

Pc= control mortality (all in %)

3. Results

The results in table 1 and figures 1-6 indicated that, all exposure periods (6-96 hrs) to volatile fumes of garlic aqueous extract caused significant mortality to *Callosobruchus maculatus* as compared to the control. Effects were dose and time (exposure period) dependent with average percentage mortality caused by the concentrations 0.1 % range from 34% after 6 hours exposure to 94% after 72 hours. The respective maximum and minimum values for the 72 hours period were 93% and 96% (table 1). On the other hand effects caused by the concentration 5% following 24 hours continuous exposure can reach 94% with a range 61-100% (table 1). The control sets did not exceed 19.3% even after 96 hours of continuous exposure with a range 3.5%-19.3% (table 1).

Table 1. Effects of various exposure periods to garlic aqueous extracts on the mortality of *Callosobruchus maculatus*

Exposure period (hrs)	Mortality (%)	Controls			Concentration of garlic extrcat (%)			
		Untreated	Treated with solvent	Treated with distilled water	0.1	1	5	10
6	Average	(8.94)^{b} 17.25	(5.80)^{b} 13.92	(4.29)^{b} 10.08	(33.60)^{a} 38.13	(46.00)^{a} 42.67	(44.80)^{a} 41.03	(52.30)^{a} 46.33
	Minimum	(0.57)^{a} 0.01	(0.57)^{a} 0.10	(0.57)^{a} 0.10	(8.80)^{a} 17.25	(16.80)^{a} 24.18	(13.10)^{a} 21.15	(19.3)^{a} 26.07
	Maximum	(13.10)^{b} 21.15	(13.10^{b} 21.15	(11.70)^{b} 19.95	(73.50)^{a} 59.01	(87.1)^{a} 68.85	(93.30)^{a} 75	(93.3)^{a} 75
SE±		3.68	3.63	3.27	18.8	20.4	23.3	21.4
12	Average	(4.68)^{b} 9.27	(1.81)^{b} 6.54	(3.54)^{b} 8.24	(41.08)^{a} 39.53	(41.84)^{a} 39.96	(48.12)^{a} 43.93	(63.40)^{a} 59.43
	Minimum	(0.57)^{b} 0.01	(0.57)^{b} 0.10	(0.57)^{b} 0.1	(13.07)^{b} 21.38	(44.50)^{a} 41.83	(41.20)^{a} 39.80	(63.30)^{a} 52.78
	Maximum	(10.00)^{b} 18.44	(6.70)^{b} 15.03	(16.4)^{b} 23.85	(57.4)^{a} 49.22	(88.4)^{a} 70.08	(98.9)^{a} 83.85	(98.9)^{a} 83.85
SE±		2.73	1.87	4.86	12.9	15.1	18.2	11.9
24	Average	(7.07)^{c} 12.15	(4.18)^{c} 9.09	(9.54)^{c} 17.14	(46.10)^{b} 42.75	(49.23)^{b} 44.53	(94.00)^{a} 75.86	(96.60)^{a} 79.23
	Minimum	(0.57)^{b} 0.01	(0.57)^{b} 0.01	(0.57)^{b} 0.01	(19.30)^{b} 26.07	(26.5)^{b} 30.99	(60.70)^{a} 51.15	(77.9)^{a} 61.92
	Maximum	(16.40)^{b} 23.85	(13.10^{b} 21.15	(16.4)^{b} 23.85	(80.7)^{a} 63.93	(93.3)^{a} 75	(100)^{a} 90	(100)^{a} 90
SE±		4.59	3.72	4.58	17.8	19.6	12.2	6.87
48	Average	(7.75)^{b} 15.04	(7.15)^{b} 13.68	(6.70)^{b} 15.03	(74.50)^{a} 59.65	(83.90)^{a} 66.35	(80.40)^{a} 67.89	(91.00)^{a} 72.54
	Minimum	(2.40)^{b} 8.92	(1.20)^{b} 6.21	(6.70) ^{b} 15.03	(53.50)^{a} 47.01	(56.79)^{a} 48.85	(62.00)^{a} 51.93	(67.02)^{a} 55.08
	Maximum	(13.10)^{b} 21.15	(13.10^{b} 21.15	(6.70)^{b} 15.03	(90.70)^{a} 72.29	(98.90)^{a} 81.15	(100)^{a} 90	(100)^{a} 90
SE±		3.09	3.44	0.0	10.8	12.3	11	9.84
72	Average	(19.30)^{b} 26.07	(15.70^{b} 23.36	(17.80)^{b} 24.96	(94.40)^{a} 76.35	(97.60)^{a} 81.15	(100)^{a} 90	(100)^{a} 90
	Minimum	(19.30)^{b} 26.07	(15.70^{b} 23.36	(16.30)^{b} 23.85	(93.30)^{a} 75.00	(97.60)^{a} 81.15	(100)^{a} 90	(100)^{a} 90
	Maximum	(19.30)^{b} 26.07	(16.30^{b} 23.85	(19.30)^{b} 26.07	(95.50)^{a} 77.71	(97.60)^{a} 81.15	(100)^{a} 90	(100)^{a} 90
SE±		0.0	0.2	0.866	0.635	0.0	0.0	0.0
96	Average	(16.40)^{b} 23.85	(15.70^{b} 23.36	(19.30)^{b} 26.07	(91.30)^{a} 72.78	(95.50)^{a} 77.71	(100)^{a} 90	(100)^{a} 90
	Minimum	(16.40)^{b} 23.85	(15.70^{b} 23.36	(19.30)^{b} 26.07	(91.30)^{a} 72.78	(95.50)^{a} 77.71	(100)^{a} 90	(100)^{a} 90
	Maximum	(16.40)^{b} 23.85	(15.70^{b} 23.36	(19.30)^{b} 26.07	(91.30)^{a} 72.78	(95.50)^{a} 77.71	(100)^{a} 90	(100)^{a} 90
SE±		0.0	0.0	0.0	0.0	0.0	0.0	0.0

Values in parenthesis represent the transformed data (by arc sine).

LSD= Least significant difference.

SE± = Standard error.

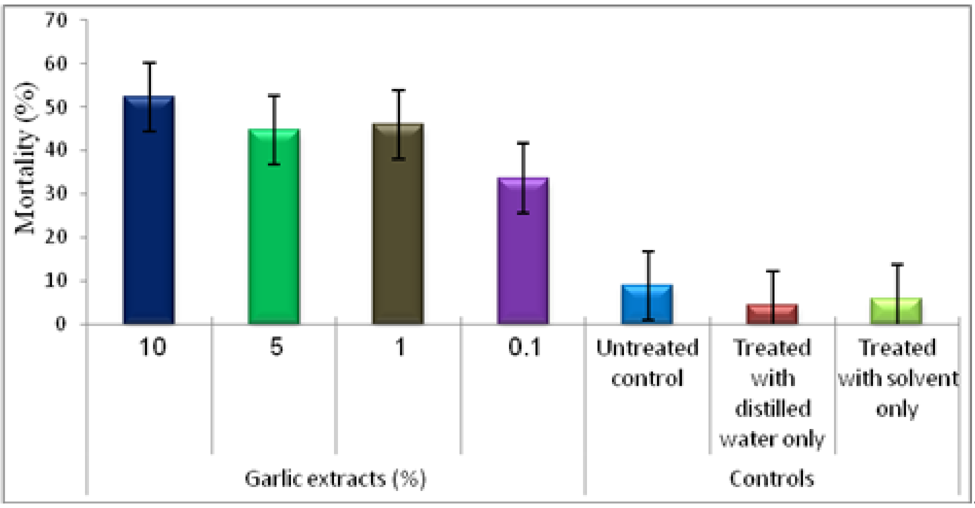

Figure 1. Average mortality of *Callosobruchus macul*atus caused by 6 hrs fumigation with garlic aqueous extract, Vertical bars represent the standard error

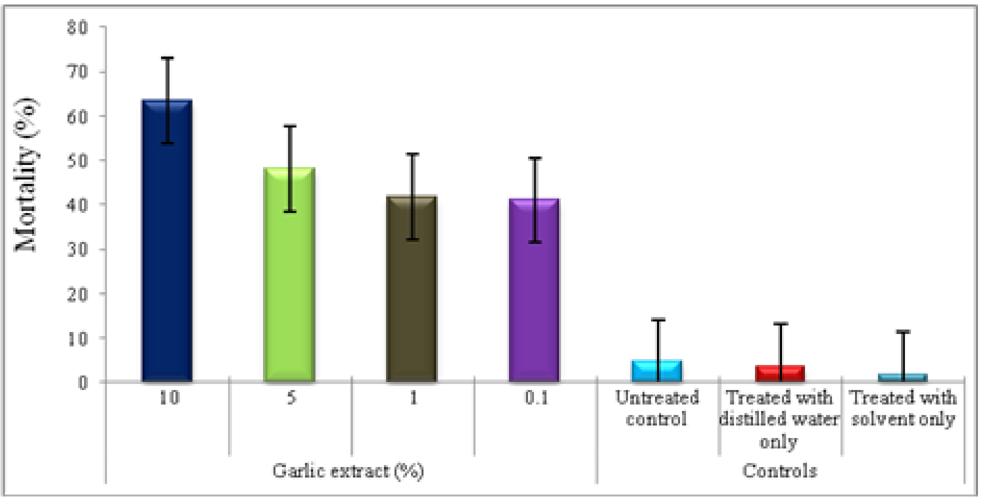

Figure 2. Average mortality of *Callosobruchus macul*atus caused by 12 hrs fumigation with garlic aqueous extract, Vertical bars represent the standard error

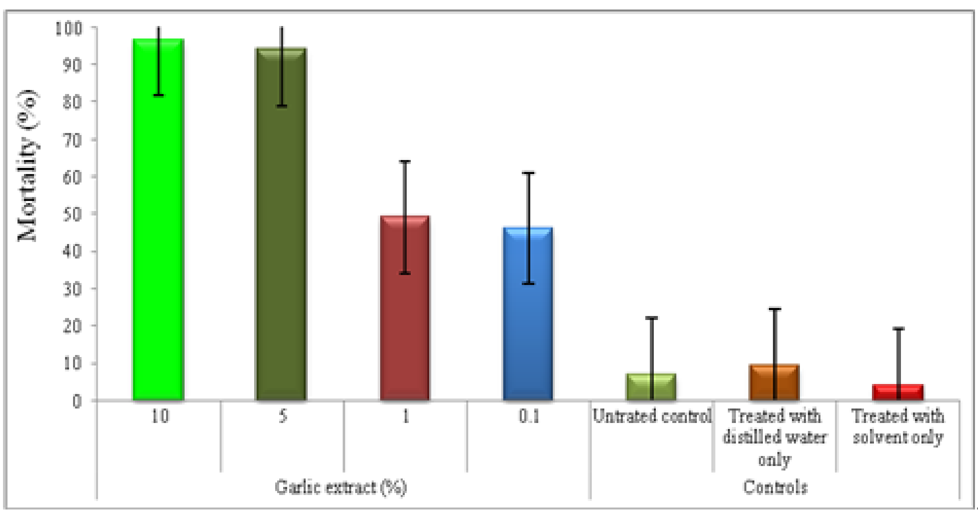

Figure 3. Average mortality of *Callosobruchus macul*atus caused by 24 hrs fumigation with garlic aqueous extract, Vertical bars represent the standard error

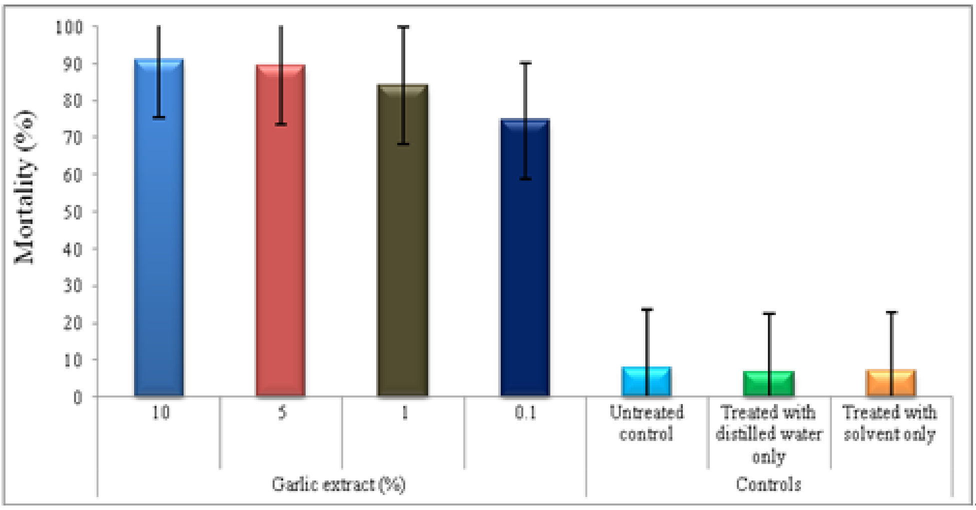

Figure 4. Average mortality of *Callosobruchus macul*atus caused by 48 hrs fumigation with garlic aqueous extract, Vertical bars represent the standard error

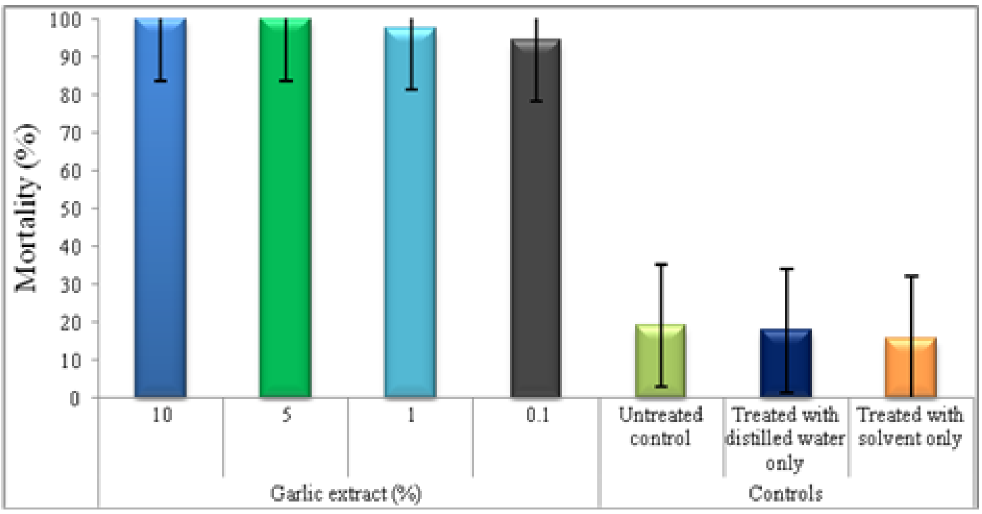

Figure 5. Average mortality of *Callosobruchus macul*atus caused by 72 hrs fumigation with garlic aqueous extract, Vertical bars represent the standard error

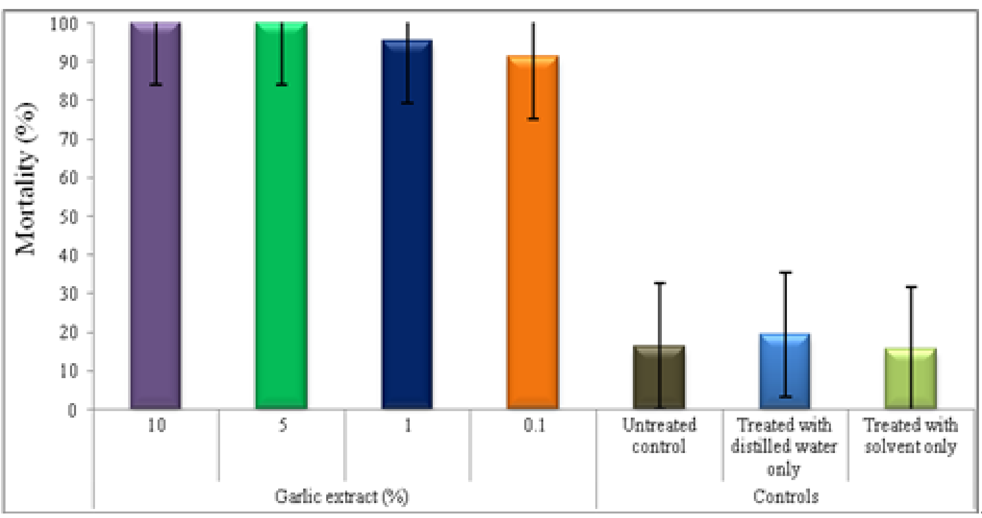

Figure 6. Average mortality of *Callosobruchus macul*atus caused by 96 hrs fumigation with garlic aqueous extract ,Vertical bars represent the standard error

3.1. Probit Analysis

Data of various exposure periods showing uniform responses were subject to probit analysis. Based on that probit analysis was not possible for the 96 hours exposure data as it did not show a uniform response covering the entire dose range. Results were summarized in the following subtitles:

3.1.1. Responses as Related to Dose

Table 2 and figures 7-11 showed the log-dose responses of *Callosobruchus maculatus* exposed to garlic aqueous extract. Result indicated that, the test insect is sensitive to garlic extract as indicated by it is low LD_{50} ranging between 0.351% for the six hours exposure period to 0.059% for the 72 hours exposure period. The corresponding LD_{90} values ranges from 14.9% to 1.20%. The slopes of the mortality regression line were steep and positive indicating the homogeneity of the population or fast action of garlic aqueous extract. Chi-square value was small (0.54-3031).

Table 2. Log-dose probit responses of *Callosobruchus maculatus* (adult) exposed to fumigant action of garlic aqueous extract for different exposure periods in plastic cups

Times (hours)	Lethal doses (%)		Fudicial limits		LD90/LD50 Ratio	χ2	Slope	df
			Lower	Upper				
6	LD_{10}	8×10^{-2}	2.18	118.0	42.51	0.54	1.27	**2**
	LD_{50}	0.35	0.0	1.38				
	LD_{90}	14.92	3.45	2065.63				
12	LD_{10}	0.27	2×10^{-2}	0.87	5.23	3.31	0.56	**2**
	LD_{50}	1.41	0.19	3.33				
	LD_{90}	7.41	3.16	68.59				
24	LD_{10}	0.04	0.0	0.18	6.31	0.87	0.62	**2**
	LD_{50}	0.26	0.006	0.68				
	LD_{90}	1.66	0.62	17.36				
48	LD_{10}	3×10^{-2}	0.0	0.06	34.01	0.68	1.19	2
	LD_{50}	0.088	1×10^{-2}	0.04				
	LD_{90}	2.99	0.54	4.937				
72	LD_{10}	2×10^{-2}	0.0	0.05	20.36	1.49	1.02	2
	LD_{50}	0.059	0.0	0.31				
	LD_{90}	1.20	0.15	15365				

χ^2 ≡ Chi-square
DF ≡Degree of freedom
LD_{10} ≡ Lethal dose that induce 10% mortality in a test population
LD_{50}≡ Lethal dose that induce 50% mortality in a test population
LD_{90} ≡Lethal dose that induce 90% mortality in a test population

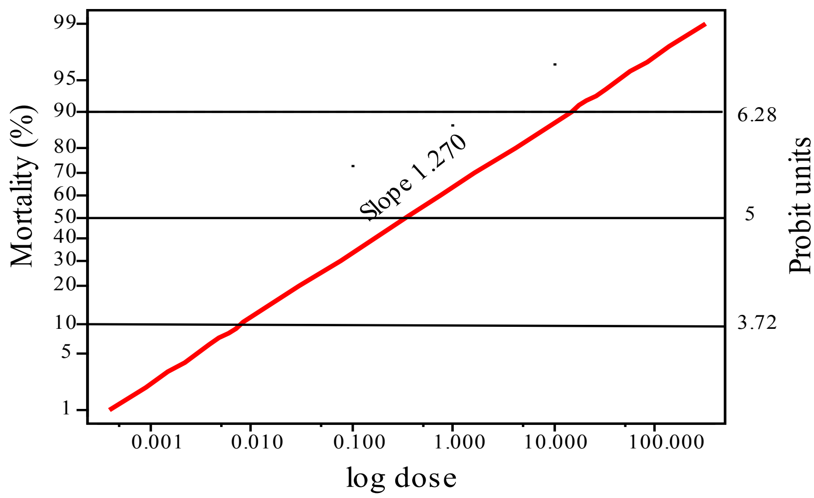

Figure 7. Log dose probit line of 6 hours response of *Callosobruchus maculatus* exposed to the fumigant action of garlic aqueous extract

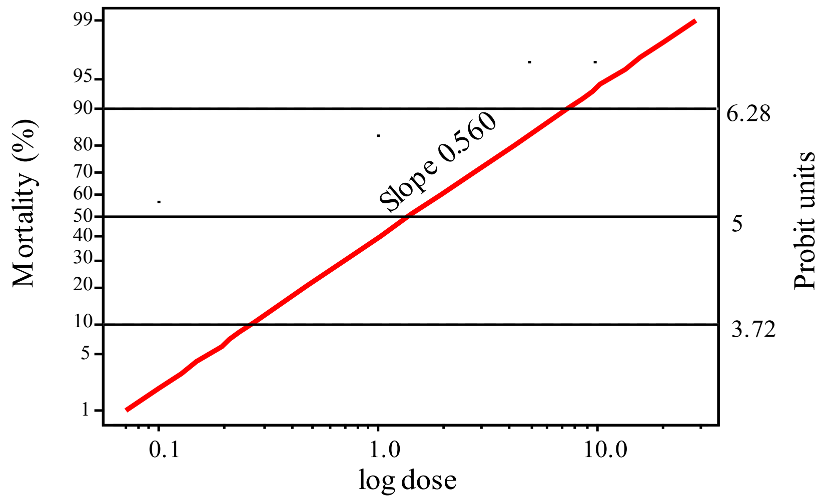

Figure 8. Log dose probit line of 12 hours response of *Callosobruchus maculatus* exposed to the fumigant action of garlic aqueous extract

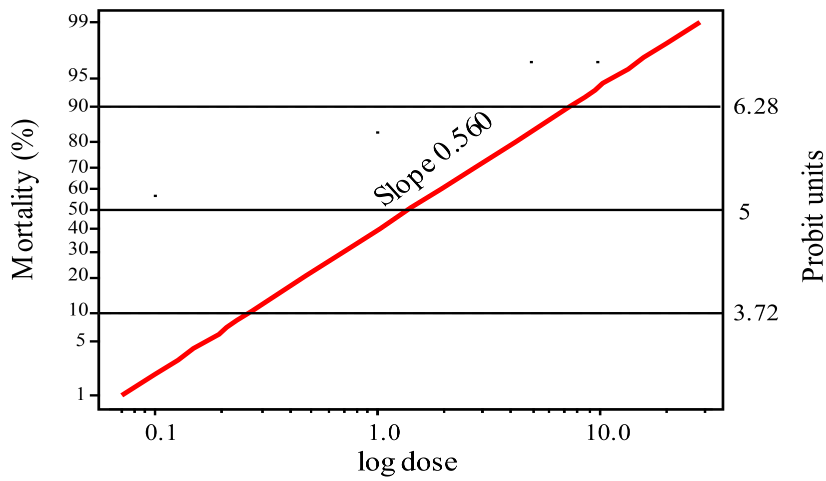

Figure 9. Log dose probit line of 24 hours response of *Callosobruchus maculatus* exposed to the fumigant action of garlic aqueous extract

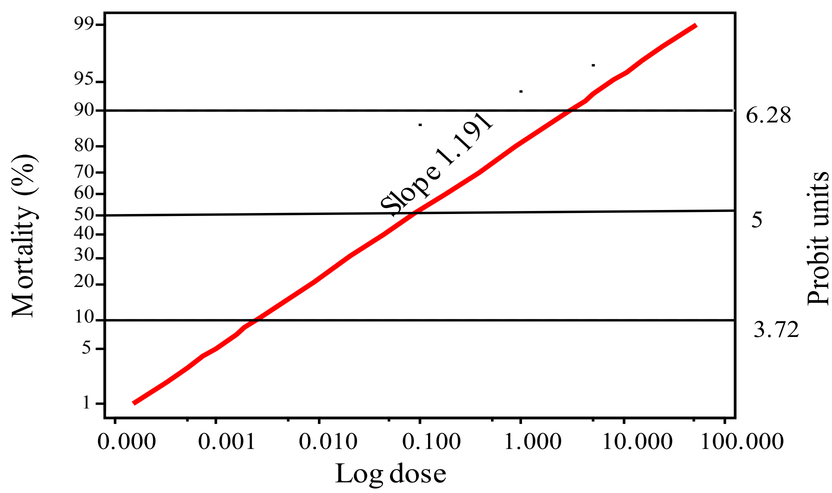

Figure 10. Log dose probit line of 48 hours response of *Callosobruchus maculatus* exposed to the fumigant action of garlic aqueous extract

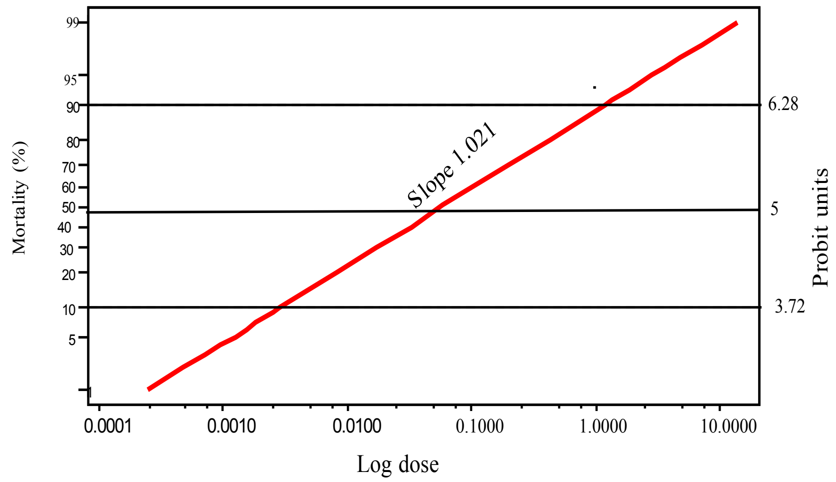

Figure 11. Log dose probit line of 72 hours response of *Callosobruchus maculatus* exposed to the fumigant action of garlic aqueous extract

3.1.2. Responses as Related to Time

Log-time probit responses of *Callosobruchus maculatus* (adult) were given in table 3 and figures 12-15. The results showed that, the test insect was sensitive to fumigant action of garlic aqueous extract at the various doses tested, as indicated by the low LT_{50} ranging from 3.56 hours at the highest dose (10%) to 50.68 hours at the lowest dose tested (0.1%). The respective values for LT_{90} range from 16.32 hours to 112.39 hours. The slopes of the regression lines were steep and positive indicating good homogeneity of the test population. The latter is also indicated by the narrow LT_{90}/LT_{50} ratio. Chi-square value is small..

Table 3. Log-time probit response of *Callosobruchus maculatus* (adult) exposed to the fumigant action of garlic aqueous extracts at different concentrations in plastic cups

Doses (%)	Lethal times (hrs)		Fudicial limits		LT_{50}/LT_{90} ratio	χ^2	Slope	df
			Lower	Upper				
10	LT_{10}	0.78	0.0	3.40	4.57	0.90	0.515	3
	LT_{50}	3.56	1×10^{-2}	8.189				
	LT_{90}	16.32	5.56	959662				
5	LT_{10}	0.99	0.0	3.84	4.38	0.60	0.50	3
	LT_{50}	4.35	1×10^{-2}	9.29				
	LT_{90}	19.09	8.68	3064.06				
1	LT_{10}	1.52	0.0	6.16	6.46	2.21	0.63	3
	LT_{50}	9.87	2×10^{-2}	20.39				
	LT_{90}	63.86	30.13	787713				
0.1	LT_{10}	22.85	2×10^{-2}	38.93	2.22	3.90	0.27	3
	LT_{50}	50.68	2.97	159.31				
	LT_{90}	112.39	68.41	356760				

χ^2 ≡ Chi-square

DF ≡Degree of freedom

LT_{10} ≡ Lethal time that induce 10% mortality in a test population

LL_{50}≡ Lethal time that induce 50% mortality in a test population

LT_{90} ≡Lethal time that induce 90% mortality in a test population

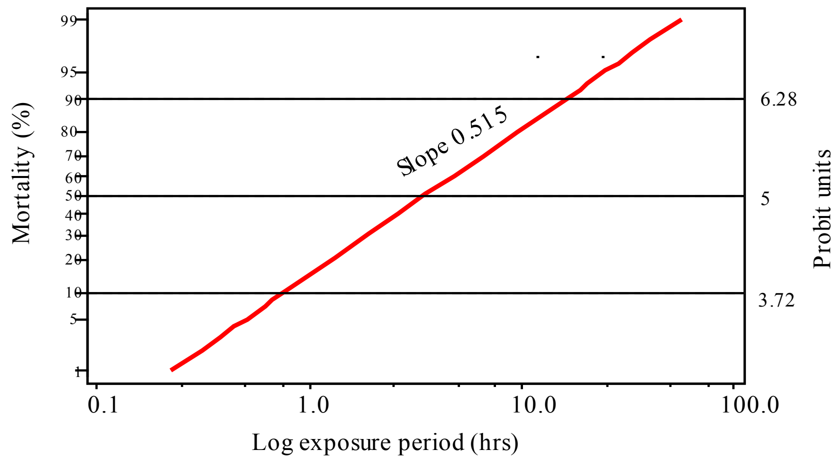

Figure 12. Log time-probit line of *Callosobruchus maculatus* exposed to fumigant action of garlic aqueous extract (10%)

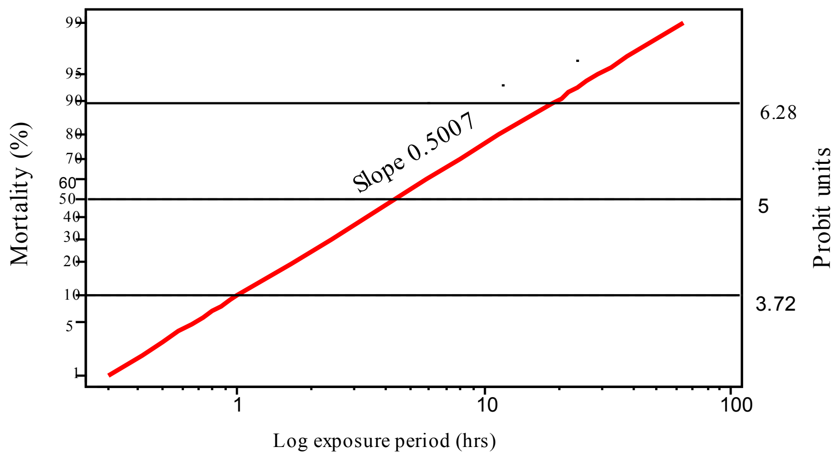

Figure 13. Log time-probit of *Callosobruchus maculatus* exposed to fumigant action of garlic aqueous extract (5%).

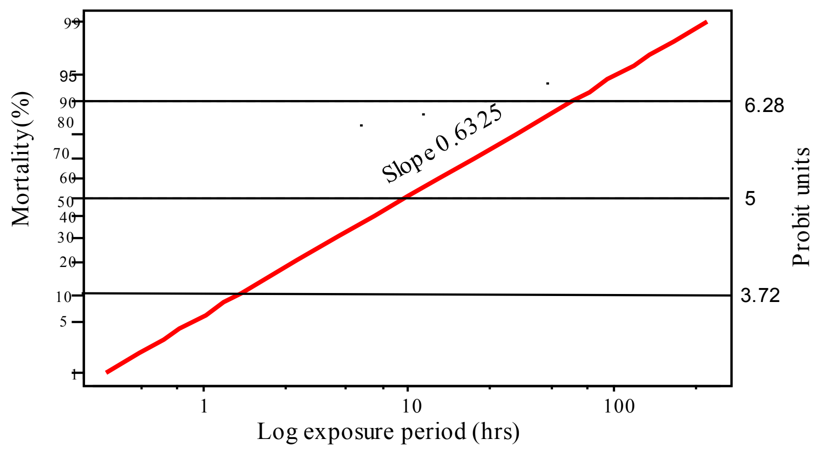

Figure 14. Log time-probit of *Callosobruchus maculatus* exposed to the fumigant action of garlic aqueous extract (1%)

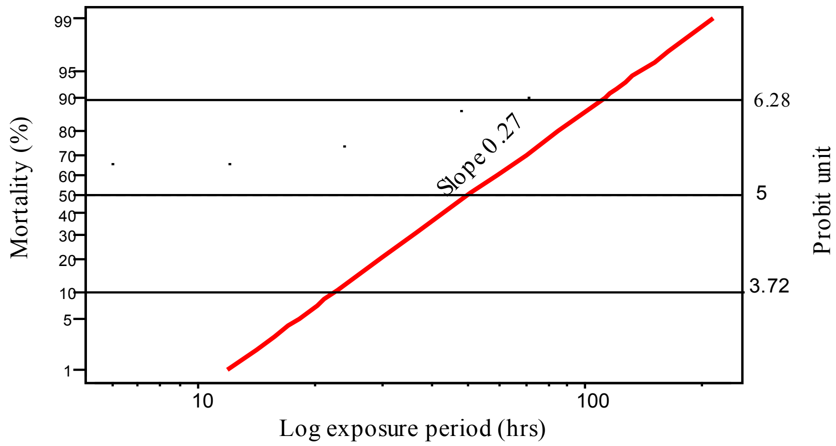

Figure 15. Log time-probit of *Callosobruchus maculatus* exposed to the fumigant action of garlic aqueous extract (0.1%)

4. Discussion

The increasing problems of pesticides especially those associated with the large-scale use of broad spectrum synthetic pesticides have directed the need for effective, biodegradable pesticides with greater selectivity. This awareness has created a worldwide interest in the development of alternative strategies, including the discovery of new types of insecticides [30]. This has led to re-examination of the century-old practices of protecting stored products using plant derivatives, which have been known to resist insect attack [31]. Plant-derived materials are more readily biodegradable, less likely contaminate the environment and less toxic to mammals.

In Sudan and other countries the previous investigations [21]; [9], [20], [22], [23] and[24] indicated the promising potential of garlic products in the control of stored product pests. These results indicated the superior sensitivity of Bruchids to various types of garlic essential oils. These previous finding were based on either readymade oils or steam distilled oils which requires a complicated process for extraction. Further the effect of the fumigation period was not examined. Stemming from this the current study aimed at the evaluation of the effects of various exposure periods on the efficacy of garlic aqueous extract, the extract was based on the simple and cheap solvent system (water). Result indicated that, the test insects were sensitive to garlic aqueous extract and the mortality can reach 94.4% after 72 hours following exposure to the lowest concentration tested (0.1%). The highest concentration (10%) and the intermediate concentration (5% and 1%) can cause 100% mortality within shorter periods; 24, and 48 hours respectively. The test also showed no significant recoveries following the various exposure periods. Generally, the comparison of efficacies of various exposure periods to garlic aqueous extract showed that, The 72 hrs exposure to the highest dose (10%) caused an average mortality ranging from 94%-100%, while shortening the exposure period to 48 hrs can cause at least 80% mortality, even at the lowest concentration tested (0.1%) compared to the controls which showed no more than 13.10% non significant mortality. The effects were dose and time related.

Data showing uniform responses were further subject to probit analysis. The results confirmed sensitivity of *Callosobruchus maculatus* as indicated by the low LD_{50} values and the steep slopes of regression lines. Effects were dose and time (exposure periods) dependent. These findings agrees with the previous results [21];[9],[22], [23] and[24], who tested the efficacy of steam distilled and/or readymade extracts from various garlic cultivars against *Callosobruchus maculatus* and other storage pests. The probit analysis of data obtained from the current experiments confirmed that the increase in time (exposure periods) increases the efficacy (decrease LD_{50} and LD_{90} values) and by the way their ratios as well as the slope of mortality regression lines which became more steeper indicating improvement in the homogeneity of responses. These findings were in line with the results of [21];[19];[9], [22],[23] and [24], who reported that increasing the pre-accumulation or exposure periods results in better efficacy.

In Sudan the knock-down and recoveries among garlic fumigated insects was first reported by[21] and [9]. The current results indicated that test insects did not recovered after a watching period of four days. These findings agree with[9] and [22], who reported that *C. maculatus* (Adult) did not recovered after four and five days respectively. The significance of watching recoveries of poisoned or treated pests is essential for better reading of the end-point mortality as reported by[26] and[32].

The results of time related responses showed that *Callosobruchus maculatus* was sensitive to garlic aqueous extract as indicated by it is low LT_{90} (14.92, 7.41, 1.66, 2.99, 1.20 hours) and LT_{50} values (3.56, 4.35, 9.87, 50.63 hours). Increasing the concentration of garlic aqueous extract increased the slope of regression lines and caused progressive improvement of homogeneity of responses. Chi-square value was small in both dose and time related responses.

The current results agree with the previous finding that vapor of garlic extracts can penetrate muslin cloth cover and cause significant mortalities to test insect inside plastic cups

[9, 21, 22, 23, 24]. The efficacy of the fumigant action of this extract in infested jute sacs deserves examination in future work.

5. Conclusion

Result indicated that garlic aqueous extract is an effective fumigant that can be further explored for the control of bruchids specially *Collosobruchus maculatus* (Adult).

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