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Efficacy of Botanical Oils on Adult Mortality, Egg Laying, Hatchability and Adult Emergence of *Callosobruchus. Maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchidae)

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ABSTRACT: The cowpea weevil *Callosobruchus maculatus* Fabricius, is the major insect pest that threatens effective legume storage. The laboratory experiment was conducted to assess the efficacy of four different botanical oils (Clove, Eucalyptus, Jojoba and Lemon) against the cowpea weevil, *C. maculatus* by using residual film technique. All the botanical oils were applied at two different concentration levels (0/50 and 0.75%). The results showed that all used oils significantly increased the mortality and reduced the mean number of eggs laying, hatchability and emergence percentages compared to the untreated control. The treatment of cowpea seeds with oils reduced slightly the germination but swelling percentage was higher in treated seeds than control. The results also indicated variation in the activity of botanical oils regarding the stage of the insect, the plant origin of the oils and concentration level. The present study suggested that botanical oils (Clove, Eucalyptus, Jojoba and Lemon) may be preferred option than conventional pesticides far the protecting stored pulse grains against stored products insect pests' infestation, especially *C. maculatus*.

KEYWORDS: Botanical oil, Control, Cowpea weevil, Stored seed grain, Toxicity.

I. INTRODUCTION

A major problem in storage is the infestation by the insects, which cause high economic losses in quality and quantity by feeding on stored food grain and endanger the public health by contamination of food (Pimentel, 1991). The cowpea weevil, *Callosobruchus maculatus* (F) is one the cosmopolitan and destructive insect pest that causes damage to various legumes in storage conditions (Demitry *et al*., 2007; Mahmoud and Mohamed, 2015). Numerous synthetic insecticides are employed to manage insect pest in storage. The repeated use of this insecticide resulted in numerous problems, including side effect in humans and adverse residual effects in foods. Owing to the drawbacks of using such insecticides in storage pest management, many researchers have encouraged the pursuit of healthier alternatives. Various botanical oils used to manage grain storage insect pests are effective and eco-friendly (Paneru and Shivakoti, 2001). Isman (2006; Kafle and Shih, 2013) contended that botanical insecticides are the best option for controlling of insect pests in storage in developing countries. Botanical oils are promising alternative to insecticides for insect pest management. Plant oils are naturally occurring insecticides and include a variety of bio-active compounds (Isman, 2000; Sammour, *et al*., 2018). Essential oils of plant exert ovicidal and larvicidal effects on insects, inhibit respiration, suppress oviposition, act as antifeedants and reduce adult emergence due to their fumigant and topical toxicity (Regnault- Roger, 1997; Sarwar and Salman, 2015; Ali, *et al*., 2017). Since the 1970s, thousands of plants have been screened as potential sources of repellents and toxicants (Sukumar *et al*., 1991). According to Jumbo *et al*. (2018), Application of clove oils in storage condition, controlled cowpea weevil damage to crop and was capable of reducing the oviposition and population growth of cowpea weevil even at sublethal dosages. Therefore, the present study aimed to evaluate the long-term efficacy of four commonly available botanical oils (Clove, Eucalyptus, Jojoba and Lemon) on toxicity and some biological aspects of the cowpea weevil under laboratory conditions.

II. MATERIALS AND METHODS

A study on the efficacy of botanical oils against *C. maculatus* was conducted in the laboratory of plant protection under the effect of two different concentration level (0.50 and 0.75%) of botanical oils (Clove, Eucalyptus, Jojoba and Lemon). The cowpea seeds variety (Vigna sinensis) obtained from the market were selected for the culture and sample preparation during the

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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.

A. Laboratory maintenance of the experimental insect

The stored-grain insect, bruchid beetle, *C. maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchidae) was used in the present experiments. cleaned and sterilized (heating at 70 ºC for 1hr) cowpea seeds samples were placed in glass jar to reabsorb moisture. Then, transferred amount 300 gm cowpea beans to depth of 5 cm to sterilized culture jar. A small population of pulse beetle with equal sexual ratio was released from previous culture under laboratory conditions on 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. After one week, the parental insects were discarded or transferred to another jar and infested cowpea seed containing eggs were transferred to fresh cowpea seed in the breading jar that was covered with pieces of cloth fastened with rubber band to prevent the contamination and escape of beetles. Adult of cowpea beetle (2-4) days after emergence ware used for experiment work, according to Hamid *et al*. (1968).

B. Botanical oils

Four plant oils (healthy and fresh) were used in this investigation. 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
Clove	*Eugenia aromatic*	Flowers	From the Market
Eucalyptus	*Cinnamomum camphora*	Leaves	″
Jojoba	*Simmondsia chinensis*	Seeds	″
Lemon	*Citrus limonun var hisso*	Fruit Peels	″

C. Residual effect of botanical oils on mortality, egg laying, hatchability and adult emergence of C. maculatus

Cowpea seeds were treated by oil at different concentration (0.5 and 0.75%) of jojoba, clove, eucalyptus and lemon oils. Treated seeds were infested with newly adults (10 pairs/ 20 gm seeds). Mortality percentages were recorded after one week. The mean number of transparent or opaque laid eggs on cowpea was counted after two weeks of infestation. Hatching and emergence percentage were also calculated. Three replicates were carried out for each treatment and control.

D. Germination tests

Germination tests were done for the treated cowpea seeds with botanical oils after one month of treatments. The results were recorded after four days.

E. Swelling tests

Swelling tests were carried out for the treated cowpea seeds with botanical oils after one month of treatments. The results were recorded after one hour of submerging.

F. Statistical analysis

All obtained data were statistically analyzed using Finney (1971) software. Comparisons among the means of the various treatments were performed, using the revised least significant different (L. S. D) at < 0.05 level. Angular transformation was done for the percentage values.

III. RESULTS

A. Residual effect of botanical oils on adult mortality, egg laying, hatchability and emergence of C. maculatus beetle

The data observed on adult mortality, eggs laying on cowpea seeds, hatchability and emergence of F1 progeny are shown in table (2).

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The maximum percentage mortality was observed on the clove oil treated treatments that at 0.75 and 0.50% concentration levels were 93.2 and 84.6%, respectively. Moderate percent mortality was recorded on jojoba oil 38.1% at 0.50% and 63.3% at 0.75% concentrations. The lowest mortality was shown by lemon oil (at 0.50 and 0.75% concentration levels) were 37.3 and 48.3% followed by eucalyptus oils 34.6 and 53.8%, respectively. Increase in concentration level resulted in an increase in average mortality. Mean percentage mortalities were significantly different ($P \leq 0.05$) with in the oils treated treatment and control, except between eucalyptus and lemon oils (table 2).

The plant oils significantly reduced the number of eggs laid by cowpea beetle on the cowpea seeds ($P \leq 0.05$) with in treatment and control (table 2). There were no eggs laid on the Clove oil treated treatment at 0.75% concentration level, but in the same oil treated treatment the mean number of eggs laid was 6.6 at 0.50% concentration. At 0.50% concentration level, significantly more eggs were laid on the eucalyptus treatment (108.7) followed by jojoba (136.6) and lemon (143.3) and still more were laid on the control. After clove oil the eucalyptus oil was more effective at 0.75% concentration, that gave 28.7 mean of eggs laid, whereas jojoba and lemon oils gave 104.0 and 118.0, respectively. The results showed that clove oils at both concentration levels used (0.5 and 0.75%) gave the lowest number of eggs laid followed by eucalyptus (at 0.75%). Jojoba and lemon oils gave the highest number of eggs laid on cowpea seeds at 0.75% concentration level. Numbers of eggs laid were reduced with increase of concentration level. However, clove and eucalyptus oils at 0.75% concentration level were more effective against *C. maculatus*, whereas the number of eggs laid reached down up to 0.0 and 28.7, respectively.

As expected, the hatchability percentage of the eggs followed a similar trend, which lowers hatchability percentages were observed on all treatments compared with control. The higher hatchability% was observed on eucalyptus and lemon oils (80.0 and 74.6%) at 0.5% concentration level, which in turn was lower than the control (table 2). The lower hatchability% was observed on clove and jojoba oils (at 0.75% concentration level) 0.0 and 26.4%. Hatchability% on lemon and eucalyptus oils at 0.75% concentration level were 51.4 and 60.3%, and clove and jojoba oils gave 25.6 and 49% hatchability, at 0.5% concentration, respectively. Hatchability% was also found to be reduced in treatment with increase of concentration levels. The mean of hatchability% were significantly different ($P \leq 0.05$) with in the oils treated treatment and control. All oils ware effective to reduce eggs hatchability, but the Clove oil was superior effective than others that gave 12.8% mean hatchability, followed by jojoba (38.1%). The eucalyptus was less effective followed by lemon that gave 70.2 and 63.0% mean hatchability, respectively.

The F1 progeny emergence of adult beetles was also found to be higher reduced by plant oils treatments. Clove and eucalyptus oils were more effective that gave 0.0 and 3.0 emerged adults (at 0.50% concentration level), 0.0 and 2.0 (at 0.75%), respectively followed by jojoba and lemon oils that 3.0 and 19.3 at 0.75%, 5.0 and 37.7 at 0.50%, respectively. A considerable reduction in the mean number of emerged adults was exhibited on the oils used treatments compared with control. Reduction percentage of adult emergence was increased with increase of concentration levels. In the reduction percentage mean followed by the same later were not significantly different at $P \leq 0.05$ (table 2).

The result of these experiments showed that different botanical oils have no similar effect on mortality, egg laying, hatchability and emergence, whereas we observed that after clove the jojoba were more effective on the mortality and hatchability than eucalyptus, but the eucalyptus wans more effective than jojoba on the egg laying and emergence.

Table 2. Residual effect of botanical oils on mortality, egg laying, hatchability and emergence of *C. maculatus* beetle

Oils	Conc. % (w/w	Mortality % after one week		Mean no. of laid eggs on cowpea		Hatchability %		Mean no. of emerged adults	Emergence %	Reduction %	
		% ± SD	mean	Total ± SD	mean	% ± SD	mean	mean	% ± SD	% ± SD	mean
Control	……	3.2±2.8	3.2[a]	238.0±9.2	238.0[e]	87.3±4.1	87.3[e]	179.0	75.1±2.1	…….	…. a
Clove	0.50 0.75	84.6±2.5 93.2±2.3	88.9[d]	6.6±0.3 0.0±0.0	3.4[a]	25.6±1.3 0.0±0.0	12.8[a]	0.0 0.0	0.0±0.0 0.0±0.0	100.0±0.0 100.0±0.0	100.0[c]

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Jojoba	0.50 0.75	38.1±2.5 63.3±4.1	50.8[c]	136.6±5.5 104.0±4.2	120.3[c]	49.8±1.7 26.4±1.3	38.1[b]	5.0 3.0	3.6±0.1 2.8±0.0	97.3±3.5 98.3±0.0	97.8[c]
Eucalyptus	0.50 0.75	34.6±1.6 53.8±3.2	44.3[b]	108.7±3.4 98.7±6.5	103.6[b]	80.0±3.7 60.3±1.1	70.2[d]	3.0 2.0	2.7±0.0 2.0±0.3	98.2±0.0 98.9±0.0	98.6[c]
Lemon	0.50 0.75	37.3±1.8 48.3±1.6	42.9[b]	143.3±3.7 118.0±6.1	130.6[d]	74.6±3.1 51.4±4.1	63.0[c]	37.7 19.3	26.3±1.3 16.3±0.9	78.9±6.1 89.2±1.9	84.1[b]
LSD	A B		4.60 5.96		8.83 5.08		2.32 0.87				3.06 2.73

*The mean followed by the same letters are not significantly different at P ≤ 0.05.
*Angular transformation was done for the percentage values.
*A LSD for the effect of different oils comparisons.
*B LSD for the effect of different concentrations comparison (0.5 and 0.75)

B. Residual effect of botanical oils on swelling and germination of cowpea seeds after one month of treatment

Result of germination and swelling of treated cowpea seeds are presented in table (3). Swelling percentage was increased with increase of concentration level and germination percentage was decreased with increase of concentration level. Mean of the swelling and germination percentage followed by the same letters are not significantly different at P < 0.05. Significant difference in germination and swelling percentage was noticed in the treated and untreated seeds.

Table 3. Residual effect of botanical oils on swelling and germination of cowpea seeds after one month of treatment

oils	Conc. % W/W	Swelling% after one hr.		Germination % after 4 days	
		% ± SD	mean	% ± SD	mean
Control	……..	39.5 ± 0.1	39.5a	100.0 ± 0.0	100.0d
Clove	0.50	63.2 ±1.9	64.3b	36.6 ± 4.5	26.6a
	0.75	65.4 ± 2.1		16.6 ± 4.5	
Jojoba	0.50	75.8 ±1.5	77.4e	83.2 ± 4.5	71.7c
	0.75	80.0 ± 2.6		60.0 ± 5.0	
Eucalyptus	0.50	65.4 ± 2.8	69.5c	86.7 ± 6.2	76.7c
	0.75	72.6 ± 2.6		66.7 ± 5.1	
Lemon	0.50	73.2 ± 2.8	74.5d	70.0 ±0.0	63.3b
	0.75	75.9 ± 3.1		56.7 ± 3.5	
LSD	A		1.88		6.21
	B		0.61		5.60

*The mean followed by the same letters are not significantly different at P ≤ 0.05.
*Angular transformation was done for the percentage values.
*A LSD for the effect of different oils comparisons.
*B LSD for the effect of different concentrations comparison (0.5 and 0.75).

IV. DISCUSSION

The botanical oils used in this experiment were considered a potent and promising as control against stored product insect, *C. maculates* and reduce the harmful to humans than other synthetic insecticides. Furthermore, studies have showed that botanical

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oils are less detrimental to non-target organisms than conventional pesticides and readily biodegradable (Tunc *et al*., 2000). Plant oils has been tested for stored product pest control by numerous investigators (Bahaduri *et al*., 1985; Uvah and Ishya, 1992; Xie *et al.*, 1995; Obeng-Ofori and Reichmuth, 1997; Rajapakse and Van Emden, 1997; Shaaya *et al*., 1997; Singal and Chauhan, 1997; Huang *et al*., 2000; Ketoh *et al.*, 2000; Lale and Mustapha, 2000; Tripathi *et al*., 2000; Tunc *et al*., 2000; Keita *et al*., 2001). Most of this work has demonstrated action against bruchid pests of cowpea or other pulses (Singh *et al*., 1978; Messina and Renwick, 1983; Pereira, 1983). This study has shown that all botanical oils investigated in this research significantly protected and reduced progeny development of *C. maculatus*, confirming the results of earlier workers**.** Despite their different properties, origin and purity all the oils tested in the present study were almost equally effective in mortality and reducing progeny development. Our results and those reported earlier indicate variations in the activity of botanical oils regarding the stage of the insect, the plant origin of the oils and concentration level.

Based on similar observations, it has been suggested that the activity of such oils against insect eggs was by a general physical property of the oil coating rather than a specific chemical action (Hill and Shoonhoven, 1981; Messina and Renwick, 1983). Most workers have observed a reduction in oviposition of bruchid beetles on oil-treated grains (Singh *et al*., 1978; Messina and Renwick, 1983), while others, especially Pereira (1983) have reported no significant action. A part from the ovicidal activity of botanical oils there is less agreement on their effects against other stages in the life cycle of insects. For example, while some workers Singh *et al*. (1978) and Tikku *et al*. (1981) reported that botanical oils had no effect on mortality or longevity of adult bruchids, others Hill and Shoonhoven (1981) and Ivbijaro *et al.* (1984) reported a high level of toxicity, against adult bruchids and *S. orvzae* respectively.

The results of the present work confirmed those of earlier reports of Qi and Burkholder (1981) and Zhang and Zho (1983) that fixed vegetable oils significantly reduce adult progeny emergence of *S. zeamais* on wheat, but the action of the oils against eggs and larvae which live within the seeds was not determined, by employing X-ray radiographic techniques in this work. Thus, any reduction in progeny development appears to have been due to effects on oviposition or on egg development. On cowpeas, only neem oil significantly reduced the expected emergence. Schoonhoven (1978) reported a reduced emergence of *Z. subfasciatus* on beans treated with cottonseed and African palm oils, while Su *et al.* (1972) reported a reduced emergence of *C. muculatus* on artificial black-eyed peas treated with peel oils of lemon, grapefruit, lime and tangerine. The ability of some oils to reduce the expected emergence while others do not, is an indication of the presence of toxic components in the former.

The reduction of germination of treated can be explained by the problem of water absorption by seeds. Water and oil are not miscible. Seeds' coat is covered of oil after treatment thus they cannot absorb enough water which is necessary for the germination. This situation can create also gas exchange problems. But in this experiment, we used residual film technique. The treatment of cowpea seeds with oils reduces slightly the germination, but swelling percentage was higher in treated seeds than control and it was increased with increase of concentration level. The results of the present research also demonstrated that the germination percentages of treated cowpea seeds with oils were decreased with increasing concentration. This is conformity with finding of Mbailao Mbaiguinam *et al*. (2006).

V. CONCLUSION

This study concludes that the tested oils reduced progeny development of *C. maculatus* and can be employed for managing of this insect in food media. Our results and those reported earlier indicate variations in the activity of botanical oils regarding the stage of the insect, the plant origin of the oils and concentration level. It is recommended the small-scale farmers or grain storage agencies must use such natural products which are not harmful to human health and environment. Therefor botanical oils can become an interesting alternative to conventional chemical control strategies.

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