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Efficacy of Some Botanical Oils against Stored-product Pest Cowpea Beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)

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Abstract
The stored pulse grain experiences major threat from insect pests. *Callosobruchus maculatus* is one of the major pests infesting stored pulses worldwide. In this study the insecticidal activity of eight botanical oils, Citronella, Clove, Eucalyptus, Jojoba, Lemon, Orange, Rosemary, Spearmint were tested against the adults of *C. maculatus* in the laboratory. The results of this study showed that all used oils have potential of protection to control storage product pests, *C. maculatus.* The highest mortality was observed on clove and jojoba followed by rosemary eucalyptus and citronella. The mortality was increased with increase of concentration levels and the duration dependent increased percent mortality was observed in all botanical oils after interval of different exposure time. The present study suggested that botanical oils (Clove, Jojoba, Rosemary and Eucalyptus) exhibited a possibility to be an alternative to the synthetic insecticides in the management of stored product pests, especially *C. maculatus.*

Keywords: efficacy, botanical oils, stored pulse grains, *callosobruchus maculatus*, mortality

1. Introduction
The pulses are considered to be an important source for fulfilling the protein needs for vegetarian and low income groups of the population in many regions of Asia and they are also referred to as poor man's meet. Cowpea, {*Vigna unguiculata* (L.) Walp.} is an important grain legume grown in Asia, the Mediterranean, Australia, Canada, the USA and Africa [1]**.** Insect pests are the major constrain in cowpea production and storage [2-4]. The cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) is a primary pest of grain [5] which is widely distributed throughout the world and also it is a major pest of economically important leguminous grains, such as cowpea, green gram, lentils and black gram [6-8]. They are important pests of pulse crops in Asia and Africa under storage conditions [9 -12]. They are causes weight losses up to 30% occurred after six month of storage which made 70% of the grain unfit for human consumption [13]. Also, after a few months of storage, 100% losses were inflicted in Benin, due to infestation by *C. maculatus* and *Bruchidus atrolineatua* (F.) [14]. This situation makes necessary the application of control measures, in order to prevent or minimize the losses caused by this pest.
Many methods have been used to prevent these post-harvest losses. Among those, chemical or synthetic insecticides have serious drawbacks, such as genetic resistance by insect species, direct toxicity to beneficial insects, human, residual toxicity, widespread environmental hazards and increasing costs of application. These serious problems of the presently used synthetic pesticides have directed the need for effective, biodegradable pesticides [15-19]. Various recommendations of storage grain protection, using different natural products to kill or keep away the infesting insect pests are advised by the researcher of yesteryears. The toxicity of large number botanical oils was assessed against several major stored product insects [20-23]. Botanical oils effectively controlled three bruchid beetles infesting stored legumes [24]. This awareness has generated worldwide interest in the development of alternative and environmental safe strategies, including the re-examination of using botanical oils and their derivatives against different stored product insect pests. Plant-derived materials are more readily biodegradable [22] and may retard the development of resistance in insects. Some are less toxic to mammals and may be more selective in action [22]. Their main advantage is that they may be easily and cheaply produced by famers and small-scale industries as crude, or partially purified extracts.
The present investigation was undertaken to determine the efficacy of eight locally available plant oils, Citronella, Clove, Eucalyptus, Jojoba, Lemon, Orange, Rosemary and Spearmint against stored grain insect pest *C. maculatus.* It was aimed to investigate the effectiveness of these oils.

2. Materials and Methods
A study on the Efficacy of botanical oils against *C. maculatus* adults was conducted in the laboratory of plant protection during March to April, 2016. Botanical oils, scientific names of plants, used parts in extraction of oils and source of oils are listed in Table 1.

Table 1: Plant oils and their scientific names, used part in extraction of oil and sources.

Oils	Scientific name of plants	Used parts	sources
Citronella	*Cymbopogon winterianus*	Leaves	From the market
Clove	*Eugenia aromatic*	Flowers	″
Eucalyptus	*Cinnamomum camphora*	Leaves	″
Jojoba	*Simmondsia chinensis*	Seeds	″
Lemon	*Citrus limonun var hisso*	Fruit peels	″
Orange	*Citrus sinensis var Valencia*	Fruit peels	″
Rosemary	*Rosmarinus officinalis*	Leaves	″
Spearmint	*Mentha viridis*	Leaves	″

2.1 Culture preparation

The whole cowpea seeds was sieved and cleaned from dust, husks or any inert materials. Samples of cowpea seeds were placed in glass jars, covered and sterilized by heating at 70 ºC for 1hr. Cowpea seeds were cooled and allowed to reabsorb moisture before use. Then amount of 300 gr cowpea seeds transferred to separately sterilized culture jar. About 150 adults of cowpea beetle, *C. maculatus* (mixed sexes) from the previous culture were added in to each jar and the jar sealed with muslin and placed at 30 ºC ± 2.0 and 75 ± 5.0 RH. After one week, the insects were sieved out, discarded or transferred to another jar. The jar contained eggs kept until emergence of adults. The period from egg laying to adult emergence was about 3 weeks. Adult insects, 2-4 days after emergence were used for experiment work.

2.2 Bioassay test

The insecticidal efficacies of different botanical oils (Table 1) were carried out by residual film technique against the *C. maculatus* beetle. The residual film was done directly on petri dish (9 cm) without any food media according to Qi and Burkholder [25]. Oil concentrations were (0.25, 0.5, 0.75%) which was prepared in acetone. One ml of each concentration was pipetted in petri dish. After evaporation of acetone, 20 adults (mixed sixes) were placed in each petri dish. Three replicates were carried out of each concentration and control. Mortality percentages were recorded after 24, 48 and 72 hours. Four Oils that caused higher mortality% after 72 hr. at 0.5% against *C. maculatus* were considered potent oils. The average mortality percentages were plotted against the concentrations mortality regression lines were eye fitted. The LC_{50} values were determined. Data were subjected to probit analysis.

3. Results

3.1 Direct toxicity of eight botanical oils on adult insects of *C. maculatus*

This experiment was conducted in order to determine the insecticidal activity of used botanical oils on *C. maculatus* adult. Residual film was done directly on petri dish (9 cm) without any food media. In all cases, considerable differences in insect mortality were shown with different botanical oils, different concentration at different exposure times. The efficacy of each treatment was determined by comparing numbers of mean mortality percentage with those in the treated control. The effectiveness of eight botanical oils (Jojoba, Clove, Eucalyptus, Lemon, Rosemary, Orang, Spearmint and Citronella) was compared with each other. Three concentration dosages; 0.25, 0.5 and 0.75% were compared for each oil and three exposure periods; 24, 48 and 72 hours were compared for each concentration, to find out the effective botanical oils and there suitable concentration dosage and exposure time for the control of cowpea beetle.

The mean percentage mortality of *C. maculatus* adult, treated with different botanical oils at different concentration and different exposure periods for each concentration are presented in Table 2, 3 and 4.

At concentration 0.25%, the highest mortality percentage was recorded in clove treated treatments followed by jojoba that, at 24 hours 98.3 and 65.5%, 48 hours 100.0 and 71.4% and 72 hours 100.0 and 75.3% mortalities. Moderate mortality percentage was observed in treatment treated with rosemary that, at 24, 48 and 72 hours of exposure times were 53.2, 58.3 and 60.7% respectively. The mean mortality percentages were lower in eucalyptus, lemon, citronella, orang and spearmint treated treatments at 24, 48 and 27 hours of exposure periods respectively (Table 2). The result showed that, the mortality percentage was increased with increase of exposure times at all treated treatment.

Complete (100%) mortality percentage at 0.5% concentration was recorded in clove treated treatment after all exposure times (24, 48 and 72 hours). Jojoba, rosemary and eucalyptus treated treatments showed 78.3, 77.2 and 75.2% mortalities at 24 hours, 88.2, 83.3 and 80.9% at 48 hours and 95.2, 59.2 and 90.8% at 72 hours of exposure periods respectively. The citronella, spearmint, lemon and orange oils also gave protection, but mean percentages of mortality were appreciable in citronella that, 33.5, 36.3 and 42.5% after interval of 24, 48 and 72 hours respectively. Spearmint, lemon and orange gave the lower percent mortalities at all mentioned exposure periods (Table 3). The duration dependent increased percent mortality was observed in all botanical oils after intervals of 24, 48, 72 hours.

Clove, jojoba, citronella, rosemary and eucalyptus oils gave higher protection at concentration 0.75%, but clove and jojoba oils appeared to be superior to citronella, rosemary and eucalyptus oils. The mortality was increased with increase of exposure time. Complete (100%) protection against the beetle was obtained by clove and jojoba at all exposure times (24, 48 and 72 hours), citronella at 48 and 72 hours and rosemary and eucalyptus at 72 hours. Appreciable mean percentage mortality was recorded in spearmint treated treatment that, 52. 3, 60.1 and 78.1% at interval of 24, 48 and 72 hours respectively. The lower percentage mortalities were given by orange and lemon (Table 4).

The results showed that, the mortality of the progeny followed a similar trend, with higher mortality observed on all treatments compared with the control. The highest mortality was observed on clove and jojoba followed by rosemary, eucalyptus and citronella. The mortality was increased with increase of concentration dosages and the duration dependent increased percent mortality was observed in all botanical oils after interval of different exposure time that, mortality was increased with increase of exposure time.

Table 2: Efficiency of some botanical oils at concentration 0.25% against *C. maculatus* at different exposure time by residual film technique.

Oils	Mortality (%) after		
	24hr ± SD	48hr± SD	72hr± SD
Control	0.0± 0.0	0.0± 0.0	0.0± 0.0
Clove	98.3± 2.1	100± 0.0	100.0± 0.0
Jojoba	65.5±3.2	71.4± 5.1	75.3± 2.2
Eucalyptus	18.1± 1.9	22.3± 4.2	33.8± 4.3
Lemon	7.1± 1.1	10.2± 2.1	15.2± 3.1
Rosemary	53.2± 3.4	58.3± 2.6	60.7± 4.3
Orange	3.4± 1.1	8.1± 2.3	12.3± 3.3
Citronella	8.8± 2.2	10.3± 3.1	11.2± 3.3
Spearmint	2.6± 1.2	4.2± 1.1	6.3± 2.2
LSD≤ 0.05	8.1	10.8	11.9

Table 3: Efficiency of some botanical oils at concentration 0.5% against *C. maculatus* at different exposure time by residual film technique.

Oils	Mortality (%) after		
	24hr ± SD	48hr± SD	72hr± SD
Clove	100.0± 0.0	100± 0.0	100.0± 0.0
Jojoba	78.3±4.3	88.2± 2.2	95.2± 3.1
Eucalyptus	75.2± 3.4	80.9± 3.6	90.8± 3.7
Lemon	10.5± 4.1	15.2± 2.8	20.3± 3.1
Rosemary	77.2± 3.3	83.3± 3.1	95.2± 2.2
Orange	6.2± 2.1	10.3± 4.1	16.1± 2.5
Citronella	33.5± 4.2	36.3± 2.0	42.5± 3.1
Spearmint	11.2± 1.0	17.3± 1.3	25.3± 3.1
Control	0.0± 0.0	0.0± 0.0	0.0± 0.0
LSD ≤ 0.05	13.2	12.3	14.2

Table 4: Efficiency of some botanical oils at concentration 0.75% against *C. maculatus* beetle by residual film technique.

Oils	Mortality (%) after		
	24hr ± SD	48hr± SD	72hr± SD
Clove	100.0± 0.0	100± 0.0	100.0± 0.0
Jojoba	100.0± 0.0	100.0± 0.0	100.0± 0.0
Eucalyptus	76.2± 3.2	92.8± 3.8	100.0± 0.0
Lemon	12.5± 0.3	20.6± 4.1	28.2± 2.4
Rosemary	88.2± 1.5	93.2± 1.1	100.0± 0.0
Orange	18.3± 4.1	20.1± 4.4	22.1± 1.5
Citronella	94.3± 2.2	100.0± 0.0	100.0± 0.0
Spearmint	52.3± 3.1	60.1± 5.2	78.1± 5.6
Control	0.0± 0.0	0.0± 0.0	0.0± 0.0
LSD ≤ 0.05	9.4	11.2	13.8

3.2 Efficacy of four potent botanical oils against *Callosobruchus maculatus*

This study was carried out by residual film technique directly on petri dish (9 cm) without any food media, in order to determine the insecticidal activity of used potent botanical oils on *C. maculatus* adult at different concentration. Five concentration dosages; 0.20, 0.25, 0.30, 0.35 and 0.40% were compared for each oil at 72 hours of exposure time, to find out the effective botanical oils and there suitable concentration dosage for the control of cowpea beetle. The LC_{50} values were determined.

The data obtained on mortality percentage of *C. maculatus* adult treated with different potent botanical oils were combined (Table 5 and 6). The values of mean percentage mortality of *C. maculatus* adult were shown in Table 5. The most toxic botanical oil was clove that gave 26.12, 40.31, 61.22, 85.30 and 92.00% mortality at 0.20, 0.25, 0.30, 0.35 and 0.40% concentration levels respectively and the least toxic botanical oil was rosemary that gave 13.52, 25.46, 50.82, 62.44 and 74.30% mortality at 0.20, 0.25, 0.30, 0.35 and 0.40% concentration levels respectively followed by eucalyptus and jojoba. Considerable differences were recorded among all treated treatment. Parallel response to the level of concentration that the mortality percentage increased with increase in concentration level in most cases. The concentration proved able to induce maximum percentage mortality of adult 92.00, 90.00, 80.09 and 74.30 at 0.40% in clove, jojoba, eucalyptus and rosemary respectively. As shown in the same table the order of different concentration of oils on mortality of *C. maculatus* adult increased with increase of concentration level in all treated treatment. The concentration 0.40% was most toxic to *C. maculatus* and caused 92.00, 90.00, 80.09 and 74.30% mortality in clove, jojoba, eucalyptus and rosemary respectively, followed by 0.35, 0.30, 0.25 and 0.20%.

Table 5: Efficacy of the potent botanical oils against *C. maculatus* adults

Concentration%	Mortality% after 72hrs			
	Clove	Jojoba	Eucalyptus	Rosemary
0.20	26.12	24.43	20.61	13.52
0.25	40.31	38.32	35.32	25.46
0.30	61.22	52.81	47.95	50.82
0.35	85.30	77.24	58.62	62.44
0.40	92.00	90.00	80.09	74.30

The LC50 value of the botanical oils presented that jojoba oil most effective against *C. maculatus.* Clove oil is less effective against *C. maculatus* comparative with rosemary and eucalyptus oils (Table 6)

Table 6: LC50 values of the botanical oils against *C. maculatus*

Oils	Slope function	LC 50	Confidence limits		Slope	$(Chi)^2$	
			Lover	Upper		Calculate	Tabulate
Clove	1.43	1.23	0.112	0.132	6.41	2.19	7.82
Jojoba	2.08	0.139	0.116	0.68	3.08	3.08	7.82
Eucalyptus	2.14	0.155	0.133	0.178	3.02	1.09	9.49
Rosemary	1.44	0.310	0.280	0.350	6.10	7.13	9.49

4. Discussion

Botanical oils can play an important role in stored product pests control and reduce the risks associated with, the use of conventional insecticides. The toxicity of a large number of botanical oils extracted from various spices and herb plants was assessed against several major stored product insects [26, 27, 28, 29, 30, 20, 31, 21, 32, 33, 23, 22, 34].

Although an increasing number of plant oils have been screened for use in preventing post-harvest losses due to insects, most of this work has demonstrated action against bruchid pests of

cowpea or other pulses [35-37]. However, Qi and Burkholder [25] showed that botanical oils could protect wheat from the granary weevil, *Sitophilus granarius* (L.). Zhang and Zho [38] also demonstrated the effectiveness of such oils in protecting rice from *Sitophilus oryzae* (L.) and *S. zeamais*.

The result of this study has shown that all applied botanical oils significantly affected the mortality percentages of *C. maculatus* compared with control, Not all the botanical oils tested showed satisfactory activity, but the botanical oils of clove, jojoba, rosemary and eucalyptus, proved to be promising as control against stored-product insects, especially *C. maculatus*. Our results and those reported earlier indicate variations in the activity of botanical oils regarding the stage of the insect, the species of the insect the plant origin of the oils, concentration level of the oils and exposure time. For example, while some workers [35, 39] reported that botanical oils had no effect on mortality or longevity of adult bruchids, others [40, 41] reported a high level of toxicity, against adult bruchids and *S. oryzae* respectively.

In the present study with adult *C. maculatus*, applied oils caused appreciable mortality only at higher rates of concentration level and the mortality was decreased with decrease of concentration levels. This was in agreement with Qi and Burkholder [25] who demonstrated that significant mortality occurred only when adult *S. granarius* were exposed to grains freshly treated with a high rate (10 ml/kg) of groundnut oil. The present study further showed that the mortality effect at high concentrations decreased or disappeared when adults were exposed to love exposure time. It is suggested, therefore, that the very high 24 hr mortality of adult *Sitophilus* sp. on oil-treated maize grains reported by Ivbijaro *et al.* [41] was possibly a result of the insects drowning in large quantities of free surface oil.

Although the toxicity levels achieved are lower than those that would be expected from the use of commercial insecticides [42], it would seem worthwhile to explore the possible joint action of oils and conventional synthetic insecticides in simple mixtures as a means of making their use more cost effective and attractive. Such an approach would have the added advantage of minimizing the use of synthetic insecticides thereby reducing possible deleterious side effects associated with their use on stored food produce.

5. Conclusion

This study has shown that all used botanical oils significantly affected the mortality of *C. maculatus*. With adult *C. maculatus*, applied oils caused appreciable mortality only at higher rates of concentration level and the mortality was decreased with decrease of concentration levels. The present study further showed that the mortality effect at high concentrations decreased or disappeared when adults were exposed to love exposure time. Not all the botanical oils tested showed satisfactory activity, but the botanical oils of clove, jojoba, rosemary and eucalyptus, proved to be promising as control against stored-product insects, especially *C. maculatus*.

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