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Efficacy of botanical pure compounds, camphor and menthol against stored product pests, *Triboliun confusum* Jacquelin du val (Coleoptera: Tenebrionidae) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) adults

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Abstract
Stored product pest insects, *callosobruchus maculatus* and *Triboliun confusum* caused significant losses in the diverse food grains and their products through infestation. The insecticidal activity of botanical pure compound (camphor and menthol) at different concentration level (0.25, 0.50 and 0.75 w/w) and exposure periods (24, 48 and 72 hrs) was investigated against adults of *C. maculatus* and *T. confusum,* in a series of laboratory experiments. The result showed that the mortality percentage of the adult pest insects increased with increase of concentration level and with exposure periods, *C. maculatus* was more susceptible than *T. confusum* against used botanical pure compounds. In general menthol was more effective than camphor for the control of these insects. The result of the present study exhibited that botanical pure compounds can play an important role in stored grain protection. Therefore, botanical pure compounds may be preferred option than conventional insecticides for protecting stored grains and their products against stored product insect pests' infestation, especially *C. maculatus*.

Keywords: Stored grain products, control, botanical compounds, insects, insecticidal activity, mortality

1. Introduction

The global stored product grain losses by infestation of insect and other bio agent damage range from 10-40% [1]. Insect fests are a major concern which is estimated approximately 35% of crops all over the word [2]. The confused flour beetle, *Triboliun confusum* is one of the serious insect pest of grain and other food products stored in homes, grocery store, warehouses and silos which are cases considerable losses [3]. The cowpea beetle *callosobruchus maculatus* is a primary insect pest of stored grain which is widely distributed throughout the world [4] it is economically important and major pest of leguminous grain including lentil, green gram, black gram and cowpea [5, 6] they are important insect pests of pulse crops in Africa and Asia in the storage [7, 8] which are cases considerable weight losses and made 70% of the grain unfit for human consumption occurred after six months of the storage [9].

Different methods have been used to prevent these post-harvest losses such as chemical, physical and biological treatments [1]. Among those, chemical or synthetic insecticides largely used as stored grain protestant could have undesirable effects included environmental pollution, ozone depletion, toxicity to non-target organism, pesticide residues and pest resistance, in addition to direct toxicity to users and increasing cost of application [1, 10. 11, 12]. Therefore, the development of bio degradable insecticides such as plant derived material has been focused as viable pest control strategy in recent years [13, 14, 15]. Plant may provide potential alternative to currently used synthetic insecticides due to their rich source of active chemicals [16]. Plant derived compounds are known to exhibit low toxicity to mammal and have even been approved as flavoring agent in food material [14, 17]. Samir *et al*. [18] also evaluated the toxicity of the test monoterpenes varied with insect species, which they observed considerable differences in mortality of insects.

The present investigation was cured out to determine the insecticidal activity of two different botanical pure compounds, camphor and menthol against adult stage of stored product insect pests, *T. confusum* and *C. maculatus*. It was aimed to investigate the effectiveness of these compounds.

The global pest-harvest grain losses by insect damage and other bioagents range from 10% to 40%. Methods used to control stored grain insect pest included physical, chemical, and biological treatments [1]. Chemicals largely used as pesticides in crop protection could have undesirable effects such as ozone depletion, environmental pollution, toxicity to nontarget organism, pest resistance, and pesticide residues, in addition to direct toxicity to users [1]. Therefore, the development of bio insecticides has been focused as viable pest control strategy in recent years [2, 3].

Plants may provide potential alternative to currently used insect-control agents because they constitute a rich source of bioactive chemicals [4]. Aromatic plants are among the most efficient insecticides of botanical origin and essential oils often constitute the bioactive fraction of plant extracts [3].

Essential oils are secondary metabolites abundant in aro-matic plants families such as Lamiaceae and Apiaceae which

contain a large number of compounds such as monoterpens and sesquiterpenes. Essential oils are known to exhibit low toxicity to mammals, and the most terpenoids and phenols found in plant essential oil have minimal toxicity and have even been approved as flavoring agents in food [2, 5]. The insecticidal [6, 7], nematicidal [8], and antibac-terial [9] effects of coriander essential oil have previously been reported.

Coriandrum sativum (Apiaceae) is a native of the Mediterranean region and is grown in North Africa, central Europe, and Asia as a culinary herb and medicament. The essential oil of C. sativum exhibited volatile toxicity to stored product insects. L ´ opez *et al*. [6] fractionated the seeds of Coriander sativum by column chromatography and tested them in the laboratory for volatile toxicity against three stored rice pests, Sitophilus oryzae, Rhyzopertha dominica and Cryptolestes pusillus. Their experiment showed the active compound of coriander essential oil against S. oryzae was linalool, while the fractions that contained mixtures of linalool, camphor, and geranyl acetate were as active against

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2. Material and Methods

A study on the efficacy of botanical pure compound against *T. confusum* and *C. maculatus* adults was conducted during December 2016 to April 2017 under the effect of three different concentration level (0.25, 0.50 and 0.75w/w) of botanical pure compound (camphor and menthol). The wheat grain variety (*Triticum vulgare*) and cowpea seeds variety (*Vigna sinensis*) obtained from the market were selected for the culture and sample preparation during the experimental study. The conditioned samples were cleaned from husks, dust or any inert material then stored at room temperature in sealed bags in the Laboratory of plant protection until used for the experiments. Culture of insect was prepared and grown in the laboratory at a temperature of 30 ± 2 ˚C, 12: 12 L: D and 70% RH kept in plastic jar of 1kg capacity.

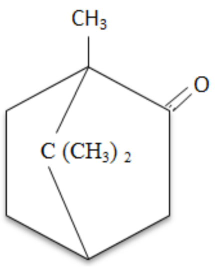
2. 1 Laboratory maintenance of the experimental insects

Two major stored-grain insects, confused flour beetle, *T. confusum* Jaquelin du Val (Coleoptera: Tenebrionidae) and the bruchid beetle, *C. maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchidae) were used in the present experiments. cleaned and sterilized (heating at 70 ºC for 1hr) wheat grain and cowpea seeds samples were placed in glass jars separately to reabsorb moisture. Then, transferred amounts 300 gm cowpea beans and 400 gm wheat grain to depth of 5 cm to separately sterilized culture jars. A small populations of flour beetle and pulse beetle with equal sexual ratio ware released from previous culture under laboratory conditions on wheat grain and cowpea seeds inside a growth chamber at 30 ºC ±2 12: 12 L: D and with 70±5 RH. The growth chamber was sealed with muslin and the beetles were allowed for matting and oviposition. Respectively after two weeks and one week, the parental insects were discarded or transferred to another jar and infested wheat grain and cowpea seed containing eggs were transferred to fresh wheat grain and cowpea seed respectively in the in the breading jars those were covered with pieces of cloth fastened with rubber band to prevent the contamination and escape of beetles. Adult of flour beetle (10-15) and cowpea beetle (2-4) days after emergence ware used for experiment work, according to Hamid *et al*. [19].

2. 2 Botanical pure compound

A- Camphor

- Chemical name: 1, 7, 7-Trimethyl bicycle [2.2. 1] heptan-2-one.
- Structural formula:

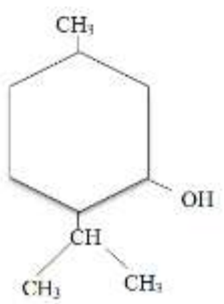


- Empirical formula: [$C_{10}H_{16}O$].
- Molecular weight: 152.23.
- Solubility: Slightly soluble in water and highly soluble in organic solvents.
- Melting point: 179.75℃.
- $LC5_0$:3000mg/kg (oral in mice).
- Physical form: White crystals.
- Source: It was supplied by E. Merck. Darmstadt Germany.

These informations were recorded from Anonymous [20].

B- Menthol

- Chemical name: 5 methyl-2-(1-methyl ethyl) cyclohexanol.
- Structural formula:



- Empirical formula: [$C_{10}H_{20}O$].
- Molecular weight: 156.26.
- Solubility: Slightly soluble in water, very soluble in alcohol, chloroform, ether, petroleum-ether, freely soluble in glacial acetic acid.
- Melting point: 41-4375℃.
- $LC5_0$: 3180mg/kg (oral in rate).
- Physical form: Transparency crystals.
- Source: It was supplied by E. Merck. Darmstadt Germany.

These informations were recorded from Anonymous [20].

2. 3 Residual film technique

Preliminary concentrations (0.25, 0.5 and 0.75%) of the botanical pure compounds (camphor and menthol) were prepared in acetone and evaluated against *T. confusum* and *C. maculatus* beetles. One ml of each concentration was put in each petri dish (9 cm). After evaporation of acetone, 20 adults were placed in each petri dish. Mortality percentages were recorded after 24, 48 and 72 hours of exposure.

Accordingly, series of different concentration of camphor and menthol were prepared and used to evaluate of the toxicity against *T. confusum* and *C. maculates* beetles. Mortality percentages were recorded after 72 hours. The LC_{50} values were calculated from concentration mortality regression lines. The slope of the concentration's mortality regression line and LC_{50} confidence limits were calculated as described by Litchfield and Wilcoxon method [21].

3. Results

3. 1 Effect of Botanical pure compound (camphor and menthol) on *T. confusum* and *C. maculatus* adults of different exposure time by residual film technique

This experiment was conducted in order to determine the insecticidal activity of used botanical pure compound on *T. confusum* and *C. maculatus* adult. In all cases, considerable differences in insect mortality were shown with different botanical pure compound, different concentration at different exposure period.

Mortality percentage of *T. confusum* and *C. maculatus* adult treated with tested botanical pure compound (camphor and menthol) with different concentrations at 24, 48- and 72-hours exposure period are shown in Table 1.

Camphor at 24 hr of exposure time gave no any number of mortality percentages of *T. Confusum* adult at all used different concentration (0.25, 0.50 and 0.75%) and menthol at 0.25%. The highest value of mortality percentages (40.0 and 23.8%) were observed at 72 hr exposure time on menthol treated treatments at 0.75 and 0.50% concentration, respectively, followed by camphor (21.0%) with 0.75% concentration at 72 hr. Significant differences ($P < 0.05$) were recorded among the treatment, treated with same concentration of camphor and menthol at all used exposure period, except between camphor and menthol with 0.25% concentration at 24 and 72 hr.

Complete (100%) mortality of *C. maculatus* adult was recorded on menthol treated treatment with 0.50 and 0.75% concentration levels at all used exposure periods and camphor with 0.75% at 72hr.The lowest value of mortality percentages (9.9, 10.6, 12.4 and 12.4%) were recorded on camphor followed by menthol (12.5, 15.1 and 18.5) with 0.25% concentration at 24, 48 and 72 hr exposure periods, respectively. Significant and non-significant differences ($P < 0.05$) among the treatment, treated with same concentration of camphor and menthol at all used exposure periods are available in Table 1.

The results of this experiment showed that, mortality percentages increased with increase of concentration level and exposure periods, *C. maculatus* was more susceptible than *T. confusum* against the used botanical compounds and in general menthol was more effective than camphor for the control of *T. confusum* and *C. maculates.*

Table 1: Efficacy of camphor and menthol to *T. confusum* and *C. maculatus* adults of different exposure time by residual film technique

Oils	Conc. % W/W	*T. confusum*			*C. maculatus*		
		Mortality (%) after			Mortality (%) after		
		24 hr. % ± SD	48 hr. % ± SD	72 hr. % ± SD	24 hr. % ± SD	48 hr. % ± SD	72 hr. % ± SD
Camphor	0.25	0.0 ± 0.0	2.3 ± 1.2	8.5 ±1.9	9.9 ± 0.3	10.6 ± 0.2	12.4 ± 0.3
	0.50	0.0 ± 0.0	3.4 ± 1.1	16.9 ± 1.3	72.9 ± 6.5	78.9 ± 7.2	86.9 ± 3.5
	0.75	0.0 ± 0.0	6.0 ± 0.0	21.0 ± 0.0	83.9 ± 5.4	95.9 ± 3.8	100.0 ± 0.0
Menthol	0.25	0.0 ±0.0	6.8 ± 1.8	12.8 ± 1.5	12.5 ± 4.0	15.1 ± 5.0	18.5 ± 1.7
	0.50	8.8 ± 1.9	10.0 ± 0.0	23.8 ± 1.6	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	0.75	10.0 ± 0.0	19.4 ± 1.5	40.0 ± 1.3	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Control		0.0 ±0.0	0.0 ±0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
LSD ≤ 0.05	0.25	------	3.2	4.6	5.7	9.1	8.0
	0.50	3.3	3.3	4.6	15.3	14.1	4.9
	0.75	3.3	3.2	3.2	19.1	8.3	------

*Angular transformation was done for the percentage values.

LC50 value of the camphor and menthol showed that *T. confusum* has displayed greater sensitivity to menthol compound than camphor. Similarly, menthol is greatly toxic against *C. maculatus* comparative to camphor (Table 2).

Table 2: LC50 value of the camphor and menthol to the *T. confusum* and *C. maculatus*

Compound	*T. confusum*							*C. maculatus*						
	Slope function	LC_{50} %	Confidence limits		Slope	$(Chi)^2$		Slope function	LC_{50} %	Confidence limits		Slope	$(Chi)^2$	
			lower	upper		Calc.	Tab.			lower	upper		Calc.	Tab.
Camphor	2.07	0.62	0.08	1.0	3.16	4.6	7.82	1.44	0.48	0.30	0.73	5.18	3.6	7.82
Menthol	1.80	0.45	0.07	0.89	3.94	6.88	7.82	1.34	0.33	0.25	0.63	6.22	7.2	7.82

4. Discussion

The results of our experiment demonstrated that, *C. maculatus* was more susceptible than *T. confusum* against the used botanical pure compounds; in general menthol was more effective than camphor for the control of *T. confusum* and *C. maculatus* and mortality percentages increased with increase of concentration level and exposure periods. This was in agreement with finding of Samir *et al*. [18] who found out that, toxicity of the test monoterpenes varied with insect species and bioassay method. For example, in contact assays, geraniol, linalool and menthol were more toxic to *S. oryzae*, whereas carvone, cuminaldehyde and fenchone were more effective on *T. castaneum*. Camphene, camphor, cineole, limonene and myrcene had approximately the same toxicity against both insects. Most test compounds in fumigation assays were more effective toward *S. oryzae* and *T. castaneum*, except for camphene, camphor and geraniol that had weak activity toward both insects. Differential toxicities to *S. oryzae* and *T. castaneum* by monoterpenes have been noted previously [22, 23].

5. Conclusion

The results of our experiment demonstrated that, *C. maculatus* was more susceptible than *T. confusum* against the used botanical pure compounds. In general, menthol was more effective than camphor for the control of *T. confusum* and *C. maculatus* and mortality percentages increased with increase of concentration level and exposure periods.

6. Acknowledgment

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7. References

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