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Bioefficacy of leaf and peel extracts of *Euphorbia balsamifera* L. and *Citrus sinensis* L. against *Callosobruchus maculatus* Fab. [Coleoptera: Bruchidae]

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ABSTRACT

Experiments were conducted to evaluate the efficacy of selected plants extracts of Euphorbia balsamifera L. and Citrus sinensis L. against Callosobruchus maculatus Fab. in the laboratory under the conditions of 28 ±2°C and 70 ± 5% R.H. The test insects were reared in rearing bottles and tested on whole cowpea seeds for comparison. Different concentrations of 0.5, 1.0, and 2.0 g of the plant extracts were dissolved in 10 ml acetone and applied for mortality, seed damage and germination tests. Total (100%) mortality of adults of C. maculatus was observed after 3.67 to 6.67 days of treatment with the plant extracts. The findings of this study revealed that the plant extracts significantly (P<0.05) reduced cowpea seed weight loss. There was no (0.00%) weight loss of cowpea seeds recorded when higher (2.0 g/10.0 ml acetone) concentration of both extracts was applied. These plant extracts showed significant (P<0.05) effect on the germination capacity of cowpea seeds. The results obtained in this study indicated that extracts from C. sinensis and E. balsamifera had potentially reduced C. maculatus infestation on cowpea seeds. Hence, recommended for the control of C. maculatus attacking cowpea seeds during storage.

Keywords: Bioefficacy, *Callosobruchus maculatus*, Cowpea seeds, Plant extracts

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INTRODUCTION

Cowpea [*Vigna unguiculata* L. (Walp)] is known to be attacked by insect pests both in the field and during storage [1]. *C. maculatus* is a very serious pest of cowpea in storage in Nigeria and many other countries. This notorious pest attacks the stored pulses and has dispersed throughout the tropics and subtropics through the medium of commerce and now has become a real menace [2]. Initial infestation of cowpea seeds occurs in the field just before harvest and the insects are carried into the store where their population builds up rapidly. The female beetle lays eggs on the seed surface and the larva immediately after hatching bores into the seed. By the time it reaches the adult stage it consumes the seed cotyledons [2]. Damaged seeds are riddled with emergence holes, defaced with egg covers and have reduced viability. Heavy attack causes severe powdery and weight loss. In Nigeria alone, the dry weight loss due to *C. maculatus* exceeds 2900 tonnes each year [3]. In some cases damage in terms in holed seed can increase to 99% after 6 months of storage [4].

Farmers usually rely on chemical insecticides to prevent loss of stored products such as cowpea seeds. These chemical insecticides are so adverse to the environment, making it unfavourable for life by causing frequent environmental pollution that leads to effects on human health. All these necessitated build-up of storage tools that are user and ecologically-friendly [3]. As a result of these serious drawbacks, there is need to develop alternative, cheap and safe methods of insect pest control [5]. Some of these alternative methods include the use of plant

extracts. Many of the plant extracts applied were found effective in controlling insect pests of storage [1, 3, 6]. Most of the plant extracts were reported to be efficacious in adult mortality, emergence and seed damage.

Investigation on the efficacy of extract of peel powder of *Citrus sinensis* (L.) and other plant powders on *S. zeamais* attacking maize grains in storage was conducted and the report revealed 70.00% adult mortality and 42.77 Weevil Perforation Index, 28 days after application of the plant extract [7]. The effectiveness of peel powder of *C. sinensis* as seed protectants of some stored soya bean against *Callosobruchus maculatus* (Fab.) was studied and the report showed 0.00% adult emergence when *C. sinensis* was used at the dose of 1.5, 2.0 and 3.0 g/30 g soya bean seeds [8]. Peels of *C. sinensis* applied as powder was also found to be toxic at higher dosage of 15 g/250 g of haricot bean grains causing 65.95% mortality after 96 hours of exposure [9].

The medicinal properties of *Euphorbia balsamifera* (L.), a very common plant in the northern Nigeria was reported, and studies on the application of phytotherapy in odontology using latex from the plant species were conducted [10]. The effectiveness of two related species, *E. candelabrum* and *E. tirucalli* in the control of ticks was evaluated [11], but its use as grain protectant against insect pests in the store is scarce. However, it was recently reported that the leaf powder of *E. balsamifera* was effective against *S. zeamais* [12]. The present study therefore, investigates the insecticidal potentials of extracts from peels of *C. sinensis* and leaf of *E. balsamifera* against *C. maculatus* on cowpea seeds during storage.

MATERIALS AND METHODS

Rearing of the Insect

Adults of *C. maculatus* were obtained from naturally infested cowpea seeds. Adult's *C. maculatus* were cultured in incubator at 28 ± 2°C and 70 ± 5% R. H. in the Biology Laboratory of the Department of Biology, Umaru Musa Yar'adua University (UMYU), Katsina. Healthy seeds used for the experiment were purchased from the Katsina Central market and disinfested in an oven at 60°C for I hour before using them as a substrate for insect rearing. Fifty pairs of *C. maculatus* were introduced into the rearing bottles containing 250 g seeds. The bottles were covered with muslin cloth and secured with rubber bands. The parent weevils were sieved out after 7 days of oviposition period. Later the seeds were kept in the incubator for adult emergence. The F1 generation was used for the experiment.

Collection and Extraction of Plant Materials

Leaves of *E. balsamifera* were collected from the bushes around Barhim village in Katsina and the peels of *C. sinensis* were also obtained from Katsina Central Market. The plant materials were rinsed with clean water and dried under shade, in a well-ventilated area in the Biology Laboratory of the Department of Biology, UMYU, Katsina for 14 days before grinding into fine powders using laboratory blender (Model: 8010ES) and sieved using 80 µm laboratory sieve. The powders were Labeled and kept separately in glass containers and stored at room temperature prior to use.

Twenty gram (20 g) of each of the plant powders were dissolved separately in 500 ml of ethanol (equivalent to 40 mg/ml) and stirred thoroughly using glass rod for 30 minutes. The mixtures were left to stand for 72 hours and shaken several times at certain intervals. The mixtures of the plant powders were filtered using muslin cloth. The filtrate was then transferred into rotary evaporator at 45°C to separate the solvent from the extract. The resulting extract was air-dried to remove traces of the solvent. The extract was stored in tightly corked bottles in a refrigerator before use. Each of 0.5, 1.0 and 2.0 g of the dried extract was dissolved in 10.0 ml acetone to prepare solutions of various concentrations (50, 100 and 200 mg/ml respectively) for the bioassays.

Mortality Test

Twenty gram (20.0 g) of the cowpea seeds were placed in each of the four Petri dishes. The seeds in the first, second and third Petri dishes were mixed with 2.0 ml of each of the concentrations (0.05, 0.10 and 0.20 g/ml respectively) of *E. balsamifera* extract, while none of the extracts was added to the fourth Petri dish, serving as the control. The same procedure as followed for *C. sinensis* extract. The extracts were thoroughly mixed with the cowpea seed with the aid of a glass rod to ensure thorough admixture. Ten insects (five pairs) were released into each of the Petri dishes. The Petri dishes were covered with muslin cloth and secured with rubber bands. The Petri dishes were arranged in a Completely Randomized Design (CRD) and kept in an incubator at 28 ± 2°C and 70 ± 5% R.H. There were four replicates for each treatment. Dead insects were removed and counted daily from each Petri dish until total mortality was obtained.

Damage Assessment

Damage assessment was done on treated and untreated grains. To determine seed damage rate, samples of 10 seeds were taken randomly from each Petri dish of the treatment. The number of damaged (grains with characteristics hole) and undamaged grain were counted and weighed. Percentage weight loss was calculated as follows:

$$\% \text{ Weight loss} = \frac{[UaN - (U+D)]}{UaN} \times 100$$

Where:
U = weight of undamaged fraction in the sample,
N = total number of grains in the sample,
Ua = average weight of one undamaged grain,
D = weight of damaged fraction in the sample.

Germination Test

For germination test 10 seeds were taken randomly from each treated and untreated Petri dishes and placed separately in Petri dishes containing moistened filter paper (Whatman No. 1). Each treatment was repeated four times. Healthy untreated seeds were used as a control. The number of emerged seedling from each Petri dish was counted and recorded after 7 days. The percentage germination was computed using the following formula:

$$\text{Viability index (\%)} = \frac{(NG \times 100)}{TG}$$

Where:
NG = Number of seeds germinated
TG = Total number of seeds tested in each Petri dish.

RESULTS

Results of the period resulting in total mortality of adult *C. maculatus* treated with the two plant extracts are given in Table 1. The Table shows that there was a decrease in the number of days resulting in total mortality from 6.67 to 5.00 days and finally 4.00 days when 0.5 of *C. sinensis* extract was raised to 1.0 and finally 2.0 respectively. There was also a decrease in the period resulting in total mortality from 6.0 to 4.67 days and finally 3.67 days when 0.5 extract of *E. balsamifera* was raised to 1.0 and finally 2.0 respectively, whereas the effects on the period resulting in total mortality of *C. maculatus* in the control was 8.33 days. The results indicate that increase in the extract concentration reduced the number of days resulting in total mortality of the adult insects.

Results on seed damage caused by *C. maculatus* in treated and untreated seeds are seen in Table 2. All the extracts significantly ($P<0.05$) reduced weight loss from the treated Petri dishes. No cowpea seeds were recorded damaged in the treatments of 2.0g/10ml acetone of both extracts of *C. sinensis* and *E. balsamifera*. The seed weight loss was higher (28.21%) from treatments with 0.5g/10ml acetone of *E. balsamifera* than that of *C. sinensis* which was 16.81%. The results also indicate that the untreated cowpea seeds got the highest (65.77%) weight loss.

Table 1: Effects of Extracts from Peel of *C. sinensis* and Leaf of *E. balsamifera* Resulting in Total Mortality of Adult *C. maculatus* on Cowpea Seeds

Plant Extract	Concentration (g/10 ml acetone)	No of Insects Introduced	Mean Period Resulting in Total Mortality (Days)
C. sinensis	0.50	10	6.67
	1.00	10	5.00
	2.00	10	4.00
E. balsamifera	0.50	10	6.00
	1.00	10	4.67
	2.00	10	3.67
Control	0.00	10	8.33
S. E.	_	_	0.73
LSD ($p<0.05$)	_	_	2.59

The germination capacity of cowpea seeds treated with *C. sinensis* and *E. balsamifera* extracts is given in Table 3. The percentage germination increased with the increase in concentration of the extracts. Higher (90.00%) seed

germination was observed in cowpea seeds treated with *E. balsamifera* extracts at the concentration of 2.0 g/10 ml ethanol, while the least (53.33%) was observed in the control. The percentage (76.67%) germination of cowpea seeds treated with 2.0g/10ml acetone of *C. sinensis* was similar to that of 1.0g/10ml acetone of *E. balsamifera*.

Table 2: Effects of Extracts from Peel of *C. sinensis* and Leaf of *E. balsamifera* on Weight Loss by *C. maculatus* Reared on Cowpea Seeds

Plant Extract	Concentration (g/10 ml acetone)	No of Insects Introduced	Weight Loss (Per cent)
C. sinensis	0.50	10	16.81
	1.00	10	4.03
	2.00	10	0.00
E. balsamifera	0.50	10	28.21
	1.00	10	10.71
	2.00	10	0.00
Control	0.00	10	65.77
S. E.	_	_	2.61
LSD (p<0.05)	_	_	4.89

Table 3: Effects of Extracts from Peel of *C. sinensis* and Leaf of *E. balsamifera* Extracts on Germination of Cowpea Seeds.

Plant Extract	Concentration (g/10 ml acetone)	No of Insects Introduced	Seed Germination (Per cent)
C. sinensis	0.50	10	66.67
	1.00	10	73.33
	2.00	10	76.67
E. balsamifera	0.50	10	56.67
	1.00	10	76.67
	2.00	10	90.00
Control	0.00	10	53.33
S. E.	_	_	4.36
LSD (p<0.05)	_	_	6.31

DISCUSSION

This study indicated that leaf extract of *E. balsamifera* and peels extract of *C. sinensis* could be used in the control of *C. maculatus* on stored cowpea seeds. The extracts applied at different rates caused 100% mortality of *C. maculatus*. The result revealed that the peel extracts of *C. sinensis* was effective in killing adult *C. maculatus* growing on cowpea seeds. Similar results were reported with 70.00% adult mortality of *S. zeamais* 28 days after the application of *C. sinensis* peel acetone extract was used [7]. The effectiveness of the orange peel was probably due to silica or silica like component, which are abrasive and the ability of the particles to adhere to the grains. There was no sufficient literature on the use of extracts from the leaf of *E. balsamifera* in reducing grain damage and hence resulting in mortality of *C. maculatus*. However, leaf powder of *E. balsamifera* was used as grain protectant against *S.* zeamais and the findings of the studies showed 90.00% adult mortality of *S. zeamais* after 28 days of treatment of 20 g sorghum grains with 1.00g of *E. balsamifera* leaf powder [13]. Similarly 21.75 days of survival period of *S. zeamais* treated with 2.0 g of leaf powder of *E. balsamifera* was reported, which was significantly ($p<0.05$) different from the control where 122.50 day was recorded [12]. Mortality of *C. maculatus* due to treatment of these plant extracts was directly proportional to the dosages used which agrees with the report that insecticidal activity of any plant extract depends on the active constituents of the plant extract [14]. The effectiveness of these plant extracts might be attributed due to their repellent and toxicity effects against the insect.

Both of the plant extracts were observed to be very effective in protecting the cowpea seeds against damage that might be caused by adult *C. maculatus.* Findings of this study indicated that the highest dose applied did not allow the weevil to cause any damage to the cowpea seeds. This does not agree with the findings of an investigation where 65.24% maize grain damage was reported 28 days after application of *C. sinensis* peel acetone extracts [7]. However, 22.00% damage of sorghum grains caused by *S. zeamais* when treated with leaf powder of *C. sinensis* was reported, while the leaf powder of *E. balsamifera* resulted in only 6.00% sorghum grain damage [12]. Presence of alkaloids, tannins, flavonoids, saponins, steroid, terpenoid, cardic glycoside and balsam was noticed in *E. balsamifera*, and also it was found that the extract of *E. balsamifera* leaves, stem and roots were mostly effective against the *Salmonella typhimurium, Psuedomonas aeroginosa, Klebsiella* spp. *Escherichia coli* and *Candida albicans* [15]. Worldwide reports indicated that when mixed with stored grains leaf, bark, seed powder, or oil

extracts of plants reduce oviposition rate and suppress adult emergence of stored product insects, and also reduce seed damage rates [16, 17, 18]. This might be due to their killing ability which did not allow the adult *C. maculatus* to stay longer in the cowpea seeds thereby feeding less in the seed and hence reducing weight loss.

The germination test reveals that the plant extracts tested against *C. maculatus* did not show any visible adverse effect on germination capacity of the cowpea seeds. The germination capacity was generally dependant on the concentrations of the extracts. Increase in the concentration increased the ability of the seeds to germinate. This means that as the plant extracts had the potentiality of killing the weevils, the seeds were less damaged and did not lose their viability, hence high germination capacity. A report indicated that about 93.00% of haricot beans germinated after treated with *J. curcas* at a dose of 15/ 150 g grain [19]. Similarly, another investigation revealed that powders of *D. stramonium, J. curcas, P. dodecondra* and *A. indica* used in the control of *S. zeamais* did not show any significant effect on the germination capacity of sorghum [20].

CONCLUSION

The findings of this study have revealed that the two plant extracts have potentiality of controlling *C. maculatus* attacking cowpea seeds during storage. The efficacy of the extracts was also found to be directly proportional to the amounts applied and also reduced the cowpea seeds damage that might be caused by the insect pest. It is therefore recommended that the peel and leaf extracts of *C. sinensis* and *E. balsamifera* could be used as alternative insecticides by our farmers.

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