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Equity Journal of Science and Technology, 2020 7(2): 100 - 104

ISSN 2354-1814; E-ISSN 2683-5961

EQUIJOST An Official Publication of Kebbi State University of Science and Technology, Aliero, Nigeria

Bioactivity of Selected Plant Parts in the Control of Cowpea Bruchid (*Callosobruchus maculatus* L.) on Stored Cowpea (*Vigna unguiculata* L. Walp.)

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Received: Sep 7, 2020: Accepted: Oct 20, 2020; Published Online: Oct 28, 2020

Abstract
Cowpea bruchid (*Callosobruchus maculatus*) is a very serious primary pest of stored cowpea grains with 30 – 100% loss potential. This study is therefore aimed at evaluating the efficiency of some plants in the management of the bruchid. The treatments evaluated were pulverized (dusts) eucalyptus leaves - *Eucalyptus globulus* Lab., neem leaves – *Azadirachta indica* A. Juss., neem stem bark, neem seed kernel, ginger rhizome – *Zingiber officinale* Rosc., pawpaw leaves – *Carica papaya* L. (all at 2 g/kg of seeds), and control (untreated). The experiment was laid out in a Completely Randomized Design (CRD) with three replicates. Data collected included mortality of adult insects, number of eggs laid, grain damage, grain weight loss, and germination percentage on SAMPEA-11 infested seeds. Data were analyzed using Analysis of Variance (ANOVA), and significantly different means were separated using Duncan's New Multiple Range Test (DNMRT) at $p \leq 0.05$. Results indicated that neem seed kernel followed by eucalyptus leaves dusts effected the highest mortality rate throughout the assessment period. The control (untreated seeds) had the highest number of F_1 progeny produced (84.7±6.0), seed damage (92.8%±1.4), and grain weight loss (33.4±0.3%). Neem seed kernel dust had the highest germination percentage (77.2%±3.3) followed by eucalyptus leaves dust (74.5%±1.7). The study showed therefore that the botanical dusts evaluated, particularly neem seed kernel and eucalyptus leaves dusts, had promising potentials in the control of *C. maculatus* in stored cowpea.

Keywords: Bioactivity, *Callosobruchus maculatus*, Eucalyptus leaves dust, Neem stem bark dust, Plant dusts

1. Introduction

Cowpea (*Vigna unguiculata* (L). Walp) is a leguminous plant which is widely cultivated in the tropics and subtropics. It belongs to the sub-family papilionaceae, and its tribe is phaseolae [1]. In Nigeria, cowpea is grown mostly in semi-arid areas because of its ability to tolerate moisture stress [2]. Cowpea has high protein content of 23 - 25% making it appropriate in the diet of many people in the developing countries who largely cannot access other more expensive protein sources such as fish and meat [3]. In Nigeria, cowpea production is low (≈3.2 million tonnes) and does not meet the rising market demand due to field and store pest infestations which causes qualitative and quantitative losses [4]. Its production is hampered by factors such as viruses, bacteria, fungi, weeds, and insects e.g., *Aphis craccivora*, *Megalurothrips sjostedti*, *Callosobruchus maculatus*[5].

Callosobruchus maculatus is the most important biotic constraint to cowpea storage. It accounts for 30 - 80% loss in Africa with a monetary value of over U.S. 300 million dollars annually [6]. In Nigeria, using traditional storage structures, the bruchids are implicated for 87 - 100% losses within storage duration of 3 - 6 months [7]. The control of *C. maculatus* is effectively achieved by spraying synthetic insecticides which are expensive for some low-income farmers who are largely illiterates and thus misapply them. Incidences of pesticide poisoning resulting from eating contaminated cowpea due to indiscriminate insecticide application have become news items from time to time in the recent past. The recent suspension of Nigerian grains from the European Union (EU) market due to high pesticide residue further buttresses the misuse of pesticides in Nigeria. To achieve food security, new methods are required to manage pests of stored cowpea. The efficacy and economic viability of plant products in pest management are well elucidated [8, 9]. Hence, the objective of this study was to evaluate the bio-pesticidal effects of some common plant dusts in the protection of stored cowpea against *C. maculatus* infestation.

2. Materials and Methods

2.1 Experimental Site

The study was conducted at the Laboratory of the Department of Crop Protection, Modibbo Adama

University of Technology, Yola, Adamawa State, Nigeria for a period of 6 months.Yola is located at Latitude 9º13’48” North and Longitude 12º27’36” East at an Altitude of 196m above sea level with temperature and relative humidity of 35±2ºC and 65 - 85%, respectively [10].

2.2 Source of Materials

Cowpea variety (SAMPEA-11) was obtained from Institute for Agricultural Research (IAR) Samaru, Ahmadu Bello University Zaria, Kaduna State, Nigeria. Cowpea weevils (*C. maculatus*) were obtained from infested cowpea from Yola South Market, Adamawa State, Nigeria. Neem leaves (*Azadirachta indica* A. Juss.) - *Dóógónyáárò̀ò* in Hausa, Neem seed kernels, Pawpaw leaves (*Carica papaya* L.) – *Gwándà* in Hausa, and Eucalyptus leaves (*Eucalyptus globulus* Lab.) – *Zaiti* in Hausa were obtained from the University garden, while Ginger rhizomes– *Chitta* in Hausa were purchased from Jimeta market, Adamawa State.

2.3 Insect Culture

Callosobruchus maculatus population was obtained from naturally infested cowpea obtained from Yola South Market, Adamawa State, Nigeria. The insects were cultured for about 10 weeks on a susceptible *V. unguiculata* (Kidney shape bean) seeds in 1 L glass bottles to provide insects of similar age for the study. Muslin cloth was used to cover the bottles affixed with rubber band to prevent escape of insects and to aid aeration. All parents *C. maculatus* in the bottle were removed after seven (7) days of oviposition and placed on another fresh medium repeatedly until sufficient number of insects of same age were obtained for the experiment. The culture bottles were kept at ambient laboratory conditions in an open-air shelf at temperature of 35±2ºC. Emerged F_1 progeny, 0 - 14 days old were used for the experiment [3, 6, 7].

2.4 Preparation of Plant Dusts

Adapting the method of Brisibe *et al*. [6], the plant parts (neem seed kernels, ginger rhizomes, neem stem bark, neem leaves, pawpaw leaves, and eucalyptus leaves) were dried under shade. They were each ground to powder using an electric powered blender and then sieved to dusts using 0.2 mm mesh size sieves.

2.5 Bioassay and Data Collection

Adapting the methods of Amusa *et al*. [3], Brisibe *et al*. [6], and Ojiako *et al*. [7]; the plant dusts were introduced into each bottle containing 100g cowpea at the rate of 2.0g/kg, except the control where no dust was added, then capped and shaken manually for 2min to achieve uniform distribution of plant powder in the entire grain mass. Subsequently, 50 adult insects were introduced into the bottles and then covered with muslin cloth fitted with rubber band to aid ventilation and prevent escape of insects. The experiment was laid out in a Complete Randomized Design (CRD) and replicated 3 times under ambient laboratory condition of temperature (35±2ºC), and relative humidity (65 - 85%).

Mortality of adults was assessed at 1, 3, 7 and 14 days after introduction of weevils by using soft camel hair brush to remove both dead and living insects in each bottle on a piece of white cloth which were then counted. With the aid of hand lens, the number of eggs on 20 randomly selected seeds was counted at 35 days after introduction of weevils. The percentage of seeds damaged was assessed using the formula;

$$Seeds\ damaged(\%) = \frac{Number\ of\ damaged\ grains}{Total\ number\ of\ grains} \times 100$$

Seed weight loss was determined using the method described by Lale [11];

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

Where;

Ua = average weight of one undamaged grain,

N = total number of grains in the sample,

U = weight of undamaged fraction in the sample,

D = weight of damaged fraction in the sample.

Germination test was conducted by randomly drawing 20 bean grain from each bottle and placed on each Petri dish with moistened filter paper. Germination percentage was then computed as;

$$Germination(\%) = \frac{No.of\ seeds\ that\ germinate}{No.of\ seeds(20)} \times 100$$

Progeny suppression over control (%) was computed as;

$$\frac{PCG - PTG}{PCG} \times 100$$

Where PCG is No. of F_1 progeny from control grains and PTG is No. of F_1 progeny from treated grains

2.6 Data Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using SAS statistical software, version 9.2 [12] while significant differences between the treatment means were separated using Duncan’s New Multiple Range Test (DNMRT) at 5% probability level.

3. Results and Discussion

3.1 Effect of Plant Dusts on Adult Mortality of Cowpea Weevils

The effect of plant dusts on mortality of *C. maculatus* in treated cowpea is shown in Table 3.1. Results indicate that all treatments significantly ($p \leq 0.05$) suppressed the survival of *C. maculatus* adults compared to the untreated control. The insecticidal effect of the dusts increased with increasing exposure interval. After one and three days of exposure, the weevils' mortality was highest on Neem Seed Kernel Dust (NSKD), followed by Eucalyptus Leaves Dust (ELD) and Neem Stem Bark Dust (NSBD). At 7 days post exposure, 100% mortality was recorded on NSKD and ELD with none of the treated seeds recording < 70% weevil mortality. At 14 days exposure, all plant dust applied resulted in 93% to 100% mortality of *C. maculatus* compared with the control which had 48% mortality.

The results demonstrated insecticidal activity of eucalyptus leaves, neem leaves, neem stem bark, neem seed kernel, ginger rhizome and pawpaw leaves, indicating they possess insecticidal properties against cowpea weevil – *C. maculatus*. The application of plant dusts resulted in significantly higher mortality percentage compared with the untreated control. Effectiveness of botanical products against field, and particularly, stored-product insects has been previously reported [13-15]. In the present study, all the plant dusts evaluated resulted in between 93% to 100% adult mortality of *C. maculatus*. Botanicals are known to exhibit acute toxicity, repellency, feeding inhibition, growth retardation, and development and reproduction suppression in insects [9, 14]. The observed high adult mortality might be due to direct toxicity to insects as a result of the active ingredients (e.g., α-pinene in eucalyptus, azadirachtin in neem, zingi-berene in ginger, and papain in pawpaw) associated with the plant materials. Previous reports have indicated the efficacy of botanicals against stored-product insects [3, 6, 7, 11].

Table 3.1: Mean Mortality (%±SE) of *C. maculatus* Adults Exposed to Cowpea Seeds Treated with 2 g/kg of Plant Dusts

Treatment	Percentage Adult Mortality (Days)			
	1	3	7	14
ELD	73.8 ± 6.3^{a}	96.3 ± 2.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
NLD	18.1 ± 2.0^{c}	77.1 ± 2.5^{b}	81.0 ± 3.1^{b}	100 ± 0.0^{a}
NSBD	63.6 ± 4.1^{b}	95.2 ± 4.0^{a}	98.8 ± 1.2^{a}	100 ± 0.0^{a}
NSKD	76.1 ± 3.8^{a}	99.5 ± 3.1^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
GRD	16.9 ± 2.9^{cd}	66.5 ± 1.2^{c}	73.8 ± 8.4^{c}	93.7 ± 2.6^{ab}
PLD	16.1 ± 4.1^{cd}	68.0 ± 5.1^{c}	70 ± 3.2^{c}	94.5 ± 3.3^{ab}
CON	9.3 ± 6.0^{e}	19.7 ± 3.1^{d}	24.0 ± 4.3^{d}	48.0 ± 1.2^{c}

Means followed by same letter(s) within a column are not significantly different at $p \leq 0.05$ using DNMRT (Duncan's New Multiple Range Test); **ELD** = Eucalyptus Leaves Dust; **NLD** = Neem Leaves Dust; **NSBD** = Neem Stem Bark Dust; **NSKD** = Neem Seed Kernel Dust; **GRD** = Ginger Rhizome Dust; **PLD** = Pawpaw Leaves Dust; **CON**=Control (Untreated).

3.2 Effect of Plant Dusts on Progeny Emergence of Cowpea Weevils

Data presented in Table 3.2 shows the effect of some plant dusts on progeny production of *C. maculatus* in cowpea grains. The Result showed that all treatments (the tested plant dusts) applied at 2 g/kg significantly ($p \leq 0.05$) suppressed progeny numbers relative to the untreated control. Fewer adult weevils (6.3±1.1 – 27.3±2.0) emerged from grains treated with these botanical dusts as compared with the control (84.7±6.0). Neem seed kernel dust was the most effective treatment, almost completely inhibiting progeny emergence (6.3±1.1). Generally, all plant dusts resulted in about 67 - 93% F_1 progeny suppression (Table 3.2).

The high suppression of progeny production observed in treated grains over control might be attributed to reduced egg production/inhibition of egg laying or ovicidal activity. Ofuya [16] reported that plant powders have the capacity to weaken insects resulting in the laying of fewer eggs. Although plant dusts used in the study significantly increased adult mortality, they could not complete prevent progeny development. This apparently suggests that higher dose rate of the plant dusts might be required for progeny inhibition.

Table 3.2: Effect of Some Plant Dusts on Progeny Production of *C. maculatus* in Treated Cowpea Seeds

Treatment	No. of Emerged F_1 Progeny (Mean±SE)	Progeny Suppression over Control (%)
ELD	15.0 ± 3.8^{c}	82.3
NLD	27.3 ± 2.0^{b}	67.8
NSBD	18.3 ± 1.0^{c}	78.4
NSKD	6.3 ± 1.1^{d}	92.6
GRD	22.0 ± 2.9^{b}	74.0
PLD	23.0 ± 4.1^{b}	72.8
CON	84.7 ± 6.0^{a}	-

Means followed by same letter(s) within a column are not significantly different at $p \leq 0.05$ using DNMRT (Duncan's New Multiple Range Test); **ELD** = Eucalyptus Leaves Dust; **NLD** = Neem Leaves Dust; **NSBD** = Neem Stem Bark Dust; **NSKD** = Neem Seed Kernel Dust; **GRD** = Ginger Rhizome Dust; **PLD** = Pawpaw Leaves Dust; **CON** = Control (Untreated).

3.3 Effect of Plant Dusts on Cowpea Seed Damage and Germination

Table 3.3 shows the effect of *C. maculatus* infestation on seed damage, weight loss and germination percentage in cowpea seeds treated with some plant dusts. Cowpea grains treated with neem seed kernel dust and eucalyptus leaves dust significantly ($p \leq 0.05$) reduced seed damage by *C. maculatus* by 34.5% and 36.4%, respectively compared with the control with seed damage of > 90% (Table 3.3). High seed damage (> 50%) was recorded in seeds treated with Pawpaw leaves dust, Ginger rhizome dust and Neem stem bark dust - 77.3, 60.1 and 53.5%, respectively. Weight loss was also highest in the untreated control (> 30%), but lowest in seeds treated with NSKD - 4.9±0.2% followed by ELD -6.7±0.2 (Table 3.3). Cowpea grains treated with neem seed kernel dust and eucalyptus leaves dust significantly ($p \leq 0.05$) resulted in germination percentage of 77.2% and 75.6%, respectively compared with the control with germination percentage of 45.6%. Cowpea seeds treated with NSKD had the highest germination percentage, although those treated with ELD and NSBD were not significantly different ($p > 0.05$) (Table 3.3).

The lower damage and weight loss in treated seeds suggests varying levels of potency of the plant dusts evaluated. The percentage germination of over 75% observed in seeds treated with neem seed kernel and eucalyptus leaves dusts after infestation by *C. maculatus* suggests that the plant dusts hardly have any suppressive effect on seed viability. Our results buttresses the findings of Sighamony *et al*. [17] which showed that botanical oils are comparatively safe for seed treatment as they do not impede germination. Enobakhare and Law-Ogbomo [18] in a study with *Sitophilus zeamais* in three maize varieties treated with *Vernonia amygdalina* also showed that, the treatments had no suppressive effect on the quality and viability of treated maize. Kasa and Tadesse [19] evaluated 17 plant powders for the management of *S. zeamais* on sorghum and found that the botanicals had no suppressive effect on germination of the seeds, and recommended that the extracts could therefore be used to protect sorghum seeds meant for sowing. According to Silva *et al*. [20], the insecticidal activity of botanicals varies according to the part of the plant from which the insecticidal metabolite was synthesized. Thus, comparative study using various plant parts would help identify the most suitable plant part for use as botanical insecticide. The current study however showed that, the plant dusts evaluated, particularly neem seed kernel and eucalyptus leaves dusts, have great potential in the management of *C. maculatus* on cowpea grains.

Table 3.3: Effect of *C. maculatus* Infestation on Seed Damage, Weight Loss and Germination Capacity in Cowpea Seeds Treated with Some Plant Dusts

Treatment	Seed Damage (%) (Mean±SE)	Weight Loss (%) (Mean±SE)	Percentage Seed Germination (Mean±SE)
ELD	36.4 ± 1.9^{de}	6.7 ± 0.2^{cde}	75.6 ± 1.7^{a}
NLD	47.2 ± 1.7^{d}	10.5 ± 0.2^{cd}	62.2 ± 3.5^{b}
NSBD	53.5 ± 11.9^{bc}	8.2 ± 0.5^{de}	70.6 ± 6.0^{ab}
NSKD	34.5 ± 0.2^{de}	4.9 ± 0.2^{f}	77.2 ± 3.3^{a}
GRD	60.1 ± 5.0^{c}	11.1 ± 0.2^{bc}	64.0 ± 2.8^{b}
PLD	77.3 ± 0.5^{b}	13.5 ± 0.4^{b}	62.3 ± 3.5^{b}
CON	92.8 ± 1.4^{a}	33.4 ± 0.3^{a}	45.6 ± 1.7^{c}

Means followed by same letter(s) within a column are not significantly different at $p \leq 0.05$ using DNMRT (Duncan's New Multiple Range Test); **ELD** = Eucalyptus Leaves Dust; **NLD** = Neem Leaves Dust; **NSBD** = Neem Stem Bark Dust; **NSKD** = Neem Seed Kernel Dust; **GRD** = Ginger Rhizome Dust; **PLD** = Pawpaw Leaves Dust; **CON** = Control (Untreated).

4. Conclusion

This study demonstrated the potential of some common plant parts against *C. maculatus* infestation in stored cowpea. The plant dust evaluated, particularly of neem seed kernel and eucalyptus leaves; induced higher adult mortality and progeny suppression. For the containment of progeny development, however, higher dose of the dusts might be required. The dusts apparently showed no adverse effect on seed germination and therefore could be used to protect seeds meant for sowing. Aside evaluating different parts of the plants assessed against *C. maculatus*, there is need for further research with increased dose rates and varied target insect species, as well as field validation in order to substantiate their usefulness as safer and cost-effective alternatives to synthetic insecticides.

Acknowledgements

The authors are thankful to Mr. Samuel Anami and Mrs. John Jonathan for their technical assistance during the bench work.

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