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Toxicity and reproduction inhibitory effects of some monoterpenes against the cowpea weevil *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae: Bruchinae)

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ABSTRACT

The present investigation was carried out to determine the fumigant and admixing toxicity of four monoterpenoid compounds namely eugenol, isoeugenol, carvacrol and thymol against the cowpea weevil *Callosobruchus maculatus* (F). Biological characteristics such as number of deposited eggs, number and percentage of hatched eggs, number of emerged adults and progeny reduction percentage were determined after treatments with monoterpenes. Finally, the loss percentage of seeds was calculated after the emergence of the first generation. Fumigant toxicity of eugenol and carvacrol presented the highest toxice effect with LC_{50} values of 34.58 and 37.34 mg/l air, respectively after 72h exposure time, while thymol achieved moderate toxicity with LC_{50} values of 45.32 mg/l air. For the second method, admixing with cowpea seeds test, thymol and carvacrol achieved the highest toxicity values with LC_{50} of 0.46 and 0.53 mg/g seeds after one week of exposure time, followed by eugenol (1.75 mg/g). The data indicated that isoeugenol was the lowest effective compound. Also, thymol detects the highest efficacy when it was admixed with seeds than fumigant. In contrast, eugenol achieved the high activity as fumigant rather than admixing method. Finally, isoeugenol had the lowest insecticidal activity when it was evaluated by two tested methods. The data indicated that with the increase of monoterpenes concentrations, the values of biological parameters and losses percentage decreased which, mean high significant effect of these monoterpenes against *C. maculatus.* According to these results, monoterpenes could be better than other insecticides in controlling of stored product insects.

Keywords: Monoterpenes, Cowpea weevil, Fumigant, Admixing, Biology, Losses

Introduction

Cowpea *Vignaun guiculata* (L.) is one of the most economic legume seeds, with high source of protein, vitamins and minerals (Singh *et al*., 2003). Cowpea weevil *Callosobruchus maculatus* (F) is considered as one of the most devastating bruchid species to several legumes including cowpea in tropic and sub tropic regions in the world wied (Babarinde *et al*., 2016).The larval and pupal stages develop inside the seeds.The larval bore into the seed and eat up the endosperms (Adenekan *et al*., 2018). It damages 25%, of seeds consequeutly reduces germination up to 13% and reduces market value after storage for 6 months (Haile, 2006). The insect begins infested mature dry pods in the field (Huignard *et al*., 1985). Conventional insecticides such as organophosphates, pyrethroids, carbamates caused inconvenience of environment, insect resistance and lethal effects on non-target organisms and direct toxicity to users (Abo-El-Saad *et al*., 2011). Synthetic fumigants such as methyl bromide or phosphine are still one of the most effective methods for the control of stored product pests

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(Manzoomi *et al*., 2010; El Nagar *et al*., 2012), however, their hazards effects causing many environmental problems like ozone depletion, environmental pollution, toxicity to non−target organisms (Isman*,* 2006). Based on the insect resistant strains to phosphine in Africa and Asia, it should endeavor to find alternatives friendly chemicals and one of these are monotrepenes (Bell and Wilson, 1995). Plant essential oils widely used flavors in the food industries so most of essential oil relatively has low mammalian toxicity (Koul *et al*., 2008). Monoterpenes, are the main component of essential oils that were shown to have insecticidal activity. Their components are highly volatile strongly toxic to insect pests. Monoterpenes were reported to possess acute contact and fumigant toxicity (Abdelgaleil *et al*., 2009), antifeedant effect (Hummelbrunner and Isman, 2001), growth inhibitory effects (Gunderson *et al*., 1985) and repellent activity against insects-pest (Ogendo *et al.,* 2008). Phenolic monoterpenes are characterized by the presence of a hydroxyl ($^{-}$OH) group, attached to a benzene ring or other complex aromatic ring structures (such as thymol and eugenol). They range from simple phenol to polyphenols such as flavonoids and tannins (Pavela, 2011). Current research objectives were to evaluate the phenolic monotrepenes toxicity as admixing and fumigant materials against the Cowpea weevil, *C. maculatus* moreover, the present study involved the evaluation of these monoterpenes and their inhibitory effect on progeny and seeds loss.

Materials and Methods

Chemicals

Four essential oil monoterpenes, eugenol (99%), isoeugenol (98%), carvacrol (98%) and thymol (98.5%) were purchased from Sigma–Aldrich Chemical Co. (St Louis, MO, USA) and were evaluated in the present study against the cowpea weevil.

Insect culture

Cowpea weevil was reared under laboratory condition (27±1°C and 65±5% R.H) using sterilized cowpea seeds, in 1-liter glass jars, which were covered by fine mesh cloth for ventilation (Chakraborty and Mondal, 2015). Adult insects used in toxicity tests were about 1-3 days old. All experimental procedures were carried out under the same conditions as culture.

Fumigation toxicity bioassay

Vapour toxicity of the four evaluated monoterpenes against the adults of *C. maculatus* was investigate by transferring twenty adultsinto glass jars (250ml) containing 10g of cowpea seeds and exposed them to vapors with different doses of monoterpenes dissolved in (100µl) acetone and applied to filter paper (9cm diameter). The treated filter papers attached to the inner surface of screw lid of the jar using adhesive tape, which were made air-tight, after allowing the solvent evaporate for 5 minutes. Control jars were treated with acetone alone. All treatment and control were replicated three times (Qi and Burkholdes, 1981; Broussalis *et al*., 1999). Mortality percentage (M %) were determined after 24, 48, 72 hours and LC_{50} values were calculated according to Finny (1971).

Admixing monoterpenes with cowpea seeds

Sterilized cowpea seeds were admixed with the evaluated monoterpenes according to Su *et al.* (1972). Seeds (10g, each) were mixed manually in glass jars (250ml) with different concentrations of these compounds after being dissolved in acetone (100µl). The treated seeds were infested with newly emerged adults (10 pairs) after acetone evaporation (5 minutes). Mortality percentages (M %) was recorded after one week. Three replicates were done in each treatment and check (control). LC_{50} values were calculated according to Finney (1971) and the mean numbers of deposited eggs on cowpea seeds after two weeks of treatments, hatched eggs, hatchability percentage and emerged adults for the 1st generations were counted. Loss of seeds weight was also assessed.

Biological parameters

The numbers of deposited , hatched eggs and hatchability percentage were counted after two weeks of treatments and when adult emergence began (27 days after laying eggs), the number of emerged adults was counted every day until the end of the emergence. Data on adult's emergence were used to determine the effect of fumigation and seeds admixing with monoterpenes on the fate of the deposited eggs. Adult reduction percentage was also calculated.

Loss of cowpea seeds weight as infested by *C. maculatus*

Weight losses of cowpea seeds were determined by weighing the seeds before adult's infestation and after the first-generation emergence and it was calculated according the equation of Khare and Johari (1984).

Results

Fumigant and admixing toxicity against *C. maculatus*

The toxicity of phenolic monoterpenes compounds vapors was tested against the cowpea weevil. The value of LC_{50}, LC_{90}, 95% confidence limits and slopes were estimated after 24, 48 and 72 hours after treatment as shown in Table (1). The results indicated that all the evaluated monoterpenes, eugenol, isoeugenol, carvacrol and thymol, showed fumigant activity against *C. maculatus* adults. Results observations showed that the toxicity of all compounds was increased by increasing the exposure time. Carvacrol was the most effective compound after 24h exposure time with LC_{50} of 44.53 mg/l air followed by thymol (LC_{50} =49.24 mg/l air), while isoeugenol recorded the lowest effect with LC_{50} =130 mg/l air. Insecticidal activity of eugenol and carvacrol showed the highest toxicity with LC_{50} values (34.58 and 37.34 mg/l air), respectively after 72h. Thymol also had relatively strong toxicity after 72h ($LC_{50}$of 45.32mg/l air). Nevertheless, eugenol was more toxic (LC_{50} = 44.97 and 34.58 mg/l air, respectively) than thymol (LC_{50} = 46.13 and 45.32 mg/l air, respectively) after 48 and 72h, while the results of isoeugenol possessed the lowest fumigant activity (LC_{50}=75.07 mg/l) after 72h bioassay.

Table 1: Fumigation toxicity of some monoterpenes against *C. maculatus* adults

Monoterpene	Time exposure (hrs)	LC_{50} mg/l air	Confidence limits	LC_{90} mg/l air	Confidence limits	Slope b ± S.E.	χ^2
Eugenol	24	71.81	66.78-78.16	164.92	139.29-209.65	3.55±0.33	8.41
	48	44.97	42.58-47.45	79.63	73.24-88.50	5.16±0.36	8.52
	72	34.58	31.05-38.10	101.28	82.32-139.69	2.75±0.33	7.40
Isoeugenol	24	130.19	100.8-199.7	1095.7	533.40-4313.61	1.39±0.22	2.57
	48	79.65	71.10-91.11	355.45	265.75-524.78	1.97±0.17	8.24
	72	75.07	50.16-66.24	335.65	236.77-557.64	1.66±0.17	10.72
Carvacrol	24	44.539	40.41-29.30	133.49	11.87-170.91	2.69±0.22	7.04
	48	39.40	33.32-49.95	172.02	111.61-363.52	2.00±0.28	2.64
	72	37.34	30.73 -47.85	334.85	183.95-1011.97	1.34±0.21	0.148
Thymol	24	49.24	44.57-55.02	170.34	135.37-234.10	2.38±0.21	6.80
	48	46.13	40.98-51.68	161.20	124.71-242.18	2.36±0.29	7.00
	72	45.32	42.87-47.93	57.96	68.81-87.32	5.71±0.56	1.96

Seeds admixing toxicity assays of phenolic monoterpenes on *C. maculatus* presented in Table (2) revealed that thymol and carvacrol had the highest toxicity with LC_{50} values of 0.46 and 0.53 mg/g. Results also indicated that there were no significant differences between thymol and carvacrol (overlap of confidence limits at the 95% level). Data clearly indicated that eugenol had moderate toxicity giving LC_{50} value of 1.75 mg/g, while isoeugenol was the lowest effective evaluated compound. The results of fumigation method are in the same trend as those of admixing monoterpenes with seeds as presented in Table (2) which showed that isoeugenol had weak insecticidal activity. In contrast, thymol had highest activity when admixed with seeds than it has used as a fumigant. Eugenol achieved the lowest activity when it was assayed by admixing method than fumigant, also, carvacrol showed moderate insecticidal activity with the two evaluated application methods.

Table 2: Toxicity of some monoterpene's compounds evaluated against *C. maculatus* adults by admixing with seeds assay

Monoterpenes	LC50 mg/g	Confidence limits	LC90 mg/g	Confidence limits	Slope b ± S.E.	χ^2
Eugenol	1.75	1.31-1.87	10.81	7.32-19.92	1.53±0.19	3.75
Isoeugenol	2.32	2.00-2.70	9.74	6.89-17.8	2.06±0.3	5.46
Carvacrol	0.53	0.42-0.64	4.09	2.94-6.50	1.45±0.14	6.22
Thymol	0.46	0.36-0.60	3.57	2.16-8.17	1.44±0.19	1.95

Fumigation with monoterpenes and their effect on *C. maculatus*

Data in Table (3) represent the effect of four monoterpenes; thymol, carvacrol, eugenol and isoeugenol prepared as five concentrations (10, 20, 30, 40 and 50 mg/l) and compared with control. Mortality percentage (M %) and other four biological characters of *C. maculatus* were determined during the current investigation. Concerning the M% after one-week exposure period at high concentration (50mg/l), it was ranged from 44.7±5.98% to 69.5±4.35% (isoeugenol) to 69.5±4.35% (thymol) with a mean of 57.1±5.16% comparing with control which was 10.0±2.30%. The mortality increased by increasing of monoterpenes concentrations. Analysis of variance showed that there were significant effects between isoeugenol and the other compounds, while data detect no significant variation between eugenol and carvacrol with $LSD_{0.05}$= 3.02, and between carvacrol and thymol at the high concentration. On the other hand, analysis of variation between concentrations showed significant effect with $LSD_{0.05}$ = 3.02.

The number of deposited eggs was determined by using 10 g of cowpea seeds that act as oviposition site for 20 adults of *C. maculatus.* Control indicated that the highest value of 188.0±4.61 eggs and vice versa relationship was detected between the monoterpenes concentrations and mean number of deposited eggs. No deposited eggs were maintained for thymol, carvacrol and eugenol at the high concentration, 50 mg/l, followed by 2.0±1.54 eggs recorded to isoeugenol under the same concentration. The mean number of deposited eggs was decreased using thymol (10 mg/l) (73.0±4.61 eggs) showing a reduction percentage of 61.1 as compared with control (188.0±4.61 eggs), while, with the carvacrol was 76.0±3.46 eggs with 64.4% reduction comparing with control. On the other hand, eugenol and isoeugenol showed high values of number of deposited eggs (174±3.46 and 160±5.77 eggs, respectively) and the reductions were 7.5 and 14.9%, respectively. At the lowest concentration of 10 mg/l. High significant variation were obtained between control and all the tested monoterpenes concentrations with $LSD_{0.05}$=3.05 and the data indicated that the mean number of deposited eggs was almost doubled using eugenol and isoeugenol comparing with thymol and carvacrol as shown in Table (3).

The effect of the tested monoterpenes on the number and the percentage of hatched eggs and number of emerged adults is illustrated in Table (3). The treatment of thymol showed decrease of these characters with the increase of concentrations 10 mg/l. The number of hatched eggs and number of emerged adults were58.0±7.50 and 42.0±3.70, respectively, while at the highest concentration of 50 mg/l, number of hatched eggs and number of emerged adults were zero due to the absence of number of deposited eggs. When comparing carvacrol with thymol, the data showed decrease in both values nearly half and almost 25% of control. Eugenol showed the highest values for both characters comparing with other monoterpenes (130±5.77 eggs and 86.0±1.15 adults) at the concentration of 10 mg/l and these values decreased to 0.0 at 50 mg/l for both characters.

Finally, for isoeugenol, the concentration of 10 mg/l showed 77.0±4.61 eggs and 31±2.30 adults and all the values decreased with the increase of concentration. The data showed that the lowest mean number of emerged adults at 10 mg/l was 31±2.30 when treated with isoeugenol and the highest value was 86.0±1.5 using eugenol. High significant variations were observed between the monoterpenes and their different concentrations in both biological characters. Based on the fumigation method using four monoterpenes at five different concentrations, the reduction percentage recorded in Table (3) ranged from 26.5±0.98% (10 mg/l, eugenol) to 100% (50 mg/L) for all monoterpene compounds. Carvacrol recorded highest reduction (100%) at 40 and 50 mg/l. Eugenol showed 26.5±0.98 at 10 mg/l as minimum value 100% at 50 mg/l. Finally, isoeugenol showed progeny redaction percentage that ranged from 73.5±3.29 (10 mg/l) to 100% 50 mg/l. These data indicated that the four monoterpenes showed significant effect against *C. maculatus* used at different concentrations and these data showed that the monoterpenes are promising in controlling *C. maculatus* in the future.

Table 3: Fumigation with monoterpenes and their effect on certain biological parameters of *C. maculatus*

Monoterpene compounds	**Concentration mg/l**	**Mortality (%± SE) (after one week)**	**Mean No. of deposited eggs ± SE**	**Mean No. of hatched eggs± SE**	**Hatchability %± SE**	**Mean no. of emerged adults± SE**	**Reduction% ± SE**
	Control	**10.0±2.30**	**188.0 ± 4.6**	**148.0±2.30**	**78.75±1.39**	**117.0±0.66**	**0.0 ±0.00**
Thymol	**10**	14.7±,8.48	73.0 ± 4.61	58.0±7.50	79.41±4.13	42.0±3.70	64.1±2.06
	20	18.5±1.86	67.0 ± 8.71	44.0±5.20	67.27±8.47	36.0±5.80	69.2±2.63
	30	26.9±4.35	56.3 ± 1.7	36.0±6.92	64.12±7.12	32.0±4.05	72.6±8.81
	40	42.8±2.34	18.3 ± 1.76	15.0±3.05	81.49±5.57	11.0±4.15	90.6±9.04
	50	69.5±4.35	0.0 ±0.00	0.0±0.00	0.00±0.00	0.0±0.00	100.0±0.00
Carvacrol	**10**	28.5±6.42	67.0 ± 3.5	31.0±3.46	46.15±2.25	26.0±2.90	77.8±5.80
	20	40.0±5.23	50.0 ± 8.08	22.0±2.30	44.34±3.80	19.3±1.76	83.5±3.52
	30	44.0±6.52	47.0 ± 2.30	4.0±1.15	8.70±3.90	1.0±0.00	99.1±0.00
	40	56.6±7.28	0.0 ±0.00	0.0±0.00	0.00±0.00	0.0±0.00	100.0±0.00
	50	66.7±0.00	0.0 ±0.00	0.0±0.00	0.00±0.00	0.0±0.00	100.0±0.00
Eugenol	**10**	16.7±4.72	174.0 ± 3.5	130.0±5.77	74.69±0.91	86.0±1.15	26.5±0.98
	20	32.3±7.33	100.7 ± 9.8	59.0±3.46	58.72±1.16	44.0±2.30	62.4±1.96
	30	36.7±7.06	65.0 ± 6.92	24.0±3.46	36.84±0.70	14.0±2.30	88.0±1.96
	40	54.0±10.96	24.0 ± 4.6	7.0±2.30	30.67±7.87	4.0±1.15	96.6±0.98
	50	63.9±3.78	0.0 ± 0.00	0.0±0.00	0.00±0.00	0.0±0.00	100.0±0.00
Isoeugenol	**10**	20.0±0.0	160.0 ± 5.7	77.0±4.61	48.10±0.57	31.0±2.30	73.5±3.29
	20	23.0±7.02	105.0 ± 6.9	44.0±5.77	41.81±1.37	20.0±2.30	82.9±4.39
	30	30.0±4.72	58.0 ± 6.92	25.0±2.30	43.05±0.41	23.0±1.15	80.3±2.19
	40	43.3±3.84	37.0 ± 2.30	22.0±1.15	59.77±4.99	16.0±0.00	86.3±1.09
	50	44.7±5.98	2.0 ± 1.54	1.0±0.00	38.88±20.05	0.0±0.00	100.0±0.00
L.S.D=$_{0.05}$ (treatments)		**3.02**	**3.05**	**2.14**	**5.32**	**1.53**	**2.15**

Admixing monoterpenes with cowpea seeds and their effect on of *C. maculatus*

The second technique for evaluating monoterpenes is admixing them with cowpea seeds using different concentrations (0.1, 0.5, 1.0, 2 and 3 mg/g seeds). Data in Table (4) showed that thymol (2 and 3mg/g) and Carvacrol (3mg/g) caused 100% mortality, followed by carvacrol (2mg/g) (85 ± 0.0%). On the other hand, the lowest mortality % recorded for isoeugenol (0.1 mg/g), eugenol (0.1 mg/g), thymol (0.1 mg/g) and carvacrol (0.1 mg/g) (0.0, 5.0 ± 0 and 25 ± 5.77, respectively).

No deposited eggs were found using carvacrol and Eugenol at the high concentration (2 mg/g), the lowest number of deposited eggs was ranged from 1.67±0.66 eggs (isoeugenol at 2 mg/g) to 12±1.15 eggs (eugenol at 2 mg/g). Number of hatched eggs showed decrease with the increase of monoterpenes concentrations. For thymol the mean number of hatched eggs was 109.7±9.39 (0.1 mg/g), then reduced to 30.7±2.40 (0.5 mg/g) and no hatched eggs were found at the concentration of 2 and 3 mg/g

The values of hatched eggs for carvacrol decreased to 60.0±4.62 (0.1 mg/g) as compared with eugenol (88.0±2.30) (0.1 mg/g) and 43.7±2.52 for isoeugenol (0.1 mg/g). Also, the concentrations of 3 mg/g showed no hatched eggs in addition to 2 mg/g for thymol. The materials decreased the number of hatched eggs nearly by 50% comparing with the number of deposited eggs. The high concentrations (1, 2 and mg/g) showed the lowest values of number of emerged adults ranged from 0.0 to 11.0±1.15 adults comparing with control (105.0±6.35 adults). On the other hand, with the low concentrations of 0.1 mg/g, number of emerged adults ranged from 31.3±1.76 (isoeugenol) to 74.3±4.64 adults (thymol) (Table 4). Number of emerged adults indicated that there are high significant effects of monoterpenes comparing with control and the number of emerged adults decreased from 25 to 75%.

Under the high concentration of 1, 2 and 3 mg/g, the reduction ranged from 89 to 100%, while under the lowest concentrations (0.1 and 0.5 mg/g), adults redaction ranged from 29.4±2.30 (thymol) to 80.1±1.15 (isoeugenol). The data indicated high reduction percentage after treatment with monoterpene compounds.

Table 4: Effect of admixing monoterpenes with cowpea seeds on certain biological parameters of *C. maculatus*

Monoterpene compounds	**Concentration mg/g**	**Mortality (%± SE) after one week**	**Mean no. of laid eggs± SE**	**Mean No. of hatched eggs± SE**	**Hatchability % ± SE**	**Mean No. of emerged adults± SE**	**Reduction% ± SE**
	Control	**8.0±0.00**	**197.7±9.32**	**160.3±5.45**	**81.20±2.33**	**105.3±6.35**	**0.0±0.00**
thymol	**0.1**	25.0±5.77	131.1±3.05	109.7±9.39	83.67±2.58	74.3±4.64	29.4±2.30
	0.5	50.0±4.05	62.3±3.52	30.7±2.40	49.21±1.59	13.3±1.76	87.4±1.15
	1.00	75.0±0.00	15.7±2.40	5.3±1.76	34.33±6.17	3.3±1.15	96.9±0.66
	2.00	100.0±0.00	0.0±0.0	0.0±0.00	0.0±0.00	0.0±0.00	100.0±0.00
	3.00	100.0±0.00	0.0±0.0	0.0±0.00	0.0±0.00	0.0±0.00	100.0±0.00
carvacrol	**0.1**	25.0±5.77	132.7±6.56	60.0±4.62	45.30±2.71	44.0±4.61	58.3±2.30
	0.5	45.0±0.66	45.0±39.20	35.7±2.40	79.33±2.40	17.0±2.30	83.9±0.66
	1.00	68.7±4.22	47.3±8.10	18.0±1.76	38.82±4.67	11.0±1.15	89.6±6.35
	2.00	85.0±0.00	10.0±2.30	3.0±1.31	30.83±3.62	1.0±0.00	99.1±9.99
	3.00	100.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	100.0±0.00
eugenol	**0.1**	5.0±0.00	144.0±1.15	88.0±2.30	71.98±2.71	52.0±3.46	50.6±0.10
	0.5	29.0±4.61	75.0±2.30	54.0±4.61	41.11±4.84	19.3±1.76	81.7±1.76
	1.00	35.0±5.77	34.66±5.20	14.0±1.15	27.68±1.67	4.0±1.15	92.2±1.03
	2.00	48.3±8.81	12.0±1.15	3.33±0.66	57.63±0.37	1.0±0.00	99.1±1.57
	3.00	66.7±8.81	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	99.5±0.00
isoeugenol	**0.1**	0.0±0.00	83.3±2.90	43.7±2.52	46.10±2.99	31.3±1.76	70.5±0.00
	0.5	10.0±0.00	40.7±2.14	26.0±1.15	61.38±3.87	21.0 ±1.32	80.1±1.15
	1.00	26.7±6.66	12.0±1.15	7.33±0.66	66.66±16.98	3.0±0.00	97.2±0.66
	2.00	37.5±7.63	1.67±0.66	1.0±0.00	1.0±0.00	1.0±0.00	99.1±0.00
	3.00	55.5±9.58	0.00±0.00	0.0±0.00	0.0±0.00	0.0±0.00	99.0±0.00
L.S.D=0.05 (treatments)		**2.6**	**5.0**	**2.5**	**33.99**	**1.3**	**1.2**

Weight loss percentage of cowpea seeds infested by *C. maculatus* by admixing monoterpenes compounds with seeds.

In the present study, 10g of cowpea and 20 adults of *C. maculatus* were used to determine the losses until the observation and full emergence of the first generation. Two bioassays methods were used, the first method fumigation with monoterpenes showed high losses comparing with the admixing method (Table, 5 and Figures, 1). Data showed that increase in concentrations in both methods, the losses decreased. No losses were observed or detected at the concentrations of 50mg/l and 3.0mg/g in both methods. The highest losses were observed at the low concentrations (10mg/l, 20mg/l and 30mg/l in fumigation and 0.1mg/g, 0.5mg/g and 1mg/g in admixing method). High significant variations were observed between the monoterpenes compounds with $LSD_{0.05}$ (014) and (0.27) for both methods in respect. These data recommended the usage of the admixing method with monoterpenes compounds to control the *C. maculatus* based on their effects on biological and losses data.

Table 5: Weight loss percentage of cowpea seeds infested by *C. maculatus* and assayed by two bioassay methods (fumigation and admixing, with seeds).

Concentration	**Treatments and materials**			
	Fumigation with monoterpenes compounds			
	Thymol	**Carvacrol**	**Eugenol**	**Isoeugenol**
Control(0.0mg/l)	22.5±0.36			
10	16.8±0.48	12.5±0.30	15.4±0.28	9.3±0.11
20	12.5±0.29	10.2±0.35	7.4±0.28	6.4±0.32
30	10.4±0.34	3.0±0.40	5.6±0.25	5.2±0.01
40	4.4±0.25	0.0±0.00	2.3±0.19	3.4±0.28
50	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
$LSD_{0.05}$	**0.14**			
Seeds admixing with monoterpenes				
Control(0.0mg/l)	21.0±0.32			
0.1	15.0 ±0.144	6.7±0.45	7.3±0.21	7.5±0.30
0.5	7.31±0.23	3.9±0.64	4.3±0.33	5.4±0.51
1.0	1.3±0.28	2.2±0.0.19	1.4±0.28	2.5±0.19
2.0	0.0±0.00	0.22±0.12	0.22±0.03	0.21±0.17
3.0	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
$LSD_{0.05}$	**0.27**			

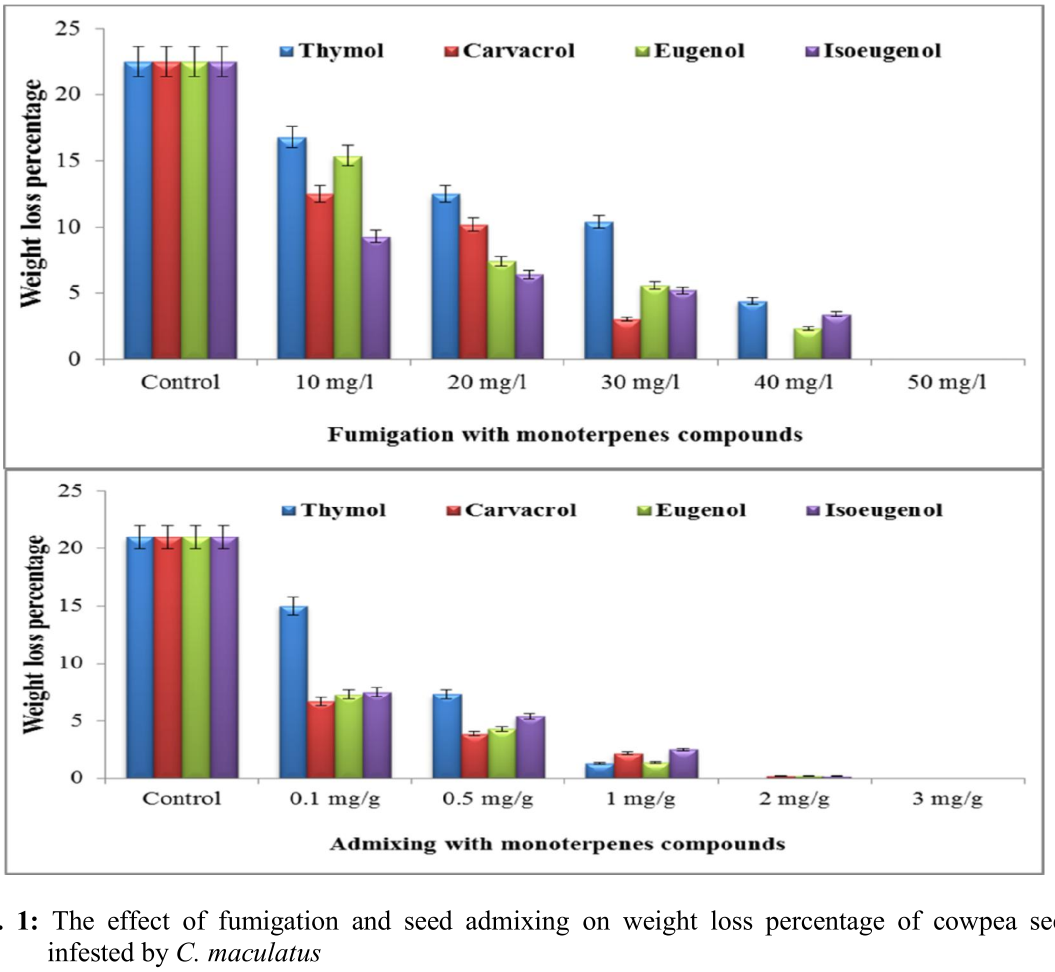

Fig. 1: The effect of fumigation and seed admixing on weight loss percentage of cowpea seeds infested by *C. maculatus*

Discussion

The present results are agreeing with previous studies, which demonstrated that the toxicities of essential oils extracted from various plant samples were mainly related to their major components. Many researchers have been assayed essential oil compounds to demonstrate their efficacy against a variety of stored product insects such as Rastegar *et al*. (2011), Tandorost and Karimpour (2012), Saglamand Ozder (2013), Abdelgaleil *et al*. (2016) and Jarrahi *et al*. (2016). The current data in a line with those who used and evaluatedpure phenolic monoterpenes against the cowpea weevil and they showed the effectiveness of these oils (Oliveira *et al.,* 2017; Kordali *et al*., 2017). Our results of fumigant and admixing with seeds toxicities indicated that the insecticidal activity of the monoterpenes was highly depended on the bioassay method. For example, eugenol was the most potent fumigant and the third most potent in seeds admixing toxicity and these results are similar to those reported by Brari and Thakur (2015) who detected that eugenol and thymol have potent fumigant toxicity against *C. analis, S. oryzae, Stegobium paniceum* and *T. castaneum*. In addition, Ogendo *et al*. (2008) found that eugenol, at 1µl/l air, caused 79%, 61% and 100% mortality of *R. dominica, O. surinamensis* and *C. chinensis,* respectively, 24 hrs after treatment and 90 -100% mortality of *T. castaneum* and *S. oryzae* was demonstrated after 168 hrs treatment. The fumigant toxicity of eugenol tested against *C. maculatus* caused complete adult mortality at 10µl /l air 24 h (Ajayi *et al*., 2014). Thymol and carvacrol exhibited the highest admixing toxicity (LC_{50} = 0.46 and 0.53 mg/g) while they induced a moderate fumigant activity (LC_{50} = 45.32 and 37.34 mg/l air, respectively after 27h). Other founding are similar with our results are those of Kanda *et al.* (2017) who stated that thymol was most toxic to *T. castaneum* and *R. dominica* compared to carvacrol and eugenol in the contact assay. The results of Erler (2005) are agreeing with our results who found that cavracrol and thymol are most active fumigant. Furthermore, isoeugenol was lowest toxic when it was used as fumigant (LC_{50} = 75.07 mg/l air) after 27h or in admixing assay (LC_{50} = 2.32 mg/g). Monoterpenoids are typically volatile and rather lipophilic compounds that can penetrate into insects rapidly and interfere with their physiological functions. Therefore, the admixing with seeds and fumigant toxicity of monoterpenes against stored-product insects has been previously recommended (Lee and Coats, 2003). These results indicated that the assay method has effect on the insecticidal activity of these compounds. The present study has shown that the monoterpenes gave good protection to cowpea seeds by suppressingre production (F1 progeny). However, Ajayi and Wintola (2006) reported that, Clove (*Syzgium aromaticum* (L.) had its effect in suppressing oviposition, egg hatchability and progeny emergence of *C. maculatus* at the rates of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0g/ 20g cowpea seeds. Clove reduced oviposition of *C. maculatus* when tested as pre-treated application compared with control. Consequently, F1 progeny emergence was significantly affected compared with control and egg mortality was highest. In addition, Tapondjou *et al*., (2002) stated that powder of leaves of *Chenopodium ambrosioides* inhibited F1 progeny production and adult emergence of *C. chinensis* and *C. maculatus*. The results obtained from this study indicated that weight loss in cowpea seeds are related to the number of survival insects. This finding is also in agreement with (Asawalam and Hassanali, 2006). There was no weight loss recorded in the 750 mg/250g grain of the *Vernonia amygdalina* essential oil against infestation by *S. zeamais.*In conclusion, the tested monoterpenes such as eugenol, isoeugenol, carvacrol and thymol are more effective on adult'smortality and reproductive development and they showed complete protection at lower concentrations against *C. maculatus* in both assay methods (fumigation and admixing with seeds). Monoterpenes can be used as alternative insecticides because of their safe, effective, readily available and environmentally friendly materials that can be used for insect control.

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