BIOEFFICACY OF PLANT POWDERS IN THE MANAGEMENT OF COWPEA WEEVIL, Callosobruchus maculatus (F.) IN STORED COWPEA, Vigna unguiculata (L.) Walp.

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SUMMARY

The study evaluated the insecticidal efficacy of leaf powders of four locally available plants namely, Indian jujube (Ziziphus mauritiana), Christis thron (Ziziphus spinachristi), Camel's foot (Piliostigma reticulatum) and Tallow tree (Detarium senegalense) against Callosobruchus maculatus (cowpea weevils) infesting cowpea seeds (Vigna unguiculata L.). Bioassay was done by direct application of each plant leaf powder using three concentrations of 1, 3 and 5 g/100 g (1%, 3% and 5% w/w, respectively) of cowpea seeds. Permethrin was added at 0.12 g/100 g cowpea seeds as standard check and untreated treatment with no plant leaf powder or synthetic insecticide served as control. Treatments were laid out in completely randomized design (CRD) with three replications. Data were subjected to analysis of variance using Genstat 17th Edition. Significant treatment means were separated using Student Newman Keuls (SNK) test at 5% level of probability. The results showed significant increase in mortality of C. maculatus at 3 % w/w treatment application (80-83%) after 7 days' exposure to leaf power of Z. mauritiana and Z. spina christis compared to the control (0.07%). Similarly, leaf powders of Z. mauritiana and Z. spina christis at 3 % w/w treatment application was the most effective in reducing oviposition (27.00), thereby bringing about a significant reduction in adult emergence (1.11-0.55), number of emergence holes (0.67) and grain damage (1.11), while it competed favourably with the synthetic insecticide (permethin). All the four plant leaf powders did not exhibit any significant negative effect on viability of the cowpea seeds tested. The results from present study showed that the plant leaf powders could be recommended for adoption by local farmers against C. maculatus infestation and damage to their stored cowpea seeds.

Key words: Bioefficacy, plant powders, Callosobruchus maculatus, Vigna unguiculata

Cowpea, Vigna unguiculata L. (Walp) is one of the most commonly stored food commodities in the tropics and sub-tropics (30). In west and Central Africa, the crop constitutes the cheapest source of dietary protein for low-income sector of the population, thus helping to alleviate protein malnutrition in human (35). It is also an important cash crop for many poor farmers

that make up part of the export commodities for the countries cultivating it. Nigeria and Niger account for 66% of the world cowpea production (172). Other countries with significant production in West Africa include Barkina Faso, Ghana, Senegal and Mali (40).

Cowpea production is affected by insect pest infestations and infection by plant pathogens which lead to economic losses. Insect damage is the major constraint to cowpea grain production in most cowpea producing nations (40). The major insect pest that attack cowpea right from the field to the store is cowpea weevil (Callosobruchus maculatus F.) (Coleoptera: Chrysomelidae). It is polymorphic and has been confused with C. analis, which has non serrate antennae; C. chinensis which has a more angular outline: and C. subinotatus which lacks the dark spots on the elytra and is larger (21). Eggs are laid by the female bruchids on cowpea seeds or pods and the hatched larvae bore into the seed where the entire immature life is spent. The adults emerge out through circular holes they made leaving the seeds damaged. Under favorable conditions, up to 18 adult bruchids can emerge from a single seed (14) and each window left means that one-tenth (1/10) of a seed has been lost (13). A global conservative estimate puts grain legume losses during storage at between 15-25 percent annually (36). Damage of up to 40 percent of cowpea in storage has been recorded in several parts of Africa (41), but Ahmed (3) reported that C. maculatus particularly accounts for over 90 percent of cowpea seed damage. Infestation of cowpea by C. maculatus also leads to loss in weight and quality of seeds. It also causes discoloration, changes of flavor, mould formation, reduced nutritional value due to lowered protein levels and poor germination of seeds due to embryo damage (32). Other damaging effects of C. maculatus infestation to stored cowpea include esthetic contamination of seeds by dead insects, larvae and pupal cocoons and the integument of the insect has been found to contain various carcinogenic compounds such as ethyl, methyl and methoxy quinines which cannot be denatured by boiling or baking (19).

Over the years, the destructive activities and menace of storage pests have been effectively suppressed with synthetic organochlorine and organophosphate compounds (1). However, the application of these chemicals as pest control agents is associated with problems such as high persistence of the compounds, resurgence and genetic resistance of pests, negative effects on non-target organisms, poor knowledge of application by farmers, direct toxicity to the users, non-availability of the chemicals and increasing costs of application (10:37). These led to the searches for alternatives to the synthetic pesticides that are safe, cost-effective and eco-friendly (44). The present study was therefore, aimed at investigating the insecticidal potentials of Camel's foot (Piliostigma reticulatum), Tallow tree (Detarium senegalense), Indian jujube (Ziziphus mauritiana) and Christis thorn (Z. spina-christi) leaf powders against C. maculatus development and damage to stored cowpea seeds.

MATERIALS AND METHODS Experimental site

The experiment was conducted in the Entomology Laboratory of the Department of Crop Protection, Faculty of Agriculture, Bayero University, Kano during the months of September – December 2019.

Source of cowpea seeds and their preparation

A local cowpea variety (Kananado) was purchased from Dawanau International Grains Market, Kano. The seeds were visually checked to ensure that they were not infested. The seeds were then placed in freezer for 3 days to ensure that all stages of insect present were destroyed by the cold.

Maintenance of *C. maculatus* culture *The initial culture of C. maculatus* were obtained from already infested cowpea

seeds maintained in the laboratory and was used to establish new cultures on fresh uninfested cowpea seeds. The stock cultures of *C. maculatus* were raised by placing 100 unsexed adults in 1 Kg of disinfested kananado cowpea seeds contained in two-litre jar. Muslin cloth was used to cover the top of the jar so that cowpea weevils could not escape. This was allowed for mating, oviposition and further multiplication of the insects until the required numbers were obtained after six weeks of inoculation. The F₁ progenies which emerged from the cultures were used for the experiment.

Collection and preparation of plant materials

The plant leaves from camel's foot (Piliostigma reticulatum), tallow tree (Detarium senegalense), Indian jujube (*Ziziphus mauritiana*) and Christis thorn (*Z*. spina-christi) used as treatments in the study were collected from the vicinity of Bayero University Kano (New Site). The plant leaves from these plants were separately washed and dried under shade for seven days in the laboratory and ground to fine powders using electric blender (Master Chef MC-B144). The powders were further sieved through 1 mm² perforation. The powdered materials were separately kept in air-tight containers, labeled, and stored at room temperature until needed for the experiment.

Source of synthetic chemical

Permethrin powder (0.6 a.i.) was purchased from Sabon Gari market, Kano State. The expiry date was checked properly to ensure that the chemical used was not an expired product.

Bioassay

The first experiment comprised of a study on the evaluation of insecticidal potentials of the botanicals. Hundred grams of cowpea seeds were placed in a 500 ml glass jar and then thoroughly mixed with the various plant leaf powders at three dosage/concentration levels of 1, 3 and 5 g/100g of cowpea seeds (1 %, 3 % and 3 % w/w, respectively), while a synthetic insecticide (permethrin) was applied at 0.12g/100g of cowpea seeds as standard check, and untreated cowpea seed served as control. Five (5) pairs of newly emerged adults were then introduced into each container of cowpea using entomological aspirator. The containers were covered with muslin cloth and tightly secured with rubber bands. The experiment was arranged in completely randomized design (CRD), replicated three times, and kept on laboratory bench for eight weeks and the following data were recorded:

Mortality rate: At 7 days after infestation, dead insects in each treatment and replicates were removed, counted and recorded. Insects were probed three times with a tip of pen to confirm mortality (44). Mortality rate was calculated using the formula:

Mortality rate =

Number of dead C. maculatus x 100

Total number of *C. maculatus*

Fecundity of *C. maculatus*: The insects were allowed to mate and oviposit for 10 days, after which the number of seed with eggs were counted with the aid of dissecting microscope and recorded from each of the experiment unit, while the seeds from each treatment were examined individually (7).

Egg Hatchability and Progeny Development of *C maculatus*: After 10 days of oviposition, 30 treated and untreated seeds that contain eggs were selected and placed into a new plastic container containing clean uninfested cowpea seeds. Adult that emerged were recorded 30 days after the exposure (28).

Percentage adult emergence was calculated from each of the treatments and replicates,

using the formula:
% Adult emergence =
Number of adult emerged x 100
Number of eggs laid

Number of holed cowpea grains: At the end of the experiment, the number of exit holes was assessed by counting the number of holes which appeared on each seed with the aid of dissecting needle. The seeds were turned upside down and from side to side to ensure that no hole was left uncounted

Percentage seed damage: At the end of the experiment, 30 seeds were randomly selected from the sample bottles and the seeds were separated into damaged and undamaged categories and each was counted. Thus percentage seed damage was calculated using the formula:

% Seed damage =
Number of seed damaged x 100
Total number of seeds

Loss in grain weight: At the end of the experiment, treated and untreated treatments were sieved to remove dead insects and other debris in the cowpea seeds and the seeds were weighed to obtain the final seed weight. Percentage seed weight loss was calculated using the formula (38):

% weight loss =

<u>Initial weight-Final weight</u> x100

Initial weight

Germination percentage: At the end of the experiment, 10 seeds were randomly picked from each jar in all the treatments and replicates and placed in Petri dishes lined with moistened filter paper. These were left on the laboratory bench at ambient temperature and relative humidity for 7 days after which germination percentage was calculated using the formula after Olisa *et al.* (33):

% Germination =
Number of germinated seed x 100
Total number of seed planted

Residual Toxicity

Adult mortality: Data on residual toxicity

were collected by counting the number of dead insects from the F₂ progeny produced by the parents at 60 days after treatment.

Larval and pupal mortality: Larval and pupal mortality was obtained at the end of the experiment by opening the cowpea seeds with a scalpel and a pair of forceps. Dead larvae and pupae inside the seeds were counted separately and each expressed as percentage of the seed examined (7).

Data Analysis: Data collected were subjected to analysis of variance (ANOVA) using Genstat 17th Edition computer software. Significant treatment means were separated using Student Newman Keuls (SNK) test at 5% level of probability.

RESULTS

Treatment effects on mortality

Table 1 shows that the synthetic chemical had significantly higher mean adult mortality (0.9) rate, but was statistically similar with cowpea seed treated with *Ziziphus mauritiana* and *Z. spina-christi* leaf powders at 3 and 5g concentrations. Lowest mean mortality was recorded in the untreated control (0.07), except that this was not significantly (P<0.05) different from the mortalities recorded in grains treated with *Piliostigma reticulatum* and *Detarium senegalense*, irrespective of the rate of application.

Treatment effect on fecundity of *C. maculatus* on stored cowpea seeds

All the tested plant materials caused reduction in the mean number of eggs laid by *C. maculatus*. Cowpea seed treated with *Z. mauritiana* leaf powder at 5g/100 cowpea seed concentration had the lowest mean number of eggs (26.67), but was not significantly different from that of *Z. spina*-

christi at 3 and 5 g/100g cowpea seed; and synthetic chemical at 0.12 g/100 cowpea seed (25.33). The untreated control had the highest mean number of eggs (100.67) and was significantly (P<0.05) higher than all other treatments (Table 1).

Treatment effect on adult emergence of *C. maculatus* on stored cowpea seeds

All the plant leaf powders irrespective of their concentrations significantly affected adult emergence of C. maculatus 30 days after treatment (Table 1). Z. spina-christi leaf powder at 3 g/100 g cowpea seed concentration recorded mean of 0.00 adult emergence, although this did not differ significantly (P<0.05) with Z. mauritiana and Z. spina-christi leaf powders at 3 and 5g/100 cowpea seed, respectively and the chemical control (permethrin) at 0.12 g/100 g cowpea seed concentration. The untreated control recorded the highest mean number of adult emergence (36.11) and was significantly different from all other treatments.

Treatment effect on seed perforation

Table 1 shows the effect of plant materials on number of holed cowpea seeds caused by *C. maculatus*. Treatments with synthetic pesticide powder (permethrin) at 0.12 g/100 cowpea seeds had zero (0.00) seed perforation, although this was not significantly different from treatments with *Z. mauritiana* and *Z. spina-christi* leaf powders at 3 and 5g/100 g of cowpea seeds, respectively. The highest percentage seed perforation (76.00%) was in the untreated control seeds, and this was significantly (P<0.05) higher than the seed perforations in all the other treated seeds.

Effects of treatments on percentage cowpea seed damage caused by adult C. maculatus: There was significant difference (P<0.05) in percentage cowpea seed damage among all the treatment compared with the untreated control (Table

1). Seeds treated with *Z. mauritiana* at 5 g/100 g cowpea seeds and permethrin at 0.12 g/100 g cowpea seeds had 0.00% mean cowpea seed damage, although this were not significantly (P<0.05) different from treatments with *Z. mauritiana* leaf powder at 3 g/100 g cowpea seeds and *Z. spina-christi* leaf powder at 3 and 5g/100 g cowpea seed concentrations (1.11%). However, the untreated control which sustained the highest mean cowpea seed damage (43.33%) was significantly different from other treatments.

Treatment effects on seed weight loss

The percentage seed weight loss caused by adult *C. maculatus* on cowpea seeds treated with plant leaf powders revealed that the synthetic chemical powder (permethrin) at 0.12 g/100 g cowpea seeds had 0.00% seed weight loss but was not significantly different (P<0.05) from the weight loss (0.33%) in treatments with plant leaf powders of *Z. mauritiana* and *Z. spina-christi* leaf powders at 3 and 5g/100 g cowpea seeds concentrations. The control treatment had the highest seed weight loss (32.33%) and was significantly higher than the weight loss in other treated grains (Table 1).

Treatment effects on germination percentage

Table 1 shows the results of germination of cowpea seeds treated with various leaf powders. Seeds treated with Z. mauritiana leaf powder at 5 g/100 g cowpea seeds and Z. spina-christi leaf powder at 1, 3 and 5g/100 g cowpea seeds had the highest mean germination percentage of 96.67%, although these were not significantly different from the germination percentages in grains treated with the synthetic pesticide (permethrin). The untreated treatments had the lowest germination percentage (6.67%) and was significantly (P<0.05) different from other treatments.

Table 1: Effect of plant leaf powders on adult C. maculatus mortality, insect development, seed damage and percentage seed germination of cowpea

Treatment	Concentration (% Adul	on (% Adult	Number	% Adult	Number	% Cowpea seed	% Cowpea	% Seeds
	w/w)	mortality	of eggs	Emergence	of holed	damage	weight	germination
		(Rate)	laid		cowpea		loss	
					seeds			
Z. mauritiana		0.37b	63.67d	10.00c	19.00e	20.00d	5.00c	63.33b
	3	0.80a	27.00e	1.11d	0.67f	1.11e	0.33d	93.33a
	5	0.83a	26.67e	0.55d	0.33f	0.00e	0.33d	96.67a
Z. spina-christi		0.33bc	62.00d	11.11c	19.00e	26.66c	5.33c	66.67b
,	3	0.83a	28.00e	0.00d	0.67f	1.11e	0.33d	96.67a
	5	0.83a	27.67e	0.55d	J. 10.67f	1.11e	0.33d	96.67a
P. reticulatum		0.17d	77.33c	29.44b	62.67d	36.66b	29.00b	16.67c
	3	0.23cd	80.33bc	29.44b	64.00cd	35.55b	28.00b	13.33c
	5	0.17d	84.33b	30.55b	63.67cd	36.66b	28.67b	6.67c
D. senegalense		0.10d	81.00bc	30.55b	67.67b	36.66b	28.67b	13.33c
	3	0.20cd	80.33bc	30.00b	67.00b	36.66b	28.00b	10.00c
	5	0.10d	81.00bc	31.77b	65.67c	36.66b	28.00b	10.00c
Permethrin	0.12	0.9a	25.33e	0.33d	0.00f	0.00e	0.00d	96.67a
Control	0.00	0.07d	100.67a	36.11a	76.00a	43.33a	32.33a	6.67c
SE+		0.0398	1.373	0.855	0.638	1.784	0.512	3.87

Effects of plant leaf powders on the mortality of adult, larval and pupal F₂ generation of C. *maculatus*:

Mortalities of larval, pupal and F_2 generation of *C. maculatus* were affected by the plant leaf powders, and results on Table 2 showed significant (P<0.05) differences among treatments. Seeds treated with *Z. spina-christi* leaf powder at 3% w/w had the highest mean adult mortality (7.33), followed by *Z. mauritiana* at 3 and 5% w/w and *Z. spina-christi* at 5% w/w, although there were no significant differences among them. The lowest F_2 mean larval mortality of *C. maculatus* (0.33) was recorded at

treatments containing *D. senegalense* at 1% w/w and the synthetic pesticide (permethrin) at 0.12 g/100 g cowpea seeds, except that these were not significantly different from treatments with *P. reticulatum* at 5% w/w. Similarly, seeds treated with *Z. mauritiana* leaf powder at 3 and 5% w/w recorded high pupal mortality (4.00 and 3.00, respectively) compared to permethrin and untreated control. Lower larval mortality was achieved on seeds treated with *P. reticulatum* leaf powder (0.00), but was statistically similar with other leaf powders and significantly different from the untreated control.

Table 2: Mean mortality of adult, larval and pupal F₂ generation of *C. maculatus* sixty days after treatment with leaf powders

		F	Residual To xicity	
Treatment	Concentration (% w/w)	Adult Mortality	Larva Mortality	Pupal Mortality
Z. mauritiana	1	2.67bc	0.67bc	1.00c
	3	6.67a	0.67bc	4.00a
	5	6.67a	0.67bc	3.00ab
Z. spina-christi	1	2.33bc	0.33bc	1.33c
	3	7.33a	0.67bc	3.00ab
	5	6.67a	1.00ab	2.00bc
P. reticulatum	1	1.00ef	1.00ab	0.33c
	3	1.67cd	0.00c	0.76c
	5	0.67eg	0.33bc	0.33c
D. senegalense	1	0.33g	0.33bc	0.33c
	3	1.67cd	0.67bc	0.67c
	5	1.00ef	0.33bc	0.33c
Permethrin	0.12	0.33g	0.33bc	0.33c
Control	0.00	3.33b	2.33a	2.00bc
SE+	-	0.384	0.351	0.385

DISCUSSION

Plant leaf powders of Z. mauritiana and Z. spina-christi used in this research showed significant (P < 0.05) effect on the mortality and fecundity of adult C. maculatus as well as damage caused on cowpea seeds. All the leaf powders were observed to have affected the mortality of C. maculatus at varying amounts applied. The results of this study conform with the report of previous workers (34;27;1;19) who observed that

certain botanicals were effectively toxic against storage insect pests including *C. maculatus*. The resultant mortality rates of *C. maculatus* in this investigation could be attributed to the insecticidal effects of the chemicals in the tested plant species.

The high toxicity of *Z. mauritiana* and *Z. spina-christi* could be attributed to the presence of phenolic and alkaloid metabolites which act as insecticidal, repellant and antifeedants against insects

(12;6;4). The present study is in line with the findings of Urbbi and Dipsikha (42), who reported the effect of phenolic extracts of *Ziziphus jujuba* leaves on *Aedes aegypti* (Diptera: Culicidae). Earlier report revealed that *Z. jujuba* has been effective against *Culex pipiens* larvae in which the petroleum ether extract and oil had caused pathological effect on pupa and adult (16).

Several researchers have worked on plants and naturally derived pesticides that are nontoxic to human and other animals (45;44;24). This serves as useful tool for the development of a safer, effective, sustainable and environmentally friendly bruchids control tactics. Promising results obtained in this study showed the insecticidal effect of *Z. mauritiana* and *Z. spina-christi* against *C. maculatus*. The identified compounds which are naturally occurring in most plant materials are known to be pesticidal, bactericidal and fungicidal in nature, thus conferring the pesticidal activity to plants (5).

The present study confirmed that leaf powders of Z. mauritiana and Z. spinachristi had relatively caused high mortality of C. maculatus on stored cowpea seeds. This corroborates the findings of Bhagat and Tripatri (11) and Lucy et al. (23), who reported an increasing efficacy of neem leaf powder as concentration increased from 1-3g/100 g seeds. On the other hand, the plant extracts might have interfered with the normal embryonic development by suppressing hormonal and biochemical processes. Similar physiological interferences were observed by Ofuya et al. (31) and Jayakumar et al. (20). Similarly, oviposition and adult emergence were suppressed in grains treated Z. mauritiana and Z. spina-christi leaf powders. This is similar with the findings of Yusuf and Ahmed (43), who reported that the ground

parts of some plants (Neem, Chinaberry, Eucalyptus and Chilli) were effective in suppressing the emergence of the maize weevils *S. zeamais* on stored maize grains.

The effect of all the treatments with the exception of the control (untreated grains) proved effective in reducing the number of emergence holes, suggesting low amount of perforated grains treated with Z. mauritiana and Z. spina-christi leaf powders. This agrees with earlier findings of Asawalaam and Emosairue (9), who reported that powdered form of Piper guineense and pirimiphos-methyl were effective in controlling weevil perforation caused by S. zeamais on stored maize grains. Grain damage caused by bruchids on cowpea seeds was highly reduced in all the treatments. The leaf powders of Z. mauritiana and Z. spina-christi appeared to be more promising as it compared favourably with the synthetic insecticide (permethrin) treatment. These findings corroborate Dike et al. (15), who reported that phenolics are well known to be directly involved in protection of grains against insects through antixenosis and antibiosis mechanisms.

On the other hand, grains treated with *Z. mauritiana* and *Z. spina-christi* leaf powders showed no significant effect on loss in grain weight. This is similar to the findings of Yusuf (44), who reported that powdered form of five plant materials (neem, chinaberry, eucalyptus, chilli and mahogany wood ash) were effective in reducing grain damage and loss in grain weight caused by *S. zeamais* on stored maize grains.

Application of *Z. mauritiana* and *Z. spina-christi* leaf powders as treatments showed no significant effect on the germination of the seed after 60 days of treatment. This was similar to the findings of Obengofori and Dankwah (29), who reported that neem leaf

powder and actellic 25EC did not affect the germination of Bambara nut seed after 60 days of treatment. The finding from the present study was also in agreement with that of Mariappan *et al.* (26), who reported that seeds protected with *Jatropha curcas* pelleted with pungan leaf powder and *Pongamia pinna* seeds pelleted with neem leaf gave high germination percentage due to effective protection from fungal and insects attacks, thus increasing percent germination of the treated seeds.

Similarly, leaf powders of Z. mauritiana and Z. spina-christi were also effective in reducing adult, larval and pupal emergence of the F₂ progenies after sixty days. This corroborates with the findings of Asawalam and Anaeto (8), who reported that plants such as neem (Azadirachta indica), chili pepper (Capsicum annum), clove (Syzygium aromaticum), Ethiopian pepper (Xylopia aethiopica), etc. possess secondary metabolites which act as antifeedants, oviposition deterrents, larvicidal and insect growth regulators. These plants are known to repel insects and the products from these plants have been reported to be toxic to insects (2). Application of products of such plants, fresh or dried materials, extracts or oil to stored products have been shown to effectively protect stored products against C. maculatus infestation (22). Oparaeke and Dike (34), recorded success using plant oils from calabash nutmeg (Monodora myristica) and Onion (Allium cepa) as protectants against C. maculatus infesting stored cowpea seeds.

CONCLUSION

Ziziphus mauritiana and Z. spina-christi leaf powders performed better than camel's foot (*Piliostigma reticulatum*) and Tallow tree (*Detarium senegalense*) in reducing damage to treated cowpea seeds by C. maculatus irrespective of their concentrations and their activities were similar to that synthetic chemical (permethrin). However, higher adult mortality of C. maculatus; reduction in oviposition and seed damage; reduction of weight loss and inhibition of progeny emergence were achieved by using both the Z. mauritiana and Z. spina-christi leaf powders at 3 and 5% w/w. Therefore, based on the results from present study, it can be recommended that the use of Z. mauritiana and Z. spina-christi leaf powders at 3% w/w could be used for the management of C. maculatus on stored cowpea. Further investigations should also be carried out to determine the specific chemical substances responsible for the insecticidal activity of the plants used in the present study.

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