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INSECTICIDAL ACTIVITY OF STEM BARK AND POD OF LOCUST BEAN (*Parkia biglobossa*) AGAINST COWPEA WEEVIL (*Callosobruchus maculates* F.) CAUSING DAMAGE IN STORED COWPEA

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ABSTRACT
Laboratory experiment was carried out to evaluate the efficacy of Stembark and pod powders of locust bean (*Pakia biglobossa)* and their appropriate concentration for the management of cowpea weevils (*Callosobruchus maculatus* F.). Spintor (0.125%) and dust (0.125 g) was used a positive control and untreated cowpea seeds serving as negative control. The experiment was laid in Completely Randomized Design (CRD) comprising of Stem bark powder (1 g, 3 g and 5 g), Pod powder (1 g 3 g and 5 g), spintor 0.125% Dust (0.125 g) and control. These were applied to 100 g healthy cowpea seeds and repeated three times. Contact activity of the plant products were evaluated and data collected were subjected to analysis of variance (ANOVA) using SPSS software (version 23) and means were separated using NDMRT at 1% and 5% probability level. The results showed that higher concentration (5 g/100g) of plant products significantly ($P \leq 0.05$) caused mortality of *C. Maculatus* after 24 (2.33), 48 (2.66), 72 (2.66) and 96 hours (3.00) by contact toxicity compared to other treatments. Similarly, higher concentration (5 g/100g seed) of the pod powder significantly ($P \leq 0.05$) decreased oviposition (3.67), adult emergence (3.66), percentage seed damage (1.73%), number of exit holes (6.33) and weight loss (1.03%) compared to untreated cowpea seed that record 58.00, 54,67, 19.67%, 56.33 and 13.87%, respectively. It was concluded that germination of the treated seeds was not affected negatively. The Pod powder of *P. biglobossa* could be recommended as suitable alternatives to chemical pesticides to be used as contact protectant against *C. maculatus* on stored cowpea seeds.

Keyword: *Callobruchus maculatus,* Control, *Parkia biglobossa,* Pod, Spintor Dust, Stem bark.

INTRODUCTION
Among the most important cultivated legumes, cowpea (*Vigna unguiculata* (**L**.) Walp) has shown several agronomic, environmental and economic advantages, contributing to the improvement of peasant farming income across Africa, Asia and South America (Hall, 2012; and FAO, 2014). Cowpea has its origin from the southern African region but has spread and is now cultivated in more than 100 countries between 40^{o}N and 30^{o}S latitudes (Vasconcelos, 2010; Fang, 2007; and Xu, 2010). Cowpea is as sources of food to the animals as fodder and pod are being consumed by the animals (Beck and Blumer, 2014). Due to its recognised nutritional value (high protein and low fat content), which is related to the prevention of diverse metabolic and cardiovascular diseases, the whole above-ground cowpea plant is used as multipurpose crop, being consumed for its leaves, green pods, green beans, mature beans, or processed into paste or flour and used as a food ingredient (Vasconcelos, 2010; and Enyiukwu *et al*., 2018).

However, Storage of the grains can be problematic in Africa due to potential infestation by post-harvest pests (Nasiru *et al.,* 2016).Severe *Callosobruchus maculatus* infestations can affect 100% of the stored peas and cause up to 60% grain loss within few months (Kang *et al.*, 2013). The most common methods of protection involve the use of insecticides, and the main pesticides used were carbomates, synthetic pyrethoids and organophosphates (*Kupferschmid et al*., 2013). However, their increasing use in recent years has created a range of ecological problems such as bio-magnification, resurgence and the development of insecticide tolerant strains of pest species (Radha and Susheela, 2014). Traditional methods of protecting stored grain include using the insecticidal properties of Neem extracts, mixing the grain with ash or sand, using vegetable oils, combining ash and oil into a soap solution or treating the cowpea pods with smoke or heat. Certain plant species were tested for their insecticidal properties under laboratory conditions against *C. Maculatus* (Kathirvelu *et al.*, 2019) reported that the formulation manifested the control over test insects.

Parkia biglobosa occurs in the belt between 5^{o}N and 15^{o}N from the Atlantic coast in Senegal to southern Sudan and Northern Uganda (Campbell-Platt, 1980). The tree is not normally cultivated but can be seen in the wild in the savannah region. It has a wide distribution ranging from the Sudan and Guinea savannah ecological zones. *Parkia biglobosa* is found in nineteen African countries: Senegal, The Gambia, Guinea Bissau, Guinea, Sierra Leone, Mali, La Côte d'Ivoire, Burkina Faso, Ghana, Togo, Benin, Niger, Nigeria, Cameroon, Chad, Central African Republic, Zaire, Sudan and Uganda (Agroforest Database, 2013). In Ghana the tree grows wild throughout the savannah to (Thiombiano *et al.*, 2012).

The extracts from *P. biglobosa* have been used as natural pesticides against different pests and diseases due to the phytochemicals present in different parts of the plant. Phytochemicals are certain non-nutritive, biologically active plant chemicals which have some disease and insect preventive properties. They provide plants with colour, flavour and natural protection against pests (Pankaja, 2013). Fawole and Abikoye (2002) discovered that *Fusarium* wilt of cowpea could be managed with *Parkia* husk ethanolic extract. The effectiveness of *P. biglobosa* ethanolic seed extract in controlling *Meloidogyne incognita* infestation in tomato plants was reported (Bawa *et al*., 2014). Also, Sani (2014) discovered the antifeedant effect of petroleum ether and ethanolic seed extracts of *Parkia biglobosa* against cowpea bean storage pest (*C. maculatus*). Bukar *et al*. (2010) determined the phytochemical composition of aqueous extracts of leaves and pods of *P. biglobosa* and found out that aqueous extracts of the pods had the highest phytochemical composition. Sherah *et al*. (2014) compared the phytochemical composition of the plant's stem bark and seed husk methanolic extracts and found that the seed husk had higher phytochemical composition Udobi and Onaolapo (2009) reported that the leaves, stem bark and roots of the plant contain relatively equal number of phytochemicals.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at Agronomy Laboratory of the Faculty of Agriculture and Agricultural Technology Abubakar Tafawa Balewa University Bauchi. Bauchi State is located between latitude 10^{o}22'E and 9^{o}48'E and situated at 690.3m above the sea level in Savannah zone of Nigeria.

Sources and Rearing of *Callosobruchus maculatus*

Samples of *C. malulatus* were collected from previously infested stored Cowpea seeds obtained from Rail Way Market in Bauchi Local Government Area (LGA) of Bauchi State, Nigeria. The insects were brought to the laboratory and kept at room temperature 28 $\pm$ 2 ^{0}C and relative humidity 70 $\pm$ 5%. 10 kg of sound uninfected Cowpea seed seeds was weight and transferred into an earthen clay pot. Thereafter, Cowpea weevils were transferred into the earthen clay pot. The top of the earthen clay pot was covered with white muslin cloth, tightened firmly with a rubber band and *C. malulatus* were allow to oviposit under crowded conditions in a light:dark regime of 12L:12D at room temperature and relative humidity for one week. After one week, dead and live parent stocks were completely sieved out to await the emergence of F_1. The same procedure was used with F_1 generation to obtain F_2 generation used for the experiment.

Disinfestation of Cowpea Seeds

The Cowpea Seeds were manually sorted to remove broken seeds with holes and other contaminants; they were sieved again using sieve 0.25 mm to remove insects and remaining contaminants. The sorted seeds were fumigated with Phostoxin tablets for 48 hours under airtight conditions to exclude any possible infestation that may affect the seeds. After 48 hours, the fumigated cowpea seed were spread on a clean polythene mat covered with a cloth to remove any residual effect of the fumigant and to exclude re-infestation. This was done for 48 hours.

Collection and Preparation of Locust Bean Pod and Stem Bark

The locust bean pod and stem bark were collected from the locust bean trees from Gubi campus of Abubakar Tafawa Balewa University Bauchi. The plant materials were washed with water to remove dirty material and it was dried under the shade to prevent the denaturation of chemical substance for about two weeks. They were grounded, sieve through 0.25 mm mesh and were stored in a polythene bag in the laboratory.

Treatment and Experimental Design

The experiment consisted of pod (1 g, 3 g and 5g), stems bark (1 g 3 g and 5 g), positive control {Spintor Dust (0.125 g)} and negative (untreated cowpea seeds). These were applied to 100 g healthy cowpea seeds and repeated three times. The experiment was laid out in completely Randomized Design (CRD) and means were separated using New Duncan Multiple Range Test (NDMRT).

Contact toxicity

Five (5) pairs of laboratory reared F_2 generation of the Cowpea weevils (*C. maculatus*.) were introduced into the treated and untreated Cowpea seeds already contained in plastic jars (measuring 4 x 6 x 8 cm). The open end of each plastic container covered with a fine muslin cloth and tied firmly with rubber band and kept at room temperature (29 –32^{o}C) in the laboratory. Observation on adult mortality was recorded at 24 hours, 48 hours and 72 hours after exposure to the treatment as described by (Liu and Ho, 1999).

Evaluation of Oviposition

Ten cowpea seed containing laid eggs were randomly selected from treated and untreated seed. Numbers of eggs were counted with the aid of magnifying hand lens and percentage oviposition deterrence was calculated using the formula (% Deterrence $=\frac{Cs-Ct}{Cs}$x100) as described by (Vanmathi *et al*., 2010).

where;

Cs = number of eggs laid on control seed,

Ct = Number of eggs laid on treated seed

Evaluation of Adult Emergence

All the treated and untreated seed were subjected to incubation at room temperature in the dark for eight weeks to monitor the emergence of adult weevil from the seeds. The number of adult weevils emerged by the weevil were recorded. The percentage adult emergence deterrence was calculated using the formula (% Deterrence $=\frac{Ac-At}{Ac}$ x 100) as described by (Vanmathi *et al*., 2010).

where;

Ac = Number of adult emerged on control,
At = Number of adult emerged on treated seed

Evaluation of Percentage Seed Damage

Ten seeds were randomly selected from each treatment and examined the number of exit holes. Seeds containing three or more holes were considered as damaged seeds. Number of damaged and undamaged seeds were counted and recorded for each repetition using the formula as described by (Awoke *et al*., 2014):

$$\% \text{ Seed Damage} = \frac{G1}{G2} \text{x} 100 \qquad \ldots (1)$$

where;
G_1 = Number of seed with holes
G_2 = number of seed without holes

Evaluation of Weight loss

At the end of the experiment, the cowpea seeds were sieve to remove the dead insect and powder, and reweight to obtain the weight loss using the formula as described by (Awoke *et al*., 2014):

$$\% \text{ Loss} = \frac{initial\ weight - final\ weight}{initial\ weight} x100 \qquad \ldots (2)$$

Evaluation of Viability Test

In the study, 10 uninfected seeds were randomly selected from each treatment to evaluate germination test. The seeds were arranged on a moisten Petri dishes, the germination process lasted for a period of seven days after which the number of germinated seeds in each Petri-dishes was counted and recorded. The germination percentage was calculated using the formula as described by (Awoke *et al*., 2014):

$$GP = \frac{number\ of\ seed\ germinated}{\text{Total number of seed planted}} \times 100 \qquad \ldots (3)$$

Data Collection and Analysis

Data were collected on Adult Mortality, Number of seed with holes, Number of eggs laid, Number of weevils that emerge, Number of germinated seed, Weight loss and Percentage grain damage. All data collected were subjected to statistical analysis of variance (ANOVA) using SPSS software (version) and New Duncan's Multiple Range Test (NDMRT) was used to separate the difference between treatment means.

RESULTS AND DISCUSSION

Table 1 showed eff ect of Stem Bark and Pod on adult mortality of *C. maculatus.* The results showed that all treated cowpea seed significantly ($P \leq 0.05$) cause mortality of adults *C. maculates* by contact toxicity compared to the untreated cowpea seed. Cowpea seeds with 5 g pod powder per 100 g recorded the highest mortality of adult *C. Maculates* after 24 (2.33), 48 (2.66), 72, (2.66) and 96 (3.00) hours of exposure compared to other treated seeds. The highest mortality recorded might be due to the higher concentration of phytocompounds in pod powder that might prevent the insect from feeding and respiration which resulted to dead by starvation or blockage spiracles that hinder respiration. Silva *et al*. (2012) and Devappa *et al*. (2010) have earlier reported that, the insecticide activity of plant varies according to the part of the plant from which toxic metabolite was synthesized. In the present study, cowpea seeds treated with 5 g pod powder recorded the highest mortality of adult *C. Maculatus* and mortality rate observed was high within three to four days after exposure to the treatments. This is in conformity to the findings of Ashamo *et al*. (2013) who reported that extract of *Zanthoxylum zanthoxyloides* caused 100% mortality of *C. maculatus* when applied at higher concentration

1.5% and 2.0% in three to four days post exposure which he attributed to the higher phytocompounds in the extract of the tested plants.

Similar result was also observed by Abdullahi (2011) who reported that, highest mortality (100%) of adult *C. Maculates* was recorded on cowpea seed treated with leaf powders of *Balanites aegyptiaca* applied at the concentration of 2 g and 1.5 g/20g seed after 24hours of treatment and which is comparable to chemical pesticide (Actellic dust) that was considered as positive control in his research. He further stated that, the lowest (0.5 g) mortality of the insect was observed on the seeds treated with lower concentration of the leaf powder of *B. Aegyptiaca* which recorded 100% mortality but after 120 hours of treatment and this was found to be better than the mortality recorded on the control seeds.

Table 1: Mean mortality of adults *callosobruchus maculatus* exposed to Cowpea Seeds Treated with Different Concentration of Stem Bark and Pod Powder of *Parkia Biglobossa*

		Mean Adult mortality (Hours)					
Treatment	**Conc.(g/100g seed)**	**24**	**48**	**72**	**96**	**168**	**336**
Stem Bark	1	1.00 b	1.00 d	0.33 c	2.33 ab	0.66 c	1.33 c
	3	0.33 cd	1.00 d	1.00 c	1.33 cd	2.66 ab	2.67 a
	5	0.66 c	2.00 bc	2.00 ab	1.66 bc	2.66 ab	2.00 b
Pod	1	0.66 c	1.00 d	1.33 b	1.66 bc	2.33 ab	1.66 bc
	3	0.33 cd	1.66 c	2.00 ab	2.33 ab	3.00 a	2.67 a
	5	2.33 a	2.66 ab	2.66 a	3.00 a	2.66 ab	0.00 d
Spintor 0.125% Dust	0.125	2.66 a	3.00 a	1.66 b	1.66 b	0.00 d	0.00 d
Control (untreated)		0.00 d	0.00 e	0.00 c	0.66 d	0.00 c	0.33 d
LS		**	**	*	*	**	*
SE(±)		0.58	0.65	0.73	0.72	0.62	0.64

**Significant at 1%, and * at 5%; Means followed by the same letter are not significantly different (P≤0.01) from each other, using New Duncan's Multiple Range Test (NDMRT); LS = level of Significance; SE = Standard Error

Table 2 showed the effect of Stem Bark and Pod Powders on oviposition and percentage oviposition deterrence of *C. maculatus*. The result revealed that the highest (58.00) oviposition was recorded in the untreated cowpea seeds (control) and the number is almost doubled than those recorded in other treated cowpea seed. As observed from the result cowpea seed treated with 5 g/100g cowpea seed and Spintor Dust significantly (P≤0.05) recorded the lowest (3.66) and (4.00) number of eggs compare to other treated seeds. The reduction in numbers of eggs could be related to the early of mortality of some female or morbidity seen on male *C. maculatus*. The present finding is in line with that by Frank *et al*. (2018) who reported that, *Piper guineense* performed better when compared with the control which he attributed to high mortality of *C. maculatus* that could not oviposit.

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Table 2: Effect of Stem Bark and Pod Powder of *Parkia biglobossa* on Number of Eggs and Deterrence of *Callosobruchus maculatus* on Treated and Untreated Cowpea Seeds

Treatment	Conc. (g/100g seed)	Number of eggs	Deterrence (%)
Stem Bark	1	19.33^{b}	66.72
	3	10.67^{d}	81.55
	5	7.67^{e}	86.72
Pod	1	15.67^{c}	72.93
	3	7.67^{e}	86.72
	5	4.00^{f}	93.62
Spintor 0.125% Dust	0.125	3.66^{f}	93.62
Control (untreated)		58.00^{a}	0.00
LS		**	
SE(±)		2.09	

**Significant At 1%; Means followed by the same letter are not significantly different (P≤0.01) from each other, using New Duncan's Multiple Range Test (NDMRT); LS = level of Significance; SE = Standard Error

Table 3 showed the effect of stem bark and pod powder on adult emergence and percentage adult emergence deterrence of *C. maculatus*. The result showed that untreated cowpea seed recorded the highest (54.67) adult emergence of *C. maculatus* compare to other treated cowpea seeds. The application of 5 g pod powder and Spintor Dust significantly (P≤0.05) have lowest (3.33) and (3.67) adult emergence of adult *C. maculatus* compare to other treated seed. The lowest progeny emergence recorded might be attributed to toxicity in the plant materials and chemicals composition in Spintor Dust which rendered eggs impotent that prevent the eggs from hatching.

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Table 3: Effect of Stem Bark and Pod Powder of *Parkia biglobossa* on Adult Emergence and Percentage Deterrence of *Callosobruchus maculatus* in Treated and Untreated Cowpea Seeds

Treatment	Conc. (g/100g seed)	Number of Adult emerge	Deterrence (%)
Stem Bark	1	17.67[b]	67.67
	3	10.00[d]	81.70
	5	7.00[e]	87.20
Pod	1	13.67[c]	74.99
	3	6.67[e]	87.80
	5	3.33[f]	93.90
Spintor 0.125% Dust	0.125	3.67[f]	93.20
Control (untreated)		54.67[a]	0.00
LS		**	
SE(±)		2.06	

**Significant At 1%; Means followed by the same letter are not significantly different ($P \leq 0.01$) from each other, using New Duncan's Multiple Range Test (NDMRT); LS = level of Significance; SE = Standard Error

The results of Table 3 agrees with that of Adenakan *et al*. (2013) and Adebiyi and Tedela (2012) who reported that, the active ingredient in plant caused different toxicity properties to insects either by contact, strong odour or respiratory poison which could inhibited the eggs from hatching even when laid. Ashamo *et al*. (2013) earlier reported that the weakening of adults by plant powder may cause insects to lay fewer eggs than normal. Oigiangbe *et al*. (2010) and Ashamo *et al*. (2013) also reported that inability of the insects to emerge may be due to the death of the insect larval that caused by inability of the larval to fully cast off their exoskeleton which typically remained linked to the posterior part of their abdomen.

Table 4 showed the effect of stem bark and pod powder on percentage seed damage and number of exit holes. The result showed that, there is significant ($P \leq 0.05$) difference between treated and untreated cowpea seeds with respect to percentage seed damage and number of exit holes. The percentage seed damage (19.67%) and number of exit hole (56.30) was high in the untreated control compared to other treated cowpea seeds. Cowpea seeds treated with 5 g pod powder recorded the lowest (1.73%) seed damage and number of exit holes (6.00) which is comparable to Spintor Dust that recorded 2.20% seeds damage and 6.66 exit holes. This implies that the higher the concentration of the plant powders, the higher the efficacy in reducing the damage cause by *C. maculatus*. Treatment with Pod powder increased adult mortality and reduced the oviposition, adult longevity and fecundity suggesting that the phytochemicals present in *P biglobossa* interfere with the feeding and reproductive capacity of the insect.

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Table 4: Effect of Stem Bark and Pod Powder of *Pakia biglobossa* on Percentage Grain Damage and Number of Exit Holes of on Treated and Untreated Cowpea Seed after Exposure to the Treatments

Treatments	Conc. (g/100g seed)	(%) Grain damage	Number of Exit hole
Stem Bark	1	4.70[b]	16.67[b]
	3	3.60[c]	12.00[d]
	5	3.20[c]	12.67[d]
Pod	1	4.93[b]	15.33[c]
	3	2.70[c]	8.67[e]
	5	1.73[d]	6.00[f]
Spintor 0.125% Dust	0.125	2.2[cd]	6.66[f]
Control		19.67[a]	56.30[a]
LS		**	**
SE($\pm$)		0.98	1.03

**Significant at 1%; Means followed by the same letter are not significantly different ($P \leq 0.01$) from each other, using New Duncan's Multiple Range Test (NDMRT); LS = level of Significance; SE = Standard Error

From Table 4, the main action of alkaloid parkin in the plant materials appears to be at the release sites of Prothoracicotropic hormone (PTTH). This is in accordance with the earlier finding of Parekh *et al.* (2007) who reported that, plant based secondary metabolites like Phenols, Tannins and Flavonoids derivatives compounds have been shown to interface with many biological activities of insects.

Table 5 revealed the effect of stem bark and pod powder on weight loss on treated cowpea seed. The result revealed that 5 g Pod recorded the lowest (1.10%) weight loss of and is comparable to spintor Dust that recorded (1.03%). Weight loss was significantly ($P \leq 0.01$) higher in the untreated control (13.87). Weight loss decline with increasing concentration of both Stem Bark and Pod Powder of *P. biglobossa.*

Similar results were reported by Suleiman and Suleiman (2014) who reported that leaf powders of *Euphorbia balsamifera* and *Lawsonia inermis* greatly reduced cowpea seed damage 28 days after treatment. This was attributed to the limited contact of *C. maculatus* with the treated seed. This finding is also in agreement with Jose *et al.* (2014) that reported all treated plant cowpea seeds kill *C. maculatus* at the end of experiment but did not lose their weight compared to control treatment. The toxicity effect exhibited by the botanical led to the reduction in F_1 progeny, low egg and adult emergence that causes weight loss. Table 5 also showed the effect of plant powder on viability test after exposure to the treatments. The result revealed that, there is no significant difference in relation to germination percentage on all treated and untreated seed.

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Table 5: Effect of Stem Bark and Pod Powder of *Parkia biglobossa* on Percentage Weight Loss and Percentage Germination after Exposure to the Treatments

Treatments	Conc. (g/100g seed)	(%) Weight Loss	Germination
Stem Bark	1	6.90[b]	96.67
	3	5.07[b]	95.00
	5	3.73[c]	98.33
Pod	1	5.60[b]	95.00
	3	3.60[c]	98.33
	5	1.10[d]	98.33
Spintor 0.125% Dust	0.125	1.03[d]	95.00
Control (untreated)		13.87[a]	93.33
LS		**	NS
SE(±)		1.64	1.98

**Significant at 1%; Means followed by the same letter are not significantly different ($P \leq 0.01$) from each other, using New Duncan's Multiple Range Test (NDMRT); LS = level of Significance; SE = Standard Error; NS = not significant

All plant extract has no negative effect on the seed viability on the treated and untreated seed. This indicated that the Plant powders may be used for storage of cowpea seeds to be used as planting materials. This corroborated the earlier works of Abdul-Rafiu (2010) and Ojiako *et al.* (2013) that reported that, actellic did not affect the viability of stored cowpea or maize, respectively. Ogendo *et al*. (2004) and Akinkurolere *et al.* (2012) also stated that plant products generally do not affect the viability of seeds treated with them. *Jatropha curcas* L. seed extract has been reported to have no adverse effect on the viability of stored cowpea seeds (Ahuchaogu and Ojiako, 2015). However, 3g of stem bark gave 100 % germination as against the control (93.33 %), suggesting that seeds can be stored for planting purposes. This result is in consistent with earlier works of Frank *et al.* (2018) who reported the effect of *Denettia tripetala* F. and Okoye and Ebeledike (2013) who reported the effect of *Mondora myristica* (Gaertn.) Dunal on seed germination after storage for some period.

CONCLUSION AND RECOMMENDATIONS

The application of 5 g pod powder per 100 g cowpea seed were statistically similar with the chemical pesticide (Spinror 125%) Dust in all the parameters observed. Therefore, it might serve as alternatives to insecticides on rural farms in tropical and subtropical regions. Insecticides are costly and pose a great threat to the environment and non-target organisms. The use of these plant products would be cost effective and sustainable, especially considering that these plants are available in the farmer's environment. In addition, these products are safe to users and require little skill to apply.

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