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Toxic Effects of Four Plant Essential Oils alone and in Combination with Controlled modified Atmosphere on the Cowpea Beetle *Callosobruchus maculatus* (Fabricius.) (Coleoptera: Bruchidae) under Laboratory Conditions

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Abstract

Four plant essential oils alone as repellent and fumigant, and in combination with the controlled modified atmospheres against the adult of cowpea weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae) were assessed in the laboratory. These essential oils were extracted from the leaves of four source plants: *Prunus amigdalus*, *Moringa oleifera*, *Simmondsia chinensis*, and *Ricinus communis*. The repellency test indicated that *C. maculatus* adults were repelled by four essential oils. Of these essential oils, the *P. amigdalus* oil was most effective followed by *M. oleifera*, *S. chinensis*, and *R. communis*. The average repellency of the *P. amigdalus* oil against *C. maculatus* adults was significantly higher than the other three tested oils after 7 days. These essential oils had a high level of toxicity in the fumigation assay against *C. maculatus* adults. The results showed that *P. amigdalus* oil gave the highest toxicity at LC_{50} (2.08 ppm) and *R. communis* gave the lowest value of LC_{50} (55.05ppm). In results of the effectiveness of the four essential oils when combined with two controlled atmospheres concentrations, 12.5- 25% CO_2, the toxicity of plant oils was enhanced significantly against *C. maculatus* adults. The results of joint toxic action indicated that at concentration 10 ppm of the four essential oils under modified atmospheres of 12.5 % CO_2 produced an additive effect at all exposure periods, while in case of *M. oleifera* and *S. chinensis* gave additive effect at 3 and 5 days exposure periods. The same trend was found at the highest concentration 20 ppm of three essential oils *P. amigdalus*, *M. oleifera*, and *S. chinensis* under modified atmospheres of 12.5-25% CO_2 and produced an additive effect at all exposure periods, while Co-toxicity values of 20 ppm *R. communis* essential oil after the various exposer periods showed antagonism effect against *C. maculatus* adults. In conclusion, the present study revealed that the combination of the four tested essential oils with CO_2 enhanced its fumigant toxicity to stored product insect, cowpea beetle.

Key words: Cowpea beetle, *Callosobruchus maculatus*, plant essential oil, modified atmosphere, toxicity

Introduction

In stored products world-wide, insect pest infestation may cause up to 40% damage Matthews, (1993).Cowpea, *Vigna unguiculata* (L.) (Walp.), is an important food legume for millions of people throughout the semiarid regions of Africa, Asia, southern Europe, and North, Central, and South America Singh, *et al.*, (2003). The cowpea beetle , *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae), is a cosmopolitan pest of legume seeds and is among the most serious pests of stored products in tropical countries Kang, *et al.*,(2013), Massango, *et al.*,(2017). The insect larvae represent the most destructive stage, as adults cowpea bruchid do not feed Ileke, *et al.*, (2017). However, as the availability of a specific host is highly discontinuous and because these adult insects can live in hosts that are normally treated with insecticides Gbaye, *et al.*, (2012).This insect might have to face insecticidal sub-lethal exposures prior to deciding where they are going to lay eggs.

In view of the damage caused by *C. maculate*, fumigants are the most cost-effective and efficient way of managing stored product pests in many storage systems, not just because they are able to kill a wide range of pests, but due to easily penetrate the products and leave minimal residue Mueller, (1990). Phosphine is a common fumigant for these reasons Lee, *et al.*, (2004). Fumigation by phosphine which is widely used may become increasingly districted in use as it makes resistance of stored product insects to this fumigant and some arguments about the genotoxicity potential of phosphine Meaklim, (1998).Safe alternatives to replace dangerous insecticides are therefore urgently required to grow and commercialize. To protect the environment and prevent negative environmental consequences, researchers focused on innovative ways of carrying out insect pest management in grain farms. In addition, they concentrated on using organic products like pesticides, and essential plant oils. Rajendran & Sriranjini, (2008).

Essential oils are potential alternative material to currently used fumigants Lee *et al.*, (2001). Plant products, including essential and component oils, were used for fumigation because it is thought that plant extracts could benefit from low mammalian toxicity and rapid degradation, as well as local availability, compared with traditional fumigants. Rajendran and Sriranjini, (2008).Some plants with

medicinal properties contain components have ovicidal, antifeedant, repellent, sterilizing, and toxic effects in insects Isman, (2006).

Controlled atmospheres such as low oxygen levels, high concentrations of carbon dioxide, and reduced pressure are efficient ways of controlling storage insects in particular on the adult stage. Modified atmosphere treatments are safe and environmentally friendly ways to manage pests that cause harm to many stored-products. Over several years, the modified atmosphere has been used to check the control of diverse insect and mite species in the laboratory under industrial conditions. Navarro, (2006).Several stored product insects have been previously investigated for controlled atmosphere mortality Mbata *et al.*, (2009).

In the present work, the efficacy as repellents and fumigants of four plant essential oils alone and in combination with carbon dioxide modified atmospheres have been investigated against the adults of *C. maculatus* under the laboratory conductions.

Materials and Methods

- Test Insect and Rearing Conditions.

The original population of *C. maculatus* was field-collected from small farm stores in Moshtohor region (Qaliubiya Governorate, Egypt) during the year 2020, and the population was placed (25 pairs of 2-day-old male and female beetles) in 1L wide-mouthed glass Mason jars containing 100 g of cowpea seeds maintained free from pest and insecticides. The jars were placed in a rearing chamber and maintained at 28± 2°C, 70 ± 5% R.H, and 12:12 h photoperiod. Female beetles were allowed to lay eggs on the seeds for 24 hours, after which they were removed. The seeds containing eggs were kept in a rearing chamber until adult emergence. Tests were performed in the stored product pests Laboratory at the Plant Protection Department, Faculty of Agriculture, Moshtohor, Benha University.

- Plant Essential Oils.

Four plant species belonging to four different families; Rosaceae, Moringaceae, Simmondsiaceae and Euphorbiaceae; were used during these investigations. Theses essential oils were bought from Al-gomhuria Company of drugs, chemicals and medical supplies in Egypt. The fumigant toxicity of this oils were tested to the adults of *C. maculatus.* The source plants for oil extraction were: *Prunus amigdalus, Moringa oleifera, Simmondsia chinensis* and *Ricinus communis* (Table 1).

Table 1. The plant species were as follows:

Scientific name	English name	Arabic name	Family
Prunus amigdalus	Bitter almond	اللوز المر	Rosaceae
Moringa oleifera	Moringa	المورينجا	Moringaceae
Simmondsia chinensis	Jojoba	الجوجوبا	Simmondsiaceae
Ricinus communis	Castor	الخروع	Euphorbiaceae

- Bioassay

a- Repellency test

Repellency of four essential oils against *C. maculatus* was carried out using an apparatus described by Su, (1989) with some modifications. A metallic ring (6 cm diameter x 0.5 cm height) was placed in the center of a glass Petri-dish (11 cm diameter x 3 cm height) on a filter paper. The filter paper was dipped in solutions of the test materials in acetone to achieve deposits at 200, 400, or 800 µg/cm^2. Ten grams of the treated samples were placed inside the ring. Thirty adults (1-2 week old) were introduced to the sample after two days from initial treatment. Treatment samples were kept for 24 hrs then repellency rate was recorded based on the number of insects counted inside and outside the ring after 1, 2, 3, 5, and 7 days from the initial treatment, Thirty newly adults were introduced to the same sample, then repellency was recorded at each period after 24 hr.

b- Fumigant Toxicity Test

In this experiment 200 ml glass jars with tilted covers were used as fumigation chambers for the plant oil. The tested concentrations of each oil inside the jars were 2.5, 5, 10, 20 and 40 ppm. Total six jars (replicates) were set up in each glass bottle. Inside every jar one filter paper was inserted at the button. Then one ml from each oil concentration (in acetone) was taken and added to every glass jar on a filter paper. Thirty adults were put inside each jar into wire gauze cages (40 mm in diameter and 45mm in height) with a small amount of diet. The jars were well closed and incubated at 28±1 °C and 65±5% R.H. The same steps were followed in the control treatment using only acetone without oil. Mortality rate was calculated after 1, 2, 3, 5 and 7 days post treatment. Each treatment with the respective control was replicated six times.

c- Effect of modified atmosphere

Carbon dioxide was provided as pure gases in pressure steel cylinders. Each cylinder was connected with a pressure regulator. The dilution method was used to achieve the required CO_2 concentration. Modified atmospheres of 12.5, 25and 50% CO_2were prepared and tested. Carbon dioxide was monitored using Gas analyzer model 200-600 (Gow-Mac-Instrument Co., USA). Batches of Thirty adults were introduced into wire gauze cages(40 mm in diameter and 45mm in height), filled with about 10 g diet (Cowpea seeds)then the cages were covered with rubber stopper. Cages were taken and introduced it into Dreshel-flask of 0.55L.Insects in the gas tight flasks were treated for different fixed exposure periods at the aforementioned temperature and relative humidity. The flasks were aireated and the insects were transferred into petri dishes and kept it at 25°± 1° C and 60 ± 5%RH for mortality assessment.

The controlled atmosphere apparatus used in this experiment was described by Darwish, *et al.,* (1993) with some modifications.

d- Combination Toxicity Test

Ten grams of Cowpea seeds were treated with four essential oils at two concentrations 10 and 20ppm as described above. Thirty insects were introduced into each cage. Insects inside the cages were transferred into the Dreshei-flask, and exposed to two concentrations of carbon dioxide. Tests were conducted at the same temperature and relative humidity at different exposure periods and insect mortalities were assessed as described above. The essential oils and controlled atmosphere combinations used in this experiment were described by El-lakwah, *et al.,* (2000) with some modifications.

e- Calculation of joint action

For the evaluation of the joint action of four plant essential oils with the modified atmospheres, the following equation was adopted as reported by Mansour *et al.,* (1996)**:**

$$\textbf{Co-toxicity factor} = \frac{\text{Observed mortality \% - Expected mortality \%}}{\text{Expected mortality \%}} \times 100$$

This factor was used to classify the results into three categories. A positive factor of 20 or more means potentiation (synergistic effect), a negative factor of -20 or more means antagonism, and any intermediate value, i.e. between +20 and -20 was considered as additive effect.

- **Statistical analysis:**

The data were corrected using data from treatments and the control according to Abbott's formula Abbott, (1925) and the data were subjected to probit analyses using LDP line software according to Finney, (1971)to estimate LC_{50}, LC_{95}andLT_{50}, LT_{95}values of the essential oils against each stored product insect species. Mortality percentages for different exposure times were subjected to analysis of variance (one-way ANOVA) using the same statistical program (SPSS 2001) for probit analysis Steel *et al.,* (1997).Means were separated at the 5% significance level by the least significant difference (LSD) test.

Results

a- Repellency Test.

Data on repellency of four plant essential oils with three concentrations against *C. maculatus* adults are presented in Table (2) and Fig.(1). Repellency studies conducted after 1, 2, 3, 5, and 7 days of treatment, the results showed that the repellency increased with increasing concentration and reduced with increasing the period of exposure.

Table 2. Corrected repellency percentage (%) of four plant essential oils against *C. maculatus* adults at different time periods after treatment.

Source plants	Rate (µg/cm^2)	Exposure period (days)					
		1 d	2 d	3 d	5 d	7 d	The average repellency %
P. amigdalus	800	94.4	92.2	85.5	73.3	65.5	82.18
	400	85.5	81.1	76.6	68.8	53.3	73.06
	200	68.8	60	54.4	43.3	40	53.30
M. oleifera	800	84.4	75.5	61.1	50	42.2	62.64
	400	78.8	61.1	52.2	33.3	25.5	50.18
	200	52.2	31.1	22.2	13.3	13.3	26.42
S. chinensis	800	64.4	58.8	42.2	25.5	21.1	42.40
	400	43.3	38.8	32.2	20	18.8	30.62
	200	38.8	25.5	18.8	12.2	0	19.06
R. communis	800	48.8	31.1	22.2	13.3	6.6	24.4
	400	22.2	15.5	8.8	5.5	0	10.40
	200	13.3	8.8	1.1	0	0	4.64

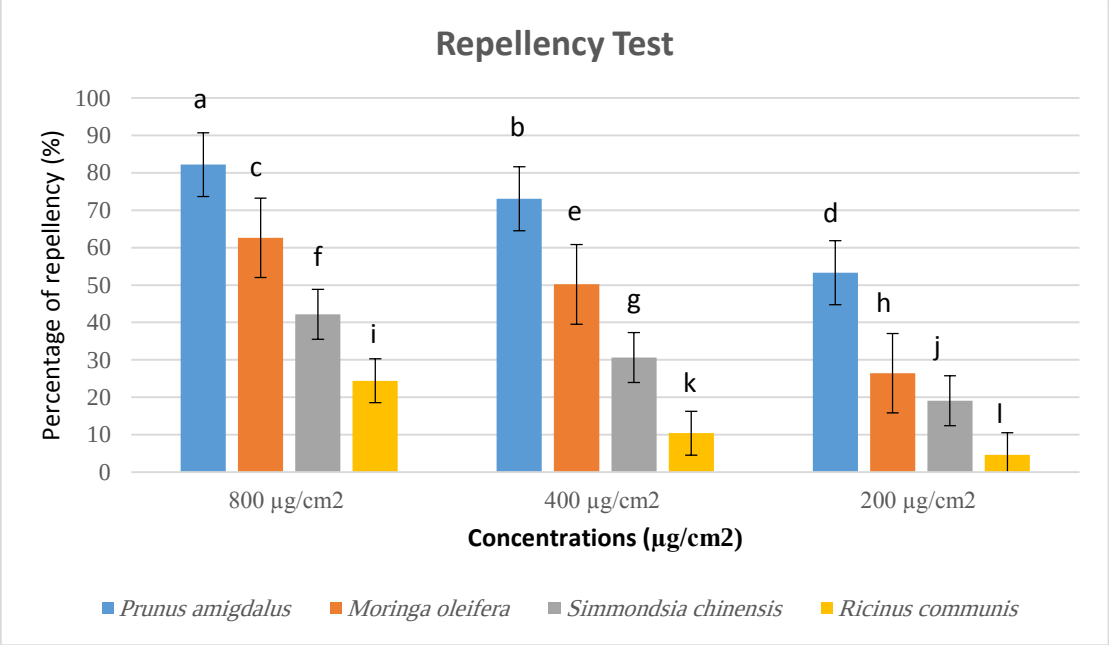

Fig. 1. Average percentage of repellency (mean ± SD) of four plant essential oils at 200, 400 and 800 µg/cm^2 against *C. maculatus* adults after day 7. Means with the same litters are not significantly different. Different superscripts denote $p \leq .05$ between treatments.

However, the repellency of the tested oils against *C. maculatus* adults at different times were significantly different. More than 50% of *C. maculatus* adults were repelled after 7 days for the *P. amigdalus* essential oil at 200, 400, and 800 µg/cm^2, and of *M. oleifera* at 400, and 800 µg/cm^2. The *S. chinensis* oil obtained 42.4% of repellency after 7 days but at the higher concentration (800 µg/cm^2). *R. communis* essential oil was the lowest repellent activity against *C. maculatus* adults at the various concentrations after the exposure periods. Within 7 days, the average percentage of repellency of the four essential oils at three test concentrations against *C. maculatus* adults were significantly different. Of all these essential oils, the *P. amigdalus* oil was the most effective followed by *M. oleifera* and *S. chinensis*. The average repellency of *R. communis* oil against *C. maculatus* adults was significantly lower than the other three tested oils within 7 days.

b- Essential oils Fumigant Toxicity Test.

The results of the four essential oil fumigation toxicity alone at five concentrations (40, 20, 10, 5, and 2.5 ppm) on the adult of *C. maculatus* at 28±1°C and 65±5% R.H. are presented in Table (3). The results showed that mortality was increased by increasing the plant essential oil concentration and period of exposure. At the highest concentration 40 ppm the mortalities were 35.5, 23.3, 15.5, and 12.2 % after 1-day exposure and increased after 7 days post-treatment to 83.3, 68.8, 54.4 and 48.8 % for *P. amigdalus*, *M. oleifera*, *S. chinensis*, and *R. communis* respectively., while at the lowest concentration 2.5 ppm the mortalities were 18.8, 12.2, 3.3 and zero % after 1-day exposure and increased after 7 days to 54.4, 38.8, 20 and 15.5 % for the same essential oils, respectively. The lethal concentrations of four plant essential oils against *C. maculatus* adults are shown in Table (4). The results showed that the lethal concentrations are exposure period dependent. The higher the exposure period was the lower the LC values. After3 days post-treatment the LC_{50} values were 18.80, 73.51, 125.67, and 169.64 ppm and declined to 2.08, 7.45, 27.81, and 55.05 ppm at 7 days post-treatment for *P. amigdalus, M. oleifera, S. chinensis, and R. communis* respectively, the LC_{95} values were 1125.91, 71549.55, 16709.60, and 11438 ppm, the corresponding values at 7 days were significantly lower and amounted 723.35, 1894.15, 2269.44, and 5447.22 ppm for the same essential oils, respectively. The results indicated clearly that *P. amigdalus* was the highest essential oil toxicity against *C. maculatus* adults followed by *M. oleifera and S. chinensis*, while *R. communis* was the least essential oil activity against the target insects.

Table. 3. Corrected percent mortality of the fumigation toxicity of four essential oils against the adults of *C. maculatus* after exposure period

Concentration PPM	Accumulative adult mortality (%) after indicated days				
	1	2	3	5	7
			Prunus amigdalus		
40	35.5	48.8	65.5	78.8	83.3
20	28.8	34.4	45.5	53.3	71.1
10	21.1	31.1	38.8	47.7	63.3
5	18.8	24.4	33.3	45.5	60
2.5	18.8	20	26.6	38.8	54.4
			Moringa oleifera		
40	23.3	37.7	44.4	56.6	68.8
20	20	28.8	36.6	42.2	62.2
10	15.5	25.5	33.3	40	54.4
5	12.2	21.1	27.7	34.4	42.2
2.5	12.2	18.8	22.2	31.1	38.8
			Simmondsia chinensis		
40	15.5	25.5	34.4	41.1	54.4
20	12.2	18.8	28.8	35.5	48.8
10	8.8	12.2	18.8	24.4	32.2
5	5.5	10	14.4	20	24.4
2.5	3.3	3.3	8.8	14.4	20
			Ricinus communis		
40	12.2	21.1	28.8	36.6	48.8
20	12.2	15.5	18.8	28.8	33.3
10	5.5	10	14.4	18.8	27.7
5	1.1	4.4	8.8	12.2	18.8
2.5	0	1.1	4.4	10	15.5

Table 4. LC_{50} and LC_{95} values of the fumigation toxicity of four essential oils against the adults of *C. maculatus.*

Plant oils	Time (days)	LC_{50} (ppm)	LC_{95} (ppm)	Slop±SD	Chi Square (χ^2)	p-Value	R.
P. amigdalus	3 d	18.80 13.79-28.94	1125.91 360.77-9606.60	0.92±0.14	2.22	0.52	0.975
	7 d	2.08 0.55-3.75	723.35 179.26-25620.37	0.64±0.14	1.83	0.60	0.956
M. oleifera	3 d	73.51 33.60-690.73	71549.55 3366.88-1238628321.1	0.55±0.14	0.21	0.97	0.992
	7 d	7.45 4.47-11.11	1894.15 396.65-74849.81	0.68±0.14	0.49	0.92	0.989
S. chinensis	3 d	125.67 58.67-712.57	16709.60 1950.13-2933582	0.77±0.16	0.35	0.94	0.993
	7 d	27.81 19.11-51.47	2269.44 572.19-35902.69	0.86±0.15	1.16	0.76	0.983
R. communis	3 d	169.64 76.70-1001.52	11438 1607.34-1113686.4	0.89±0.18	0.24	0.96	0.994
	7 d	55.05 32.75-150.81	5447.22 1012.29-202275.93	0.82±0.15	1.36	0.71	0.977

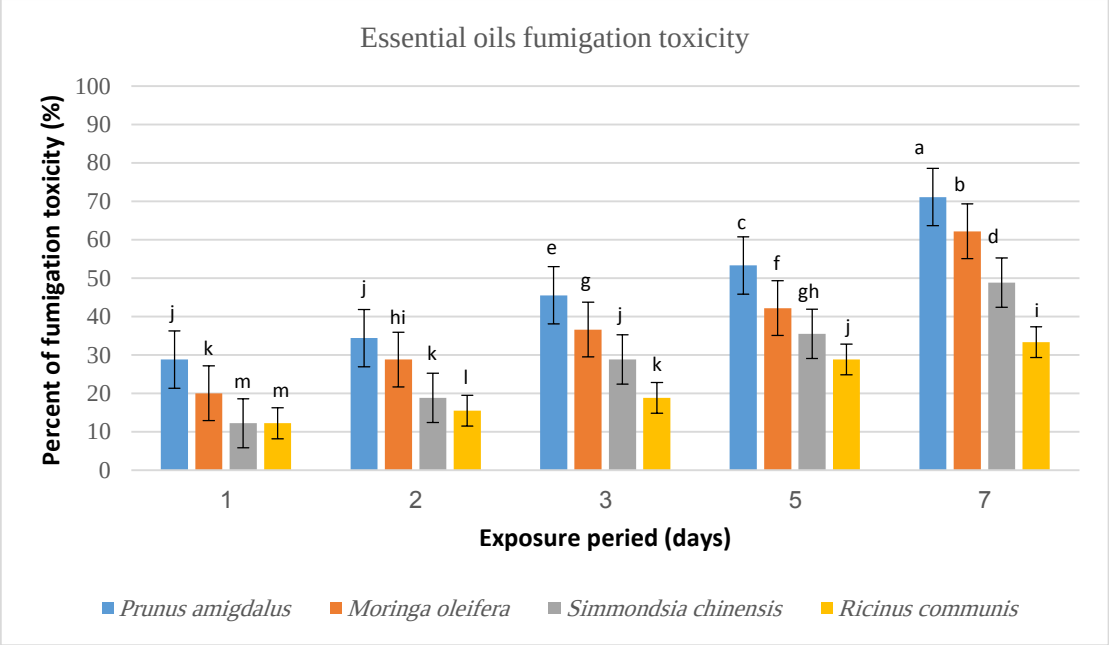

Fig. 2. Average percentage of fumigation toxicity (mean ± SD) of four plant essential oils at 20ppm against *C. maculatus* adults after exposure period. Means with the same litters are not significantly different. Different superscripts denote $p \leq .05$ between treatments.

c- Modified atmospheres toxicity Test.

The efficacy of modified atmospheres (MA) containing various carbon dioxide (CO_2) concentrations against the adults of *C. maculatus* at 28 ± 1°C and 65 ± 5% R.H. are shown in Table (5). It is obvious that using MA at concentrations 12.5, 25, and 50% CO_2after a 2-days exposure period resulted in 8.8, 10.0, and 32.2%,the mortalities were increased after 7 days exposure period to 35.5, 65.5 and 92.2% mortality for the cowpea beetle, *C. maculatus*, respectively. Elevation of mortalities was concentrations and exposure period dependent, since using the MA of 50% CO_2 produced higher mortality values. The results revealed that in Table (6), the time needed to obtain 50% mortality at 12.5, 25, and 50% CO_2 were10.92, 4.84, and 2.46 days for the cowpea beetle, *C. maculatus*, respectively. The results showed that high concentration gave mortality values more than low concentration which mean that mortality increased by increasing the CO_2 concentration.

Table 5. Efficacy of modified atmospheres (MA) containing various carbon dioxide (CO_2) concentrations against the adults of *C. maculatus*

Modified atmospheres concentration (%)	Accumulative adult mortality (%) after indicated days				
	1	2	3	5	7
50% CO_2+ 40 % N_2 + 10 % O_2	12.2	32.2	65.5	88.8	92.2
25 % CO_2 + 60 % N_2 + 15 % O_2	2.2	10.0	37.7	48.8	65.5
12.5% CO_2 + 70 % N_2 + 17.5 % O_2	1.1	8.8	18.8	25.5	35.5

Table 6. LT_{50} and LT_{95} values of the fumigation toxicity of modified atmospheres (MA) containing various carbon dioxide (CO_2) concentrations against the adults of *C. maculatus*.

Modified atmospheres concentration (%)	LT_{50} (days)	LT_{95} (days)	Slop±SD	Chi Square (χ^2)	p-Value	R.
50 % CO_2 + 40 % N_2 + 10 % O_2	2.46 1.70-3.32	8.48 7.08-20.65	3.06±0.27	8.97	0.02	0.991
25 % CO_2 + 60 % N_2 + 15 % O_2	4.84 4.31-5.55	18.79 14.09-28.62	2.79±0.28	5.97	0.11	0.984
12.5 % CO_2 + 70 % N_2 + 17.5 % O_2	10.92 8.10-18.51	89.21 41.40-379.84	1.80±0.28	1.09	0.77	0.990

d- Essential oil-carbon dioxide combination toxicity

The toxicities of four essential oils at two concentrations 10 and20 ppm in combination with two modified controlled atmospheres against *C. maculatus* adults are presented in Table (7).The data clearly showed that the tested insect was more sensitivity to the essential oils when applied under MA comparing with the essential oils alone. Complete moralities were recorded after 7-days exposure period when three essential oils *P. amigdalus, M. oleifera*, and *S. Chinensis* were used at the highest concentration under MA of 25% CO_2, while the percentage mortality was 82.2 % at the highest concentration of *R. communist* essential oil against the adult of *C. maculatus.* The lethal time values to obtain 50% mortality of four essential oils in combination with two modified controlled atmospheres against *C. maculatus* adults are presented in Table (8). The lethal time values at the lowest concentration10 ppm of four essential oils under MA of 25% CO_2were 1.82, 2.27, 3.45 and 6.20 days, while that the times needed to achieve 95% mortality were 10.37, 11.57, 21.48 and 104.45 days for same treatment, respectively. The lethal time values at the highest concentration20 ppm of four essential oils under MA of 25% CO_2were 0.99, 1.25, 1.56 and 2.66days, while that the times needed to achieve 95% mortality were 3.91, 5.81, 8.32 and 17.21 days for same treatment, respectively.

The calculated joint action of four essential oils at 10 &20 ppm in combination with two modified controlled atmosphere against *C. maculatus* adults are given in Tables (9&10).Results indicated that at concentration 10ppm of four essential oils under MA of 12.5 % CO_2 produce additive effect at all exposure periods, while in case of *M. oleifera* and *S. chinensis* at the same concentrations produced additive effect at 3 and 5 days exposure period. On the other hand, Co-toxicity values of 10ppm of four essential oils under MA of 25 % CO_2 showed additive effects with all the exposure periods. Also at the highest concentration 20 ppm of three essential oils *P. amigdalus*, *M. oleifera* and *S. chinensis* under MA of 12.5% as well as 25 % CO_2 produced additive effect at all exposure periods, while Co-toxicity values of 20 ppm *R. communis* essential oils after the various exposer periods showed antagonism effect against *C. maculatus* adults

Table 7. Corrected percent mortality of the four plant essential oils fumigation toxicity and in combination with two modified atmospheres treatments against the adults of *C. maculatus* after exposure periods

Concentration	Accumulative adult mortality (%) after indicated days				
	1	2	3	5	7
Prunus amigdalus					
10 ppm + 12.5 CO_2	28.8	35.5	53.3	73.3	84.4
10 ppm + 25 CO_2	32.2	48.8	66.6	81.1	95.5
20 ppm + 12.5 CO_2	34.4	55.5	76.6	88.8	100
20 ppm + 25 CO_2	53.3	74.4	91.1	100	100
Moringa oleifera					
10 ppm + 12.5 CO_2	21.1	30	52.2	65.5	82.2
10 ppm + 25 CO_2	25.5	33.3	64.4	78.8	88.8
20 ppm + 12.5 CO_2	41.1	58.8	74.4	85.5	94.4
20 ppm + 25 CO_2	44.4	63.3	81.1	93.3	100
Simmondsia chinensis					
10 ppm + 12.5 CO_2	14.4	18.8	33.3	52.2	64.4
10 ppm + 25 CO_2	16.6	22.2	48.8	63.3	74.4
20 ppm + 12.5 CO_2	23.3	44.4	62.2	75.5	88.8
20 ppm + 25 CO_2	38.8	51.1	68.8	84.4	100
Ricinus communis					
10 ppm + 12.5 CO_2	10	15.5	24.4	33.3	45.5
10 ppm + 25 CO_2	14.4	23.3	38.8	44.4	55.5
20 ppm + 12.5 CO_2	16.6	28.8	46.6	53.3	67.7
20 ppm + 25 CO_2	18.8	37.7	58.8	71.1	82.2

Table 8. LT_{50} and LT_{95} values of the four plant essential oils fumigation toxicity and in combination with two modified atmospheres treatments against the adults of *C. maculatus*

Plant oils	Concentrations	LT_{50} (days)	LT_{95} (days)	Slop±SD	Chi Square (χ^2)	p-Value	R.
P. amigdalus	10 ppm + 12.5 CO_2	2.40 2.02-2.79	18.51 12.68-33.45	1.85±0.22	6.27	0.99	0.962
	10 ppm + 25 CO_2	1.82 1.53-2.10	10.37 7.94-15.35	2.17±0.23	2.90	0.40	0.983
	20 ppm + 12.5 CO_2	1.55 1.28-1.80	8.23 6.43-11.81	2.27±0.24	1.34	0.71	0.966
	20 ppm + 25 CO_2	0.99 0.77-1.18	3.91 3.20-5.32	2.77±0.35	2.85	0.24	0.980
M. oleifera	10 ppm + 12.5 CO_2	2.91 2.52-3.35	18.54 13.09-31.34	2.07±0.22	4.12	0.24	0.977
	10 ppm + 25 CO_2	2.27 1.97-2.58	11.57 8.92-16.81	2.32±0.23	7.02	0.07	0.973
	20 ppm + 12.5 CO_2	1.39 1.10-1.66	9.06 6.87-13.74	2.02±0.23	1.53	0.67	0.988
	20 ppm + 25 CO_2	1.25 1.01-1.47	5.81 4.72-7.85	2.47±0.26	3.55	0.31	0.978
S. chinensis	10 ppm + 12.5 CO_2	4.78 4.05-5.94	38.02 22.90-86.98	1.82±0.23	3.31	0.34	0.973
	10 ppm + 25 CO_2	3.45 3.01-4.01	21.48 14.91-37.42	2.07±0.22	4.87	0.18	0.972
	20 ppm + 12.5 CO_2	2.22 1.91-2.54	12.52 9.44-18.94	2.19±0.22	1.36	0.71	0.992
	20 ppm + 25 CO_2	1.56 0.73-2.08	8.32 7.20-34.19	2.26±0.23	9.11	0.02	0.925
R. communis	10 ppm + 12.5 CO_2	9.27 6.77-16.33	136.92 53.31-887.74	1.40±0.23	0.78	0.85	0.989
	10 ppm + 25 CO_2	6.20 4.83-9.30	104.45 43.69-563.85	1.34±0.22	2.98	0.39	0.967
	20 ppm + 12.5 CO_2	3.97 3.35-4.88	38.86 22.61-96.12	1.66±0.22	0.98	0.80	0.992
	20 ppm + 25 CO_2	2.66 2.29-3.06	17.21 12.28-28.60	2.02±0.22	1.15	0.76	0.994

Table 9. Joint action effect of 10 ppm of four plant essential oils fumigation toxicity and in combination with two modified atmospheres treatments against the adults of *C. maculatus* after exposure periods

Essential oils treatments	Exposure period (days)	10 ppm oil alone	Adults mortalities							
			CO_2 alone		Oil+CO_2 Combination		Co-toxicity factor		Type of join action	
			CA1	CA2	CA1	CA2	CA1	CA2	CA1	CA2
P. amigdalus	3	38.8	18.8	37.7	53.3	66.6	19.12	9.49	D	D
	5	47.7	25.5	48.8	73.3	81.1	12.69	0	D	D
	7	63.3	35.5	65.5	84.4	95.5	-6.19	0	D	D
M. oleifera	3	33.3	18.8	37.7	52.2	64.4	34.29	9.15	S	D
	5	40	25.5	48.8	65.5	78.8	26.29	2.52	S	D
	7	54.4	35.5	65.5	82.2	88.8	-3.37	0	D	D
S. chinensis	3	18.8	18.8	37.7	33.3	48.8	30.67	3.45	S	D
	5	24.4	25.5	48.8	52.2	63.3	23.77	0.11	S	D
	7	32.2	35.5	65.5	64.4	74.4	5.33	0	D	D
R. communis	3	14.4	18.8	37.7	24.4	38.8	23.93	4.07	S	D
	5	18.8	25.5	48.8	33.3	44.4	-1.84	-8.37	D	D
	7	27.7	35.5	65.5	45.5	55.5	-1.54	-16.80	D	D

CA1:12.5%; CO_2 and, CA2:25% CO_2

Table 10. Joint action effect of 20 ppm of four plant essential oils fumigation toxicity and in combination with two modified atmospheres treatments against the adults of *C. maculatus* after exposure periods

Essential oils treatments	Adults mortalities									
	Exposure period (days)	10 ppm oil alone	CO_2 alone		Oil+CO_2 Combination		Co-toxicity factor		Type of join action	
			CA1	CA2	CA1	CA2	CA1	CA2	CA1	CA2
P. amigdalus	3	45.5	18.8	37.7	76.6	91.1	7.46	-12.90	D	D
	5	53.3	25.5	48.8	88.8	100	0.13	-15.90	D	D
	7	71.1	35.5	65.5	100	100	-14.5	-4.50	D	D
M. oleifera	3	36.6	18.8	37.7	74.4	81.1	0.19	-8.78	D	D
	5	42.2	25.5	48.8	85.5	93.3	0	11.26	D	D
	7	62.2	35.5	65.5	94.4	100	-8.56	-11.20	D	D
S. chinensis	3	28.8	18.8	37.7	62.2	68.8	-11.40	-13.60	D	D
	5	35.5	25.5	48.8	75.5	84.4	4.60	13.50	D	D
	7	48.8	35.5	65.5	88.8	100	-4.87	-23.80	D	A
R. communis	3	18.8	18.8	37.7	46.6	58.8	-26.5	-25.5	A	A
	5	28.8	25.5	48.8	53.3	71.1	-24.8	-34.30	A	A
	7	33.3	35.5	65.5	67.7	82.2	-28.00	-40.40	A	A

CA1:12.5%;CO_2 and, CA2:25% CO_2

Discussion

Our work has shown that there was a significant difference in repellence of essential oils against *C. maculatus* adults. *P. amigdalus* oil repellence decreased in 7 days more than the other three oil. We also found that *C. maculatus* adult fumigant toxicity was seen in all four essential oils. The mortality of adults with various oils treated with *C. maculatus* differed significantly. There was also a direct correlation between fumigant toxicity and repellent. For instance, *P. amigdalus* oil had the highest repellence effects against the adults of *C. maculatus* and its toxicity to fumigant was significantly higher amongst the four essential oils studied. Elgizawy, *et al.*, (2019) detect the chemical composition of the essential oil derived from the fruits of *Litsea cubeba* (Lauraceae).In addition, to evaluate the contact and fumigant toxicity and repellent activities of the essential oil and twomain active ingredients against the adults of two stored grain insect pests; rice weevil, *Sitophilus oryzae* (L.) and the red flour beetle, *Tribolium castaneum* (Herbst.) in the laboratory, the results showed that the essential oil, citral and D-limonene had higher fumigation toxicity on the same insects4.44, 4.89 and 16.68 µg/l, respectively. Guenther, (1948) confirmed that, while a number of chemically unrelated compounds were found in the essential oils, four main groups were possible: terpenes, straight-chain compounds, benzene derivatives, and miscellaneous. Ryan and Byrne, (1988) reported that, different experiments showed that inhibition of acety lcholinesterase may be the mode of action of the fum igant toxicity of essential oil against insects. Tembo and Murfitt, (1995) suggested that the mortality was due to anoxia. This is confirmed by our findings that when plant oils are used in combination with controlled atmosphere treatment fumigant toxicity was significantly enhanced. It is well known that the controlled atmosphere contributes to insect control two physiological and biological characteristics of stress. Donahaye and Navarro,(2000). One is the reduction in O_2 concentration, resulting in hypoxia or anoxia; another is the increase in CO_2concentration, producing hypercarbia, or a combination of both.

Our study also showed that, there were significantly different interactions between plant oil and controlled atmosphere treatment. This indicated that essential oils could exhibit maximum fumigant toxicity only in certain plant oils. Recent research by Wang *et al.*, (2001) showed that the development and reproduction of *L. bostrychophila* in such a regulated environment were successful, resulting mainly in mortality from plant oil. In combination with controlled atmosphere procedures, the increased toxicity of oils may be a result of controlled atmospheric therapies that enhancing the up-take of the plant essential oils by the insects.

For centuries, plants such as pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Schultz-Bip.), tobacco (*Nicotiana tobacum* L.), and neem (*Azadirachta spp.*) have been known to have components with insecticidal activity and used for control of agricultural pests in China Tsai,(1982) suggested that oils could be considered as efficient repellents and fumigants and also could be integrated into other pest management schemes for control of *C. maculatus* in sealed storage situations.

Conclusion

The findings of this study indicate that there were significant differences in the repellence activity of four essential oils against the adult of *C. maculatus*. Within 7 days, the *P. amigdalus* oil repellence decreased more than that of the other three oils. As well all four essential oils demonstrated some

fumigant toxicity against adults of *C. maculatus*. Mortality of adults of *C. maculatus* treated with different oils varied significantly. The use of CO_2 concentration appears to have an additive effect when combined with four essential oils against the adult of *C. maculatus* as evidenced by significant decrements in LT_{50} and LT_{95} values for the adults and the mortality percentage were enhanced. These results indicate that combination of four essential oil with CO_2 can be potential as an alternative application to the most commonly used commercial fumigants, methyl bromide and phosphine.

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التأثير السام لأربعة زيوت نباتية عطرية بمفردها وعند خلطها مع جو معدل متحكم به ضد خنفساء اللوبيا تحت ظروف المعمل

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تم تقييم التأثير الطارد والبخاري لأربعة زيوت نباتية عطريه بمفردها وكذلك عند خلطهامع جو معدل متحكم به ضدالحشرات الكاملة من خنفساء اللوبيا في المعمل، تم استخلاص هذه الزيوت النباتية من أوراق أربعة نباتات هي اللوز المر،المورينجا ، الجوجوبا، الخروع. وأشارت اختباراتالتاثير الطارد إلى أن الحشرات الكاملة لسوسةاللوبيا ثم طردها بواسطة اوراق الترشيح التي تم معالجتها بالزيوت النباتيةالاربعة. ومن بين هذه الزيوت كان زيت اللوز المر هو اكثر الزيوت فاعليةمن حيث التأثير الطارد يليه زيت المورينجا، زيت الجوجوبا ثم زيت الخروع اقلهم فاعلية. وكان متوسط التأثير الطارد لزيت اللوز المر ضد خنفساءاللوبيا أعلى معنويا من الزيوت النباتية الثلاثة الأخرى بعد سبع ايام من المعاملة.كما أظهرت هذه الزيوت مستويات عالية من سمية التبخير ضد الحشرات الكاملة من سوسة اللوبيا، حيث أظهرت النتائج أن زيت اللوز المر أعطي مستويات سمية عالية وكانت قيمة التركيز اللازم لقتل 50 في المئة من الحشرات (LC_{50}) هو 2.08 جزء في المليون، بينماكان زيت الخروع اقلهم فاعلية حيث اعطي قيم منخفضة من(LC_{50}) وهي 55.05 جزء في المليون. كانت النتائج المتحصل عليها لفعالية استخدام أربع زيوت نباتية عند خلطها مع تركيزين من جو معدل متحكم به يحتوي علي تركيز12.5–25% من غاز ثاني اكسيد الكربونتشير الي تحسن فاعلية الزيوت العطرية بشكل معنوي ضدخنفساء اللوبيا.كما أشارت نتائج التاثير السام المشترك عند تركيز 10 جزء في المليون من الزيوت النباتية الأربعة عند خلطها مع جو معدل متحكم به يحتوي على 12.5% من ثاني أكسيد الكربون اعطت تاثير إضافي عند كل فترات التعريض، بينما في حالة زيت المورينجا والجوجوبا أعطتتأثير إضافي بعدثلاثة وخمسة ايام من فترات التعريض. وفي نفس السياقوجد عند استخدام التركيز الأعلى للزيوت 20 جزء في المليون إن الزيوت النباتية الثلاثة وهي اللوز المر ،والمورينجا وكذلك الجوجوبا عند خلطهمابغاز ثاني أكسيد الكربون بتركيزي12.5– 25 % أعطي تأثير إضافي مع جميع فترات التعريض، بينما عند تركيز 20 جزء في المليون من زيت الخروع،أظهرت قيم التأثير السام المشترك بعد فترات التعريض المختلفة تأثير مضادا ضد الحشرات الكاملة من خنفساء اللوبيا.أوضحت الدراسة الحالية ان خلط الزيوت النباتية الأربعة مع جو محكم من غاز ثاني اكسيد الكربون يقوم بتحسين سمية الزيوت النباتية بشكل افضل من استخدامها بصورة منفردة عند مكافحةحشرة خنفساء اللوبياعلى الحبوب المخزونة.