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EFFICACY OF FOUR LOCAL ECOFRIENDLY PLANT EXTRACTS AS SAFE COWPEA SEED PROTECTANTS AGAINST *Callosobruchus maculatus* F. (COLEOPERA; BRUCHUIDAE)

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ABSTRACT: The current study was designed to evaluate the efficacy of four plant extracts, *i.e.*, cassia (*Acacia farnesiana* Willd), arbor-vitae (*Platychladus* [*Thuja*] *orientalis* Endl, mahogany [*Swietenia mahogany* (Linn.) Jaq], and pancratier-lily (*Pancratium maritimum* L.), as protectants against the cowpea weevil (*Callosobruchus maculatus*). Results obtained showed that at a concentration of 5 g crude extract/kg seed, there was a significant reduction in the percentage of egg hatching. The cassia and arbor-vitae extracts from leaves or seeds appear to reduce egg hatching (0.5 and 30.0%) and (4.0 and 26.0%), respectively. Complete inhibition in adult emergence was found by the ethanolic extract of cassia seeds. The extracts obtained from cassia mahogany leaves also showed strong ovicidal activity. The egg hatching percentage significantly decreased during the 8-month storage of cowpea treated with ethanol extract of cassia and arbor-vitae seeds with values of (52-53%) and (51-58%), respectively. The results also demonstrated that surface treatment of cowpea seeds with most selected extracts did not affect the germinative potential of cowpea seeds compared with the control extract the ethanol extract of cassia and arbor-vitae leaves which had 80 and 75% germination, respectively compared to the control 95%. Water absorption did not also differ significantly between all treatment and control samples. In conclusion, current study investigated that tested plant extracts can be used as cowpea seeds protectants and can be used in IPM program.

Key words: Cowpea seeds, ovicidal activity, residual activity, seed germination.

INTRODUCTION

The wide-scale commercial use of plant extracts as insecticides began in the 1850s with the introduction of nicotine from *Nicotiana tabacum*, rotenone from *Lonchocarpus* sp., derris dust from *Derris elliptica* and pyrethrum from the flower heads of *Chrysanthemum cinerariaefolium*.

The new emerged insects in plant materials as stored produced protectants can be attributed to various factors including the development of resistance to synthetic insecticides, fears over their misuse and overuse (during application), and fears about the potential effect of insecticide residue on consumers, wildlife and the environment. In addition to these problems, the increased cost of insecticide development has led to a reduction in the number of new pesticides being evaluated by chemical companies for use in storage situations (**Dales, 1996**).

Several species attack cereal and pulses in the store and cause a loss of 10-15% with germination loss ranging from 50-92% (**Adugna, 2006**). About 100% loss of pulse crops was found due to infestation by the bulse beetles, *C. maculatus* and *C. chinensis* (L.) are the major pests of cowpea in the tropics (**Raja *et al.*, 2007**). Cowpea seed beetle is a common pest of stored legumes all over the world (**Hagstrum, 2017**). In severe infestations cowpea seed beetles can cause up to 100% damage to stored products unless controlled (**Bagheri Zonouz, 1996**). Management of stored product pests relies

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heavily on the use of chemical insecticides, especially organophosphates and pyrethroids which are used to protect crops both in the field and during storage (**Fields, 2006; Tomlin, 2009**). These chemicals have many side effects, such as human health hazards, environmental pollution, undesirable effects on non-target organisms and pest resistance (**Whitford, 2002, Boyer *et al*., 2012**). To reduce or prevent the use of synthetic insecticides on commodities, plant materials have received increased interest in recent years powered leaves of some plants, plant extracts, essential oils and botanical insecticides are used in some parts of the world (**Rahman *et al.*, 2020**).

Therefore, the objective of the current study is to evaluate four plant extracts as protectants against *C. maculatus* by assessing various parameters, including the toxicity of these plant extracts and the effects on the eggs and hatchability percent of the tested insect, offspring, seed germination, water absorption, and the residual effect of the tested plant extracts during eight months and seed germination and water absorption.

MATERIALS AND METHODS

Collection and Preparation of the Plant Extracts

Plant materials

In this study, various parts of four indigenous plant species, cassia (*Acacia farnesiana* Willd), arbor-vitae (*Platychladus* [*Thuja*] *orientalis* Endl), mahogany [*Swietenia mahogany* (Linn.) Jaq] and pancratier-lily (*Pancratium maritimum* L.) were extracted by n-hexane and ethanol, where the insecticidal properties of their crude extracts were determined against *C. maculatus*.

The plants used were collected from the research farm, Faculty of Agriculture, Kafr El-Sheikh, except Soosan (*Pancratium maritimum* L.) bulbs which were collected from Baltim province, Kafr El-Sheikh. The plant parts used were dried naturally on laboratory benches at temperature of 25 - 30° C and relative humidity (r.h.) of 60-65 % for 5 - 7 days, by which time they were crisp dry, followed by further drying in the oven at 40°C for 24 hrs. The seeds of the *Acacia* and *Platychladus* [*Thuja*] species were collected from dry ones still on the plants, and were thus ready for further processing when collected. The dried materials were stored in a refrigerator until needed in a laboratory mill and then passed through a 40 mesh sieve.

Preparation of crude extracts

Each powdered plant material was extracted with n-hexane and ethanol as described by **Freedman *et al.* (1979)**. Briefly, 250 g of each dried sample was soaked in 750 ml of the solvent, in a large flask (1 liter) for 72 hrs. The flask was then shaken for one hour on a shaker, and its contents were filtered through Whatman No. 1 filter paper. The extract was concentrated by removing the solvent in a water bath, at 40° C, to obtain the crude extract. The extracts obtained, which were in the form of a crude gun, were weighted and dissolved in an appropriate volume of acetone to give stock solutions of 5 % concentration (w/v), and stirred until a smooth paste that was obtained. The stock solutions were stored in a paste was obtained. The stock solutions were in a refrigerator until needed, at which point dilutions were made freshly.

Efficacy Testing

For testing the cowpea seeds with test material, a 20 g seed sample was placed in a small glass jar (11.5 by 6 cm diameter) at which 2 ml of plant extract diluted in water or acetone, respectively, corresponding to the required concentration in the grain, was pipetted onto the glass above the grain surface. The jars were shaken vigorously by hand to mix the contents uniformly with the grains. To allow further evaporation of the solvent, the treated samples were left overnight in open jars. Samples treated with acetone or water alone served as controls.

Initial Activity

For initial activity studies, five males and five females 24 hrs old of *C. maculatus* were introduced into each jar (replicate) containing treated seeds, after 1 day of treatment. The jars were covered with muslin cloth secured with elastic bands and placed in the laboratory under constant conditions (30 ±2°C, 70 ± 5 % r.h.) for 12 – 14 days. Control and treatments had three replicates each.

Ovicidal Activity

To determine the ovicidal action of the test materials. Five sexed paris (5 males and 5 females) of *C. maculatus* adults (0 - 24 hrs. old) were released in glass Petri dishes (13 cm - diameter) containing 20 g cowpea seeds and allowed to lay eggs for 3 days. On the fourth day, the adults were removed. The eggs laid on cowpea were treated with the concentration tested for each material by spraying the seed surface up to the wetting point using 20 ml glass atomizer. 2 ml acetone solution of each diluted concentration was applied to 20 g seed sample. The seeds sprayed with acetone served as a control. At the end of the spray operation, all samples were left overnight in open petri dishes to allow further evaporation of the solvent. Three petri dishes (replicates) were used for each concentration. After 9 days post-treatment, the number of hatched eggs was recorded and the egg hatch percentage could then be calculated and corrected for control response (**Abbott, 1925**). Concentration mortality (unhatched percentage) responses were subjected to the probit analysis procedure (**Finney, 1971**) to obtain estimates of slope, LC_{50} and LC_{90} values.

Residual Activity

For residual activity studies, the seeds were treated with test material and *C. maculatus* adults were exposed to them after various periods of storage, *i.e.* 1, 2, 4, 6 and 8 months post treatment. 2 kg of cowpea seeds were taken in a 2 liter glass jar and 200 ml of the plant extract or pirimiphos-methyl, corresponding to the required concentration in the grain, was pipetted onto the glass jar above the grain surface. Jars were shacken to completely mix the medium, then left open until the solvent evaporated. Samples were transferred to paper bags enclosed in polyethylene bags and then stored in glass jars under laboratory conditions. Samples were treated with acetone alone and served as control.

Twenty grams of sample seeds were taken after 1, 2, 4, 6, or 8 months of storage in small glass jar and infested with 5 pairs of *C. maculatus*. The jars were covered with muslin secured with elastic bands and placed in the laboratory.

After 14 days, the parent adults were removed to prevent them from mixing with the first generation (F_1) of offspring.

Fecundity of the parent bruchids was determined by the total numbers of eggs laid on the cowpea seeds during 14 days. Eggs were examined under a binocular microscope and numbers of hatched and unhatched eggs were recorded. All fertile eggs hatched within 9 days under the experimental conditions described above, and No. mated adult survived for more than 10 days after the first day of oviposition (**Rajapakse and Van Emden, 1997**). The unhatched eggs, as well as those with dead embryos or larvae appeared transparent, whereas viable eggs were opaque or identifiable as having hatched (**Larson and Fisher, 1938**). Adults emerging were sieved out and recorded every other day. Counts were taken for 20 days after the first emergence.

Water Absorption

Before cowpea is cooked into various dishes, the seeds are immersed in water to soften their test and facilitate decortication (**Ivbijaro and Agbaie, 1986**). The effect of tested materials on the capacity of cowpea seeds to absorb water was determined by immersing 50 g of the seeds in the control, and 50 g of those at the highest concentration of plant extract separately in 200 ml of water as described by **Ivbijaro and Agbaie (1986).** The seeds were removed after 12 hrs., of submergence, dried partially between filter paper and reweighted. The volume of water absorbed was expressed as the difference in the weight of seeds, before and after submergence in water.

Seed Germination

Recorded of germination of the cowpea seeds in the control and those at the highest concentration used of tested materials per 20 g of cowpea were taken 6 days after placing them on regularly moistened, Whatman No. 1 filter paper in petri dishes (**Ivbijaro and Agbaie, 1986**).

Statistical Analysis

The mortality percentage in different tests cumulated in time according to the different concentrations of essential oils was analyzed using a one-way ANOVA and a subsequent least significant difference (LSD) test for mean separation at P= 5%, SPSS software program version 23 was used. The (LC50) 50% lethal concentrations, slope and 95% confidence limits (CL) were calculated based on Finney's analysis (**Finney, 1971**) using the Pc Probit software program, and significant difference between LC50 values was estimated based on 95% CL overlapping.

RESULTS

The Biological Activity of some Plant Extracts against *Callosobruchus maculatus* F.

Initial activity

The mean numbers of eggs laid by *C. maculatus*, the percentage of hatched eggs and the percentage of reduction in adult emergence (F1-progeny) were the criteria used to assess the effectiveness of tested extracts, the results summarized in Table 1, show that when seeds were treated at 2 g/kg, there was no significant difference in the mean numbers of eggs laid between the plant extract treatments and the control. However, the egg hatch was significantly reduced in the treatments of cassia seed extracts (33 and 37% hatch) and of ethanol extract of arbor-vitae seed extract (29%), compared to that of the control (70%).

The results also show that the surface treatment of seeds at a higher rate, 5 g crude extract/kg seeds, (Table 1 and Fig. 1) resulted in a significant reduction in the percentages of egg hatch in most treatments. The cassia extracts from either leaves or seeds appear to be the most effective ones in reducing egg hatching significantly (0.5–30% hatch) than that observed in the control (70%). The ethanolic extracts of seeds and leaves of arbor-vitae had a strong effect on reducing egg hatching (4 and 26% hatch, respectively).

The percentages of *C. maculatus* adult emergence (F1-progeny) were significantly reduced at the lower concentration, 2 g crude extract/kg seeds, of ethanol extracts of either cassia leaves (48 %) or pancratier lily bulbs (59%), compared to that observed in the control (80%) (Table 2 – Fig. 2). However, at the higher concentration, 5 mg/kg, most tested plant extracts had a strong effect on reducing the F1-progeny. Complete inhibition i of adult emergence was found by the ethanolic extract of cassia seeds. Strong activity by treatments of the F1-progenyproduced was also achieved by treatments of ethanol extract of arbor-vitae seeds (99.1% reduction) and of hexane extract of cassia seeds (95.3% reduction), in comparison with that of the control.

The lowest activity in this regard was observed with the treatments of hexane extracts of both leaves and seeds of arbor-vitae, mahogany leaves, and pancratier lily bulbs with low reduction percentages in adult emergence of 30.0, 47.2, 45.8, and 61.2%, respectively.

Ovicidal activity

Data presented in Table 3 show the insecticidal properties of test plant extracts (5 g/kg seeds) on eggs of *C. maculatus*. The percentages of egg hatching were significantly lower in a most plant extract treatments than those observed in the control. The productivity index of egg hatching, calculated as percent of the control, seemed to closely follow the pattern of reduction percentages in the oviposition. The ethanolic extracts from cassia seeds, arbor-vitae leaves, and arbor-vitae seeds were the most effective treatments compered to others, with productivity index of 4.5, 9.1, and 9.1, respectively, compared to the control treatment. In general, the extracts obtained from cassia leaves or mahogany leaves showed strong ovicidal activity, with productivity index of hatching ranging from 15.2 to 25.8%, relative to the control.

Residual activity

Table 4 shows data on the residual activity of selected plant extracts evaluated as cowpea seed protectants against *C. maculatus* adults over a period of 8 months. At a one-month post-treatment interval, only the hexane extract of cassia leaves and ethanol extract of cassia seeds caused a significant reduction in the oviposition by 38.0 and 48.7% compared to the control (Table 4). Subsequently, the egg hatching percentages were highly decreased by the treatments of hexane extract of cassia leaves (10%), ethanol extract of cassia seeds (24%), and also of ethanol extract of arbor-vitae seeds

Table 1. The oviposition rate and percentages of hatchability of eggs laid by *Callosobruchus maculatus* exposed to treated surface of cowpea seeds, for 24 hrs.

Plant material	Solvent	Concentration (g crude extract/kg seeds)					
		2			5		
		Mean No. of eggs laid	Mean No. of eggs hatched	% of hatchability	Mean No. of eggs laid	Mean No. of eggs hatched	% of hatchability
Cassia leaves	Hexane	337^{bc}	167^{c}	50	231^{b}	19^{c}	8
	Ethanol	380^{e}	212^{g}	56	373^{j}	113^{f}	30
Cassia seeds	Hexane	344^{bc}	113^{a}	33	324^{fg}	47^{d}	15
	Ethanol	371^{de}	138^{b}	37	188^{a}	1^{a}	0.5
Arbor-vitae leaves	Hexane	362^{de}	190^{de}	52	339^{i}	188^{h}	55
	Ethanol	331^{b}	204^{efg}	62	253^{c}	65^{e}	26
Arbor-vitae seeds	Hexane	365^{de}	194^{def}	53	335^{hi}	193^{h}	57
	Ethanol	352^{cd}	102^{a}	29	314^{ef}	12^{ab}	4
Mahogany leaves	Hexane	375^{e}	186^{d}	50	326^{gh}	135^{g}	41
	Ethanol	290^{a}	151^{b}	52	283^{d}	119^{f}	42
pancratier lily bulbs	Hexane	365^{f}	199^{d-g}	55	322^{fg}	107^{f}	33
	Ethanol	323^{b}	208^{fg}	64	311^{e}	129^{g}	41
Solvent control		380^{e}	267^{h}	70	380^{j}	267^{i}	70

Means of followed by the same letter (s) within each column are not significantly different (Duncan's multiple range test at P=0.05).

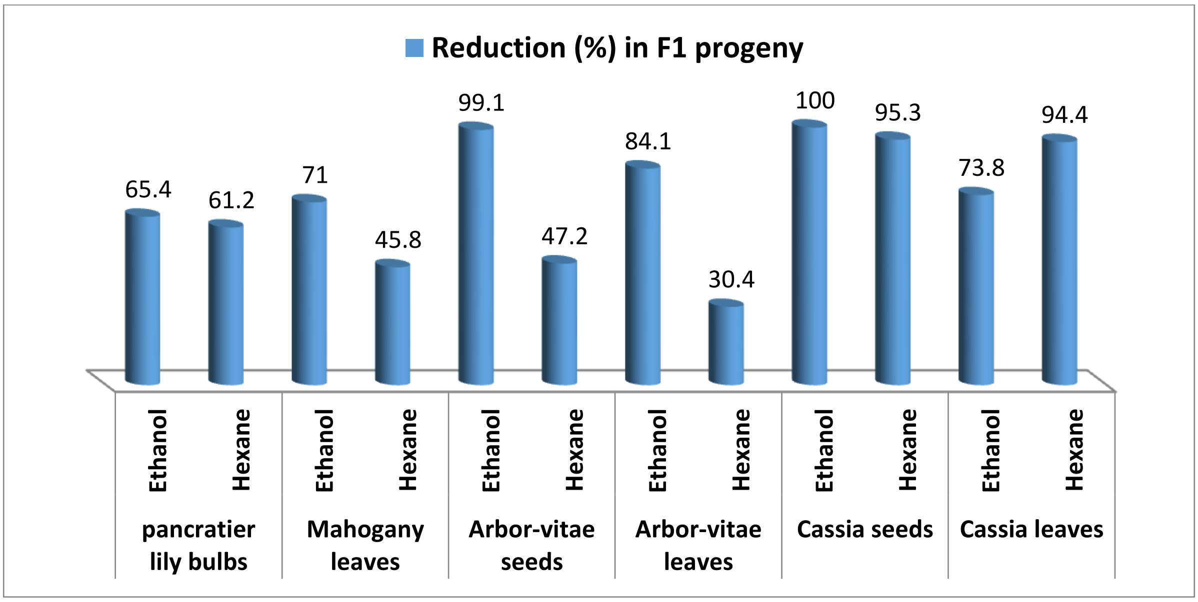

Fig. 1. Effect of surface treatment of cowpea seeds with different plant extracts (5 g crude extract/ kg seeds) on egg hatching of *Callosobruchus maculatus*

Table 2. The effect of surface treating of cowpea seeds with tested plant extracts on productivity of *Callosobruchus maculatus*

Plant material	Solvent	Concentration (g crude extract/kg seeds)					
		2			5		
		Mean No. of adult emerged	% of adult emergence [a]	% of reduction [b]	Mean No. of adult emerged	% of adult emergence [a]	% of reduction [b]
Cassia leaves	Hexane	113^{de}	68	47.2	12^{c}	63	94.4
	Ethanol	102^{bc}	48	52.3	56^{e}	50	73.8
Cassia seeds	Hexane	80^{a}	71	62.6	10^{bc}	21	95.3
	Ethanol	104^{cd}	75	51.4	0^{a}	0	100
Arbor-vitae leaves	Hexane	157^{h}	83	26.6	149^{j}	79	30.4
	Ethanol	132^{f}	65	38.3	34^{d}	52	84.1
Arbor-vitae seeds	Hexane	114^{de}	59	46.7	113^{h}	59	47.2
	Ethanol	83^{a}	81	61.2	2^{ab}	17	99.1
Mahogany leaves	Hexane	145^{g}	78	32.3	116^{h}	86	45.8
	Ethanol	94^{b}	62	56.1	62^{e}	52	71.0
pancratier lily bulbs	Hexane	129^{f}	65	39.7	83^{g}	78	61.2
	Ethanol	123^{ef}	59	42.5	74^{f}	57	65.4
Solvent control		214^{i}	80	--	214^{j}	80	--

Means of followed by the same letter (s) within each column are not significantly different (Duncan's multiple range test at P=0.05).

[a] percent of adult emergence = (No. of emerged adults / No. of eggs hatched) X 100.

[b] percent of reduction = [(No. of emerged in control – No. of emerged in treated) / (No. of emerged in control)] X 100.

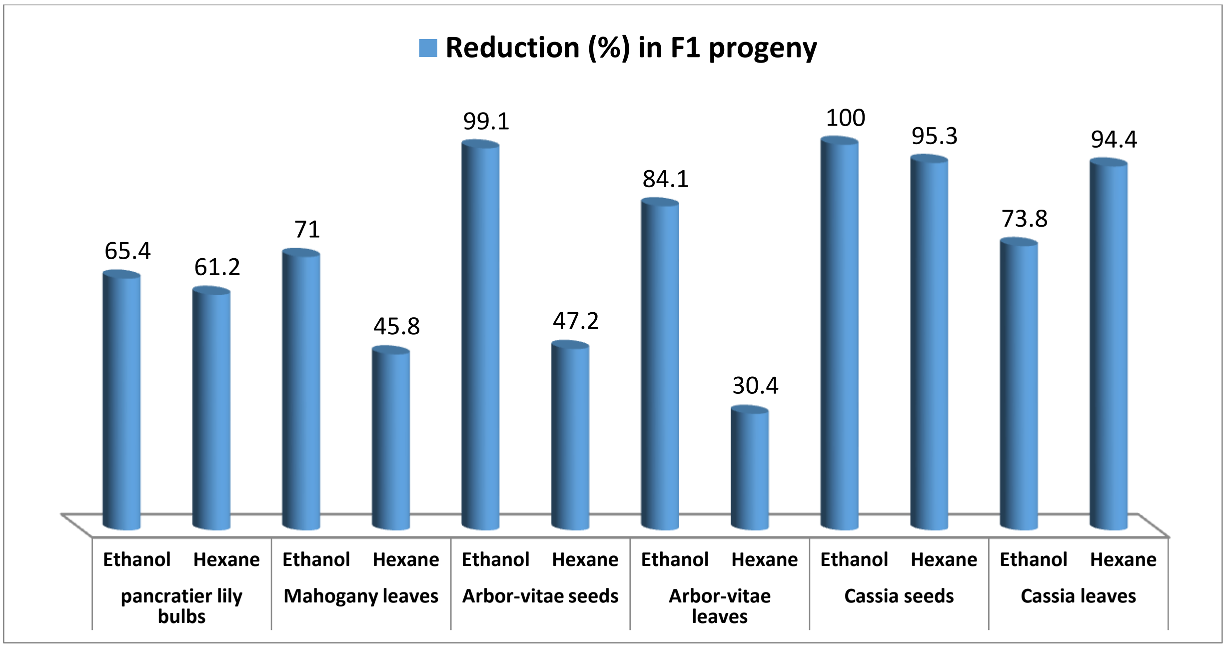

Fig.2. Effect of surface treatment of cowpea seeds with different plant extracts (5 g crude extract /kg seeds) on reduction percentage of adult emergence (F_1 progeny) of *Callosobruchus maculatus*

Table 3. Percent hatchabitly of *Callosobruchus maculatus* eggs (1-3 day-old) after surface treatment with test plant extracts (5 g crude extract/kg seeds)

Plant material	Solvent	Mean No. of eggs observed	Mean No. of eggs hatched	% of hatch	% of productivity index ^{a}
Cassia leaves	Hexane	254	25^{c}	10	15.2
	Ethanol	256	26^{c}	10	15.2
Cassia seeds	Hexane	252	23^{c}	9	13.6
	Ethanol	255	8^{a}	3	4.5
Arbor-vitae leaves	Hexane	254	68^{f}	27	40.9
	Ethanol	253	15^{b}	6	9.1
Arbor-vitae seeds	Hexane	254	26^{c}	10	15.2
	Ethanol	253	15^{b}	6	9.1
Mahogany leaves	Hexane	258	37^{d}	14	21.2
	Ethanol	253	43^{e}	17	25.8
pancratier lily bulbs	Hexane	255	147^{g}	58	87.9
	Ethanol	250	47^{e}	19	28.8
Solvent control		256	169^{h}	66	--

Means of followed by the same letter (s) within each column are not significantly different (Duncan's multiple range test at P=0.05).

^{a} percent of productivity index = (% of egg hatching in treated / % of egg hatching in control) X 100.

Table 4. Effect of surface treatments of cowpea seeds with selected plant extracts (5 g/kg) on fertility of *Callosobruchus maculatus* at various post treatment intervals

Plant material	Solvent	Storage periods post treatment (months)									
		1		2		4		6		8	
		Mean No. of eggs laid (% of reduction)^{a}	Mean No. of eggs hatched (% of hatch)	Mean No. of eggs laid (% of reduction)	Mean No. of eggs hatched (% of hatch)	Mean No. of eggs laid (% of reduction)	Mean No. of eggs hatched (% of hatch)	Mean No. of eggs laid (% of reduction)	Mean No. of eggs hatched (% of hatch)	Mean No. of eggs laid (% of reduction)	Mean No. of eggs hatched (% of hatch)
Cassia leaves	Hexane	230^{b} (38)	22^{a} (10)	299^{b} (22.7)	206^{c} (69)	310^{a} (18.8)	226^{c} (73)	-- ^{b}	--	--	--
	Ethanol	371^{e} (0.3)	260^{g} (70)	--	--	--	--	--	--	--	--
Cassia seeds	Hexane	335^{d} (9.9)	152^{d} (45)	364^{d} (5.9)	244^{d} (62)	365^{c} (4.5)	245^{d} (67)	377^{c} (0.5)	254^{c} (67)	--	--
	Ethanol	191^{a} (48.7)	46^{b} (24)	280^{a} (27.6)	149^{a} (53)	295^{a} (22.8)	155^{a} (53)	298^{a} (21.4)	156^{a} (52)	312^{a} (16.8)	164^{a} (53)
Arbor-vitae leaves	Ethanol	308^{c} (17.2)	262^{g} (86)	--	--	--	--	--	--	--	--
Arbor-vitae seeds	Ethanol	321^{cd} (13.7)	123^{c} (38)	240^{c} (12.1)	174^{b} (51)	344^{b} (9.9)	190^{b} (55)	347^{b} (8.4)	191^{b} (55)	348^{b} (7.2)	202^{b} (58)
Mahogany leaves	Ethanol	304^{C} (18.3)	197^{E} (65)	-- ^{b}	--	--	--	--	--	--	--
pancratier lily bulbs	Hexane	372^{e} (0)	262^{g} (70)	--	--	--	--	--	--	--	--
	Ethanol	315^{c} (15.3)	214^{f} (68)	--	--	--	--	--	--	--	--
Solvent control		372^{e}	269^{g} (72)	387^{e}	262^{e} (68)	382^{c}	258^{e} (68)	379^{c}	254^{e} (67)	375^{c}	278^{c} (74)

Means of followed by the same letter (s) within each column are not significantly different (Duncan's multiple range test at P=0.05). A separate analysis of variance was carried out for each parameter

^{a} percent of reduction = [(No. of eggs laid in control – No. of eggs laid in treated) / (No. of eggs laid in control)] X 100.

^{b} data ware not taken

(38%) compared to that of the control (72%). The oviposition rate began to increase after the one-month assessment point with no significant differences between the plant extract treatments selected and the control. However, the egg hatching percentages were significantly decreased during the 8 months of storage of cowpea treated with ethanol extracts of cassia seeds (52 – 53% and of arbor-vitae (51 – 58%), compared to those recorded in the controls (67 – 74%).

The results in Table 4 show that the reductions in adult emergence (F1 progeny) of *C. maculatus* in most selected plant extract treatments closely followed the pattern of reduction in the oviposition. During the 8 months of storage, treatment of cowpeas with the ethanol extracts of either cassia seeds or arbor-vitae seeds each resulted in a significant protection of seeds against *C. maculatus* as evidenced by 86.4 – 65.2% or 89.8 – 42.8% reduction in F1 progeny, respectively, in comparison with the controls. The hexane extract of cassia seeds, also, significantly suppressed the F1 progeny of *C. maculatus* during the 6 months of storage at which number of adult that emerged was reduced by levels ranging from 87.9% (at one month intervals) to 49.8% (at 6 months interval) than that of the control.

Seed germination and water absorption

The results in Table 5 show that surface treatment of cowpea seeds with most selected plant extracts, applied at 5 g/kg, had no effect on the germination potential of cowpea seeds compared with the control. However the ethanol extracts from both cassia and arbor-vitae leaves had significant decrease on the germinated potential of treated seeds, at which seed germination percentages were 80 and 75% compared to that of the control (95%).

DISCUSSION

Our results concluded that most plant extracts tested at 2 g crude extract/kg seeds had no significant effect on the fecundity of the bruchids beetle, *Callosobcuchus maculatus*. However, at this concentration, the egg hatch percentages were significantly reduced in the treatments of cassia seed-extracts and ethanol from arbor-vitae seeds compared to those of the control. At the higher concentration (5 g crude extract/kg seeds), there was a significant reduction in fecundity of *C. maculatus* due to exposure to ethanol extract of cassia seeds and hexane extract of cassia leaves. In addition, there was a significant reduction in egg hatch most treatments.

Moreover, *Lippia nodiflora* and *Piper longum* treated grains at 14 days after confinement recorded the maximum adult mortality of *S. oryzae* by 53.8 and 53.5% respectively (**Nalini *et al*., 2010**).

Hexane and ethanol extracts of *Eucalyptus camaldulensis* at 5 g/kg seeds exhibited 100 % mortality of *C. maculatus* adults after 6 days post-treatment (**Mahama *et al*., 2018**).

Additionally an aqueous extracts of *E. Caryophyllus*, *B. pinnatum*, *E. camaldulensis* and *X. aethiopica* induced insect mortality of 71.21, 81.42, 80.00 and 100.00 % respectively at 5 ml dose of aqueous plant (**Omotoso, 2008**).

Also, ethanol extract of *A. sativum* L. (Garlic), *C. millemii* Baker (Manjack), *M. myristica* (Gaetn.) (Nutmeg), *X. aethiopica* (Dunal) (Negropepper) and *Z. officinale* Roscoe (Ginger) showed lethal effects on *C. maculatus* as compared with the untreated check (**Edwin, 2017**).

Ethanolic extracts of *E. balsamifera* caused 96.67% adult mortality and *L. inermis* exerted 90.00% adult mortality of *C. maculatus* after 14 days post treatment (**Sani and Suleiman, 2017**).

Clove is widely used as spice and also shows high effect in insect control and has been demonstrate as safe for human health (**Naveena *et al*., 2006**). *Citrus sinensis* showed the highest weevil mortality on *C. maculatus* after the first day of exposure, followed by *Azadirachta indica* and the least toxic was *Cymbopogon citratus* (**Ojebode *et al*., 2006**).

100% mortality was achieved within one day after treatment with extracts of *A. calamus* var. *angustatus*, *thymus mandschuricus* (lamiaceae) against *C. chinensis* (**Kim *et al*., 2003**).

The exposure period appeared to be the most important factor affecting the toxic effects of the vapours of the Acorus oil rather than the dosage. On the other side, some plant-derived materials are found to be highly effective against insecticide-resistance pest insects (**Arnason *et al*., 1989; Ahn *et al*., 1997)**.

Table 5. Surface treatment of cowpea seeds with most selected plant extracts

Plant material	Solvent	%seed germination	Water vol. (ml) absorbed after 12 hrs
Cassia leaves	Hexane	85bc	56.5f
Cassia leaves	Ethanol	80b	55.7e
Cassia seeds	Hexane	85bc	54.2c
Cassia seeds	Ethanol	90de	51.4a
Arbor vitae leaves	Ethanol	75a	51.4a
Arbor vitae seeds	Ethanol	90de	55.0d
Mahogany leaves	Ethanol	85bc	54.3c
Pancratier lily bulbs	Hexane	90 de	51.9ab
Pancratier lily bulbs	Ethanol	85bc	54.5cd
Solvent (control)	--	95b	52.1b

Means followed by the same letter(s) within each column are not significant different (Duncun's multiply range test, P= 0.05).

Additionally, strong insecticidal activity against adults of *S. oryzae* and *C. chinensis* was obtained with extracts from *Acoras calamus* var. *angustatus, Acoras gramineus, Cinnamomum cassia* and *Cinnamomum sieboldii* (**Kim *et al.* 2003**).

Also, hexane fraction of *Callistemon rigidus* showed superior toxicity, causing 100% mortality to *C. maculatus* at 4 g/kg within only one day of exposure with LC50% of 1.02 g/kg (**Donga *et al.*, 2015)**.

Chromolaena odorata root powder recorded effective mortality on cowpea weevils which accounted for total mortality of *C. maculatus* after 72 hrs exposure period (**Osa and Georgina, 2016**).

Another research shows that, 5 g/100 g each of *Carica papaya* leaf powder and *Nona muricate* seed powder proved to be most effective in cowpea damage from *C. maculatus* (**Lawal Ibrahim *et al.*, 2018**).

The effectiveness of various plant extracts in suppressing the oviposition and egg hatching has been reported by several authors, e.g. **Maknjuola (1989)** found that neem acts not only as an oviposition suppressant but also an ovicidal. **Ivbijaro (1983)** reported a significant reduction in egg laying on cowpea mixed with neem seeds.

Plant powders and crushed leaves of *Bulinus seneglensis, Cleome viscosa* and *Hyptis sipcigera* exhibited inhibitory and ovicidal effects on the egg-laying of cowpea weevil females (**Sanon *et al.*, 2006 and Doumma, 2012**).

The inhibitory and ovicidal effects on the egg-laying of cowpea weevil females is dependent on dose of phytochemicals used, this hypothesis appears to be more important with fresh crushed leaves of *B. seneglensis* and *C. viscosa* (**Doumma, 2012**).

Cowpea seeds treated with the lowest concentration of moringa leaf powder showed a sharp decrease in number of eggs laid by the adults. This condition can be explained by the fact that using the lowest concentration of moringa leaf powder caused deterrence of the females to lay eggs (**Dimetry and El-Behery, 2018**).

The percentages of *C. maculatus* adult emergence (F_1-progeny) were significantly reduced at lower concentration, 2 g crude extract/kg seeds, of ethanol extracts of either cassia leaves or pancratier lily bulbs and hexane extract of arbor-vitae seeds, compared to that observed in the control. Moreover, at higher concentration, 5g/kg, most tested plant extracts had a strong effect in reducing the F_1-progeny. Complete inhibition in adult emergence was found by the ethanolic extract of cassia seeds. Also a strong activity in reducing the F_1-progeny produced was achieved by treatment of the ethanolic extract of arbor-vitae seeds and hexane extract of cassia seeds with 99.1% and 95.3% reduction, respectively in comparison with that of the control. Recently, **Rajapakse and Van Emden (1997)** found that no progeny of any bruchids species, *C. chinensis*, *C. maculatus* and *C. rhodesianus* emerged when the cowpea seeds had been treated with corn, groundnut, sunflower and sesame oil, at 10 ml/kg, thought the development rate of the insects was not affected by the lower dose of 5 ml/kg. **Abo El-Ghar and El-Sheikh (1987)** indicated that at concentration of 0.5%, the petroleum ether extract of *Ipomoea palmate* provided (97%) cowpea seed protection against *C. chinensis* infestation, followed by *Nerium olender* (94%), *Clerodendrum inerme* (93%) and *Atriplex lentiformis* (89%).

Many plant extracts and essential oils are known to possess ovicidal repellent and insecticidal activities against various stored product insect (**Hill and Schoonhoven, 1981; Desmarchelier, 1994**).

The ovicidal activity of tested plant extracts, at 5g/kg, showed that the percentages of egg hatching were significantly reduced in most plant extract-treatments than the observed in the control. **Pereira (1983)** showed that traditionally extracted neem kernel oil, karate oil, groundnut oil, palm kernel oil, palm oil as well as industrially extracted groundnut oil exhibited significant ovicidal activity of *C. maculatus* eggs at and above 3 ml oil/kg cowpea seeds. The ovicidal effects of the oily crude extracts tested on *C. maculatus* may by explained in terms of asphyxiation by occluding a funnel which is probably the major route of gas exchange between a thin area of the chorion and the outside (**Credland, 1992**).

The residual activity data of selected plant extracts against *C. maculatus* demonstrate that one month post treatment interval, the oviposition rate was significantly reduced in the females exposed to seeds treated with either hexane extract of cassia leaves or ethanol extract of cassia seeds than that of the control. Subsequently, the egg hatching percentages were highly decreased by the treatments of hexane extract of cassia leaves (10%), ethanol extract of cassia seeds (24%), and also of ethanol extract of arbor-vitae seeds (38 %) compared to that of the control (72%). The fecundity began to increase after one month assessment point with no significant differences between the most plant extract-treatments selected and the control. The reduction in adult emergence (F_1-progeny) of *C. maculatus* in most selected plant extract-treatment closely followed the pattern of reduction in the oviposition. In general, during the 8 months of storage, treatment of cowpeas with ethanol extracts of either cassia seeds or arbor-vitae seeds results in a significant protection of seeds against *C. maculatus*. Also, the hexane extract of cassia seeds, significantly suppressed the F_1-progeny of *C. maculatus* during 6 months of storage.

Previous studies were in the same trend of the present study where at all doses tested, hexane extracts of *Eucalyptus camaldulensis* completely inhibited progeny F_1 production of *C. maculatus*. The different plant extracts reduced significantly the rate of grain damaged and grain weight losses compared to the negative control (**Mahama *et al*., 2018**).

Ahmad *et al*. (2018) found that weight loss was observed in the seeds treated with garlic and ginger, followed by those treated with lemongrass powder, whereas the control treatment had the highest percent weight loss.

Neem kernel powder at 2.0 percent was the highly effective grain protectant based on no grain damage by these insects on gram and pigeon pea. No progeny emergence of *C. maculatus* and *C. chinensis* even after 360 DAT on treated lentil seeds (*lens culinaris*) was attributed to be oviposition inhibition (**Yadav, 1993**). **Jacob and Shelia (1993)** carried out laboratory evaluation of powders from *Datura alba*, *Calotropis procera* and Chromolaena odorata at 2.5 and 5.0 percent against *R. dominca* on rice grains at 28° C temperature, they found that all the treatments were effective with significant reduction of number of adults emerging from the grains.

Khaire *et al*. (1992) showed that No. of *C. chinensis* adults occur up to 66 days following treatment of pigeon pea with castor oil (at the 0.75 and 1.00% levels). Adult emergence was completely prevented by karanj oil at 0.75 and 1.00 %, and neem oil at 0.05, 0.75 and 1.00 % levels up to 100 days. Similarly, Periera (1983) reported that a major part of toxic activity of neem oil against *C. maculatus* was retained after 3 months of storage following cowpea seeds treatment.

The results show that surface treatment of cowpea seeds with most selected plant extracts, had no effect on the germinative potential of cowpea seeds compared with the control.

Water absorption did not also differ significantly in all selected plant extract treatments or control seeds.

Our results conclude that the plant extracts of cassia seeds and leaves and arbor-vitae seeds could perhaps be good source for further development as naturally occurring phytochemicals with the potential of protecting stored seeds from attack of cowpea weevils, provided they can be freed from constituents in the plant which are toxic to warm-blooded animals.

The effectiveness of extracts of cassia seeds and leaves probably has been attributed to presence of the phytochemicals HCN (**Janzen *et al*., 1980**) and condensed tannins (**Ramirz and Ledezma-Torres 1997**).

In addition, the active components in arbor-vitae which be belonging to flavonoids (**Kosuge *et al*., 1985**) and phenolic compounds (**Sanjay *et al*., 1993**; **Khabir *et al*., 1985**) may also be responsible to the biological activity of such plant extracts against *C. maculatus*.

From the results presented in this part and those of other workers (e.g. **Bhaduri *et al*., 1985; Makanjuola, 1989; Saxena *et al*., 1992; Ogunwolu and Odunlami, 1996**), it is clear that many plant extracts, especially vegetable oils, exhibit insecticidal properties with high activity in suppression the oviposition rate, and adult emergence against the bruchid *Callosobruchus spp*. However, the residual activity of most plant extracts never exceeds 6 months after treatment. Also, none of the plant materials has yet been tested under conditions of traditional storage (**Rajapakse and Van Emden, 1997**).

The reduction in adult emergence on seed treated with chili extract had no emergence in any treatments as compared to 20 adults emerged in control (**Tahir and Anwar, 2015**).

In concerning the important of plant powders for protecting stored grain, many studies were conducted and more data were recorded. The *Carica papaya* leaf powder and *Citrus sinensis* powder proved to be the most effective on adult emergence and adult mortality of *C. maculatus* at eight weeks of the storage (**Lawal Ibrahim *et al.*, 2018**).

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فعالية أربعة مستخلصات نباتات محلية صديقة للبيئة كمواد وقائية آمنة لبذور اللوبيا ضد (*Callosobruchus maculatus* F. (Coleopera; Bruchuidae)

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قسم الحبوب المخزنة والمنتجات الآفات – معهد بحوث وقايةالنبات – مركز البحوث الزراعية – الجيزة – مصر

تم تصميم الدراسة الحالية لتقييم فعالية أربعة مستخلصات نباتية كمواد وقائية ضد خنفساء اللوبيا (*Callosobruchus maculatus*). أظهرت النتائج التي تم الحصول عليها أنه عند تركيز 5 جرام من المستخلص الخام / كجم من البذور حدث انخفاض معنوي في نسبة فقس البيض. تظهر الأوراق أو البذور خفض من فقس البيض (0.5 و 30.0%) و (4.0 و 26.0%) على التوالي. تم العثور على تثبيط كامل لظهور البالغين بواسطة المستخلص الإيثانولي لبذور كاسيا. وأظهرت المستخلصات التي تم الحصول عليها من أوراق كاسيا ماهوجني أيضا نشاطا قويا في قتل البيض. انخفضت نسبة فقس البيض بشكل معنوي خلال 8 أشهر من تخزين اللوبيا المعالجة بمستخلص الايثانول من بذور الكاسيا وبذور الموهينجي بقيم (52-53%) و (51-58%) على التوالي.كما أظهرت النتائج أنه لم يكن للمعالجة السطحية لبذور اللوبيا بمعظم المستخلصات المختارة أي تأثير على القدرة الإنباتية لبذور اللوبيا مقارنة مع مستخلص مستخلص الإيثانول من كاسيا وأوراق الشجرة التي كانت نسبة إنباتها 80 و 75% على التوالي مقارنة مع السيطرة 95%. كما لم يختلف امتصاص الماء بشكل كبير مع جميع المعاملات والسيطرة.

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