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Potentials of Cow Dung Ash as Protectants of Cowpea Seeds against *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae)

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Abstract

Experiments were conducted to assess the protectant ability of cow dung ash (CDA) to cowpea seeds against *Callosobruchus maculatus* F. under laboratory conditions of 50-65% R.H and 28-32°C. The CDA at different rates of 0.625, 1.25, 2.50, 5.00, 10.00% (w/w) incorporated with cypermethrin powder at 0.625% were applied to 20 g of cowpea seeds in petri dishes. None of the ashes or cypermethrin was added to the control. Ten beetles were introduced into each of the petri dishes and covered with muslin cloth. The experiment was arranged in a completely randomized design (CRD) and replicated three times. Effects of the treatments on adult mortalities as well as median lethal dose (LD_{50}) of CDA against *C. maculatus* were determined. Adult emergence and weight losses in seeds treated with CDA at different doses were assessed. Findings of the study revealed that the adult mortality of *C. maculatus* among different CDA treatments differed significantly ($p < 0.05$) and ranged from 13.33 to 100.00% 72 hours after treatment (HAT). Results of the study also showed that LD_{50} of CDA against *C. maculatus* at 24 hours after treatment (HAT) was 5.51 % (w/w). No adults emerged from the treated seeds and weight losses were observed in those treated with the lowest dose and the control only. Therefore, CDA could be utilized to protect cowpea seeds from *C. maculatus* infestations during storage.

Keywords: Adult emergence, *Callosobruchus maculatus*, Cow dung ash, cowpea seed, mortality, protectants

1. Introduction

Cowpea is highly palatable, very nutritious and relatively free from anti-metabolites. Shiringani and Shimelis (2011) reported that dried seeds of cowpea provide cheap source of proteins in many diets in the tropics and sub-tropics. The report further showed that cowpea seeds contain 22-33% protein, 60-66% carbohydrates, 5-6% fibre, 3.7-4.4% ash and 1.1-3% oil.

Callosobruchus maculatus (cowpea weevil) has been reported to be the main insect pest of cowpea (*Vigna unguiculata*) (Suleiman, 2016). Ojebode *et al.* (2016) reported that *C. maculatus* is very destructive on account of its short life cycle. Their damage causes losses in weight, nutritional, commercial values of cowpea as well as viability of stored seeds (Suleiman, 2016). Larvae feed and develop inside the seed which become unsuitable for human consumption and when adults emerge they leave a neat circular exit hole. Each adult consumes approximately 25% of the seed from which it develops (Asawalam and Anaeto, 2014).

Synthetic insecticides may play a significant role in reducing storage losses due to insect pests. However, the extensive use of these chemicals poses so many problems such as insecticide resistance, health risk to consumers and environmental contamination (Sani and Suleiman, 2017). These problems have necessitated the replacement of synthetic insecticides with natural products which are eco-friendly to protect stored grains from insect infestations (Vanmathi *et al.,* 2012).

Natural products have been used for many years by small scale farmers in many parts of Africa to protect stored products (Suleiman and Rugumamu, 2017; Suleiman *et al*., 2019). Previous findings demonstrated the use of these materials as safe and effective protectants of stored cowpea against *C. maculatus* infestations (Suleiman and Suleiman, 2014; Asawalam and Anaeto, 2014). These products act by suffocating adult bruchids, restricting oviposition or even lead to the death of the insect (Sani and Suleiman, 2017).

In order to minimize the use of chemicals there is need for a search of alternative eco-friendly methods such as application of natural products like cow dung ash (CDA). Therefore, this study was aimed at evaluating the efficacy of CDA in controlling *C. maculatus* infesting stored cowpea.

2. Method

2.1 Rearing of Callosobruchus maculatus

All experiments were conducted in Laboratory 3 of the Department of Biology, Umaru Musa Yar'adua University, Katsina (UMYUK), Nigeria. Adults of *C. maculatus* were obtained from infested cowpea sold in Katsina central market. Cowpea seeds used for the rearing were brought from local market in Katsina and kept in an oven at 40ºC for 4 hours to disinfest the seeds and allowed to cool for 24 hours. Fifty pairs of adult *C. maculatus* were placed in 500 ml volume glass bottle containing 250 g of the disinfested cowpea seeds. The set up was kept on the laboratory shelf at room temperature. The parent weevils were removed by sieving after 7 days of their introduction. Seeds were kept under laboratory condition until emergence of F_1 progeny.

2.2 Preparation of CDA

Cow dung was collected from Barhim village of Katsina, Nigeria. The cow dung was sun-dried and crushed into small particles. The small sized particles were placed in the muffle furnace and heated for 3 hours. The ash collected was allowed to cool and then stored in air-tight container under prevailing laboratory condition of 50-65% R.H and 28-32ºC.

2.3 Assessment of Adult Mortality

Healthy disinfested cowpea seed (20 g) were placed in Petri dishes and treated with 0.125, 0.25, 0.50, 1.00 and 2.00 g of cow dung ash, separately. This was equivalent to 0.625, 1.25, 2.50, 5.0 and 10.0% (w/w), respectively. Cypermethrin (0.625%) was added to another 20 g seeds while no powder was added the control seeds. Five pairs of newly emerged adult beetles were introduced to each of the treated and untreated seeds in the Petri dishes. They were covered with nylon mesh and held in place with rubber hands. The set up was arranged in a completely randomized design (CRD) with three replications. This was inspected daily to remove, count and record dead insects for seven days. At the end of seven days, all beetles (dead and alive) were removed and percentage adult mortality was assessed as the number of dead beetles divided by total number of beetles introduced and multiplied by one hundred as indicated hereunder.

$$\text{Adult Mortality (\%)} = (\text{Number of Dead Insects})/(\text{Total Number of Insects Introduced}) \times 100$$

2.4 Determination of Median Lethal Dose (LD_{50}) of CDA

To evaluate LD_{50}, methods of Ebadollahi and Mahboubi (2011) were employed. The number of dead weevils in each bottle containing sorghum grains treated with the botanical powders was counted at the end of 24 hours after treatment HAT). The LD_{50} was calculated by using probit analysis with SPSS (version 16.0) software package.

2.5 Examination of Adult Emergence of Callosobruchus maculatus

The remaining insects (dead and alive) were sieved from the treated cowpea seeds immediately after mortality test. The seeds were left undisturbed under laboratory condition for adult emergence. To calculate the adult emergence direct examination was performed daily from the first emergence for seven days. All emerged individuals were removed, counted and recorded for 7 days.

2.6 Damage Assessment

Both treated and untreated seeds were sieved to remove the ash and any dust. To determine percentage grain damage, the final weight of the grain was subtracted from the initial weight of the grain, divided by initial weight of the grain and then multiplied by one hundred (100) as shown in the formula below:

$$\text{Weight Loss (\%)} = (\text{Initial Weight (g)} - \text{Final Weight (g)})/(\text{Initial Weight (g)}) \times 100$$

2.7 Data Analysis

All data were tested for normality using KS normality test and found to be non-parametric. For this reason, Kruskal-Wallis test was employed to test if there was significant difference in the effects of CDA on adult mortality and weight losses in the treated grains. Significantly different means were separated using Dunn's multiple comparison tests.

3. Results

3.1 Adult Mortality of Callosobruchus maculatus Exposed to CDA

Adult mortality of *C. maculatus* in cowpea seeds treated with different concentration of CDA varied. The mortalities were directly proportional to the varying concentrations of CDA applied and ranged from 13.33 to 53.33% among the treatments while it was 3.33% in the control at 24 hours after treatment (HAT) (Table 1). Kruskal-Wallis test showed the existence of significant difference ($KW = 18.809$; $p = 0.0045$) in adult mortality of

C. maculatus among various concentrations of CDA, cypermethrin and control. Adult mortalities at 48 and 72 HAT were observed to follow similar trend to that of 24 HAT where they were directly proportional to increase in concentration (Table 1). The treatments had significantly different ($p < 0.05$) effect on adult mortality of the insects at 24 and 72 HAT, respectively The mortalities also increased with increase in exposure periods in the order: 24 < 48 < 72 HAT.

Table 1. Mean Adult Mortality of *Callosobruchus maculatus* in Cowpea Seeds Treated with Different Doses of CDA

Dose (%)	Mean Adult Mortality of *C. maculatus* (% ± S. E.) Exposure Period (Hours)		
	24	48	72
0.625	13.3 ± 3.33^{d}	46.6 ± 6.67^{d}	83.33 ± 3.33^{b}
1.25	23.33 ± 3.33^{c}	66.67 ± 3.33^{c}	86.67 ± 3.33^{b}
2.5	26.67 ± 3.33^{c}	76.67 ± 3.33^{b}	90.00 ± 0.00^{b}
5.0	36.67 ± 3.33^{b}	90.00 ± 0.00^{a}	93.33 ± 3.33^{a}
10.0	53.33 ± 3.33^{a}	96.67 ± 3.33^{a}	100.00 ± 0.00^{a}
Cypermethrin (0.625%)	66.67 ± 3.33^{a}	93.33 ± 3.33^{a}	96.67 ± 3.33^{a}
Control (0.00%)	3.33 ± 3.33^{e}	3.33 ± 3.33^{e}	6.67 ± 3.33^{c}

Mean values with different superscripts on the same column are significantly different at $p < 0.05$

3.2 Median Lethal Dose (LD_{50}) of CDA against Callosobruchus maculatus

Median lethal dose (LD_{50}) of CDA against *C. maculatus* at 24 hours after treatment was 8.50% (w/w) with confidence limits (Cl) of 5.51 – 23.91% (Table 2). The Chi-square (X^2) goodness of fit showed that the difference was not significant ($p > 0.05$) for LD_{50} of CDA.

3.3 Adult Emergence of Callosobruchus maculatus in Treated Cowpea Seeds

No adult emergence of *C. maculatus* was observed from all the treated samples. However, the mean number of emerged adult beetles from untreated seeds was recorded as 16.67.

Table 2. LD_{50} of CDA against Adult *Callosobruchus maculatus* in Cowpea Seeds

LD_{50} (%)	95% Confidence Limits		Slope ± S. E.	X^2	*p*
	Lower Limit	Upper Limit			
8.50	5.51	23.93	2.59 ± 0.05	1.501	0.826

3.4 Weight Losses in Cowpea Seeds Treated with CDA

The only weight loss observed among the treated cowpea seeds was in those treated with the lowest concentration of CDA (0.625%) while the untreated seeds lost 11.33% of their weight to *C. maculatus* (Figure 1). Kruskal Wallis test showed that there was significant difference ($KW = 19.857$; $p = 0.0029$) in weight losses of cowpea seeds treated with cow dung ash at different doses

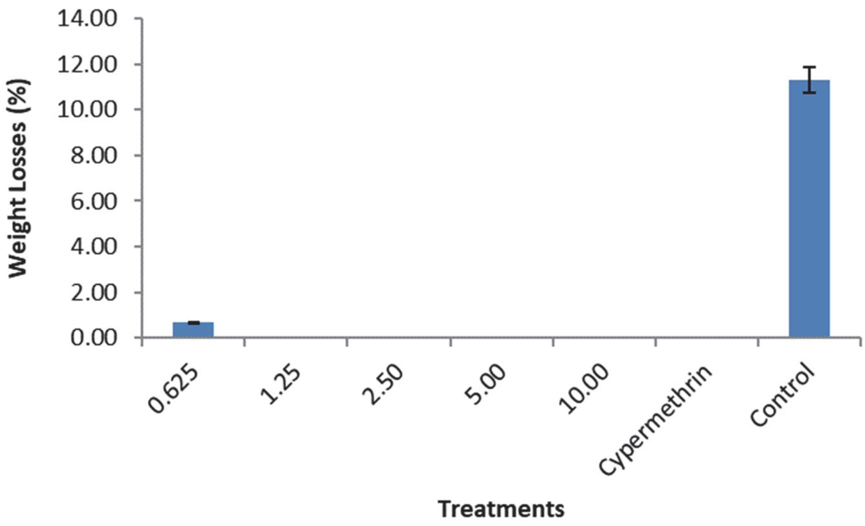

Figure 1. Percentage Weight Losses in Cowpea Seeds Treated with Varying Concentrations of CDA and Infested by *C. maculatus*

4. Discussion

4.1 Effect of CDA on Adult Mortality of Callosobruchus maculatus

Findings of this study have revealed that CDA was effective in causing high adult mortality of *C. maculatus*. This is in line with the findings of Arya and Tiwari (2013) who reported that cow dung ash applied at 2% to wheat grains resulted in 90.80% adult mortality of *Rhyzopertha dominica* Fab. Similar findings were reported by Narayana *et al*. (2019) that 2% CDA applied to maize grains caused 93.89% adult mortality of *R. dominica* at 24 HAT. It was also observed that the higher doses of CDA were almost as effective as the chemical insecticide (Cypermethrin) in killing adults of *C. maculatus*. The killing effect of CDA could be as a result of their small-sized particles that might have blocked the insect spiracle leading to impaired respiration and eventual death as observed by Suleiman and Suleiman (2014).

4.2 Effect of CDA on Adult Emergence of Callosobruchus maculatus

Results from this study indicated that the CDA was effective in reducing adult emergence of *C. maculatus* this implied that cow dung ash has a great protectant property against adult emergence of the insect. These findings are similar to earlier findings that only 2.75 adults of *Rhyzopertha dominica* emerged in grains treated with CDA (Arya and Tiwari, 2013). Similar findings were reported by Adebayo and Ibikunle (2014) that 1 g of CDA applied to cowpea seeds reduce adult emergence of *C. maculatus* from 2.80 in the control to 2.08 in the treated seeds.. Furthermore, results of this study showed that the higher doses of cow dung ash applied were as effective as Cypermethrin in reducing adult *C. maculatus*.

4.3 The Role of CDA in Reducing Weight Losses of Cowpea Seeds Infested by Callosobruchus maculatus

Finding of this study indicated that the high dose applied did not allow *C. maculatus* to cause any damage in cowpea seeds**.** This is line with the findings of Narayana (2019) which showed that only 0.19% of weight loss was recorded in maize grains treated with 2% CDA and infested by *R dominica.* Similarly, Tesema *et al*. (2015) reported that 2% of CDA gave a complete protection to chickpea against *C. chinensis.* The CDA might have inhibited the larval penetration into the and thus showed reduced levels of seed infestation and weight losses.

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