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Toxicity of two plant powders as biopesticides in the management of *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae, Bruchinae) on two stored grain legumes.

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ABSTRACT

Objective: The present study aimed to evaluate effects of leaf powder of *Chenopodium ambrosioides* (wormseed) and *Adenia cissampeloides* (snake climber) on insect populations and seeds weight loss percentage.

Methodology and Results: Two leaf powders were applied at 2.5%, 5% and 7.5% (wt/wt). All bioassays were conducted at 27±2°C and 70±5%RH. Insect mortality was evaluated after 2, 4 and 6 days of exposure and the total progeny was assessed 34 days after. *C. ambrosioides* at 2.5% showed the best efficacy, recording 69.64% of mortality in *Vigna subterranea* groundnuts and 100% of mortality in *Kerstingiella geocarpa* one's, 6 days after treatment. The lowest LC_{50} value after 6 days was obtained with *A. cissampeloides* applied at 2.37g/20g of *V. subterranea* groundnuts and with *C. ambrosioides* applied at 1.38 g/20g of *K. geocarpa* groundnuts.

Conclusion and application of findings: Because of their effectiveness, the leaf powder of these plants could be recommended as grain protectant against *C. maculatus*.

Key words: Botanical insecticides, pulses weevil, grain legumes, plant extracts, mortality rate.

INTRODUCTION

Pulses (grain legumes) are the second most important group of crops worldwide. About 870 million people are undernourished because of inadequate intake of proteins, vitamins and minerals in their diets (FAO, 2012). Pulses are excellent sources of proteins (20-40 %), carbohydrates (50-60%) and are good sources of thiamin, niacin, calcium and iron. As in many sub-tropical African countries, cereals and pulses are essential source of food for human consumption. In southern Benin, after harvest, the pulses are usually stored for long periods for seeds, trade or consumption. During storage period, they are seriously damaged by storage insect pests leading to severe losses

(Dinesh and Deepshikha, 2012). Among these pests, pulse beetle, *Callosobruchus maculatus* F. (Coleoptera: Chrysomelidae, Bruchinae) is a major pest that causes serious damage (Sharma, 1989) on stored pulse grains. In Nigeria for instance the damage due to *C.maculatus* on stored pulse reached 24 % of losses per year (Caswell, 1968). It is therefore necessary to reduce such losses by controlling pests on stored grains. When properly used, synthetic insecticides may play an important role in reducing storage losses due to insect pest (Menn, 1983; Redlinger *et al*., 1988). However, chemical pesticides have serious drawbacks such as development of pest resistance, toxic residue problems, toxicity risk on consumers and costs of application. Small-scale farmers generally use some traditional methods to protect stored foodstuffs from insect infestation in Eastern Africa (Hassanali *et al*., 1990; Poswall and Akpa, 1991). Plant materials have played an important part in those traditional methods in Africa where they have been mixed to stored grains. The mode of use and type of botanical material vary from place to place and appear to depend partly on the type and efficacy of suitable flora available in different locations. However, the number of plants that are known to possess insecticidal activity against storage insect pests is rather small. It appears necessary to develop alternative techniques to protect stored foodstuffs. Thus, this study aimed to evaluate effects of two ground leaf powder on population and damage of *C. maculatus* on stored pulse grains. *C. ambrosioides* (Chenopodiaceae) is a strongly aromatic, hairy, annual or perennial herb. It is abundant in the tropics and subtropics, especially in America and Africa (Rendle, 1983), and has been reported to have a wide variety of medicinal and insecticidal properties (Su, 1991; Quarles, 1992). *A. cissampeloides* (Passifloraceae) is a robust liana used in traditional medicine throughout tropical Africa. Most frequently recorded are the uses of an infusion or decoction of the root, stem or leaves for the treatment of gastro-intestinal complaints, such as stomach-ache, constipation, diarrhea and dysentery (PROTA, 2010).

Photo 1: Wormseed trunk, *Chenopodium ambrosioides* L. (Chenopodiaceae)
Cliche: Chougourou, 2015.

Photo 2: Snake climber liana, *Adenia cissampeloides* (Planch. Ex Hook.) Harms (Passifloraceae)
Cliche: Chougourou, 2015.

MATERIALS AND METHODS

Experiments were conducted in Laboratory of Applied Biology Research at University of Abomey-Calavi. *C. maculatus* was cultured in the laboratory at 27°C± 2%, 70%± 5% r. h. and 12h photoperiod.

Collection and preparation of plant material: Leaves of *C. ambrosioides* and *A. cissampeloides* were collected at Godomey in southern Benin during November 2012. They were dried on laboratory benches at room temperature (26°C–28°C) for 5 days. The powder was obtained by grinding the dried leaves in a coffee grinder and was sieved through a mesh of 0.5 mm size. The obtained powder was mixed to pulse grains (Bambara and kersting's groundnuts) using various doses.

Insects rearing: Adults of *C. maculatus* were reared in the laboratory under 27°C, 70% r. h. and 12/12 hours photoperiod. The original stock was obtained from stock cultures in Laboratory of Applied Biology Research of University of Abomey-Calavi. The food media used were Bambara and kersting's groundnuts. All grains used for this study were procured in Agbangnizoun, a southern Benin village.

Treatments and experimental design : The powder obtained from dried leaves of *C. ambrosioides* and *A. cissampeloides* was mixed separately with 20 g of grains in 380 ml glass jars. For each product the three dosages (treatments) were considered as follows: 2.5% (0.5g/20g of seeds), 5% (1g/20g) and 7.5% (1.5g/20g).The plant materials were thoroughly mixed for 20 min with the grains using a rotary shaker (Multifix GmbH, Germany). For each set of treatments, a non-treated seeds was considered as control. Five pairs (5 males and 5 females) 1–3 day-old adults of *C. Maculatus* were introduced into the jars with treated or untreated grains. Each treatment was replicated three times. Each jar was covered with cotton cloths to allow air circulation.

Data collection: The number of dead insects in each jar was counted 2, 4 and 6 days after treatment and the percentage mortality was corrected using the Abbott formula (Abbott, 1925):

$$P_T = \frac{P_O - P_C}{100 - P_C}$$

where P_T= Corrected mortality (%), P_O= Observed mortality (%) and P_C = Control mortality (%).

The number of damaged and undamaged grains was recorded in each treatment and weight loss due to insects was calculated using the formula of "Count and Weigh Method" (Adams and Schulten, 1978):

$$\%\ \text{Weight loss} = \frac{(U \times Nd) - (D \times Nu)}{U(Nd + Nu)} \times 100$$

where

U = weight of undamaged grains, Nu = number of undamaged grains, D = weight of damaged grains and Nd = number of damaged grains.

The F1 progeny population was assessed by keeping each sample in the laboratory until the emergence of new adults. Percentage reduction in adult emergence or inhibition rate (% IR) was calculated as follows:

$$\%\ IR = \frac{(C_n - T_n)}{C_n} \times 100$$

where
C_n is the number of newly emerged adults in the untreated (control) jar and T_n is the number of insects in the treated jar.

Statistical analysis: Data were analyzed using the SAS program version 9.2. Average means of parameters such as number of eggs laid, number of eggs hatched, dead insects, seed damage rates and progeny population were submitted to analysis of variance (ANOVA; Proc GLM; SAS Institute Inc. 2010) to compare the significance of various treatments. Means separation was performed using Student Newmann and Keuls test (SAS Institute Inc. 2010).For mortality tests, original data were corrected by Abbott's (1925) formula. Then mortality data were analyzed by probit analysis (Proc PROBIT, SAS 9.2, SAS Institute Inc. 2010) to determine LC_{50}.

RESULTS

Oviposition and adult emergence: The effects of dried ground leaves on oviposition and F1 production are given in tables 1 for bambara groundnuts and table 2 for kersting's ones. The effects of powders were evaluated by comparing the total number of eggs laid, egg hatching percentage and inhibition rates in the treated and control jars.

Table 1: Effects of different powders on oviposition and adult emergence of the pulse beetle, *Callosobruchus maculatus* fed on bambara groundnuts.

Name of plant powder	Total number of eggs laid	Egg hatched percentage (%)	Number of hatched larvae	Hatching inhibition rate (%)
C. ambrosioides				
2.5%	28.33± 3.51c*	80.69± 6.04ab	23± 4.58cd	44.04± 9.39de
5%	20± 1.00d	76.71± 2.02ab	15.33± 0.57d	62.52± 0.95c
7.5%	12± 2.00e	74.52± 4.30b	9± 2.00f	78.09± 4.37b
A. cissampeloides				
2.5%	32.33±1.52b	100± 0.00a	32.33± 1.52b	21.09±3.23f
5%	25.33±1.52c	100± 0.00a	25.33±1.52c	38.19± 2.91e
7.5%	20.66± 1.52d	100± 0.00a	20.66± 1.52d	49.59± 2.82d
Control	41± 2.00a	100± 0.00a	41± 2.00a	0± 0.00g

* Means in each column bearing the same letter (s) are not significantly different at the 5% level of probability by Student Newmann and Keuls test.

Results showed significant effect of leaf powders on the oviposition of the beetles ($p < 0.05$; Tables 1 and 2).The lowest number of eggs (12) was laid in samples treated with *C. ambrosioides* at 7.5% (Table 1).The highest oviposition inhibition rate (91.65%) was recorded in samples treated with *C. ambrosioides* at 7.5% (Table 2). Higher doses of vegetable powders severely reduced emergence, hatching and oviposition.

Table 2: Effects of different powders on the pulse beetle, *Callosobruchus maculatus* fed on kersting's groundnuts

Name of plant powder	Total number of eggs laid	Egg hatched percentage (%)	Number of hatched larvae	Hatching inhibition rate (%)
C. ambrosioides				
2.5%	24.33± 3.79c*	92.40± 7.70a	22.33± 2.52c	70.62± 3.10e
5%	16.33± 0.57d	81.74±5.53b	13.33± 0.57e	82.43± 1.19c
7.5%	12.3± 0.57e	51.49± 6.23c	6.33± 0.57f	91.65± 0.96b
A. cissampeloides				
2.5%	43.66± 2.52b	100± 0.00a	43.66± 2.52b	42.57± 1.83f
5%	22.33± 0.57c	100± 0.00a	22.33± 0.57c	70.61± 0.39e
7.5%	18.33± 0.57d	100± 0.00a	18.33± 0.57d	75.87± 0.37d
Control	76± 2.00a	100± 0.00a	76± 2.00a	0± 0.00g

* Means in each column bearing the same letter (s) are not significantly different at the 5% level of probability by Student Newmann and Keuls test.

Insect mortality and LC_{50}: Insect mortality at 2, 4 and 6 days after treatment, was evaluated at three different rates 2.5%, 5% and 7.5% (Figure 1). Mortality rates increased proportionally to the increase of the dose of powders and to with the duration of exposure time. Mortality values of *C. maculatus* for all doses of plant powders were significantly greater than the control ($p < 0.05$).

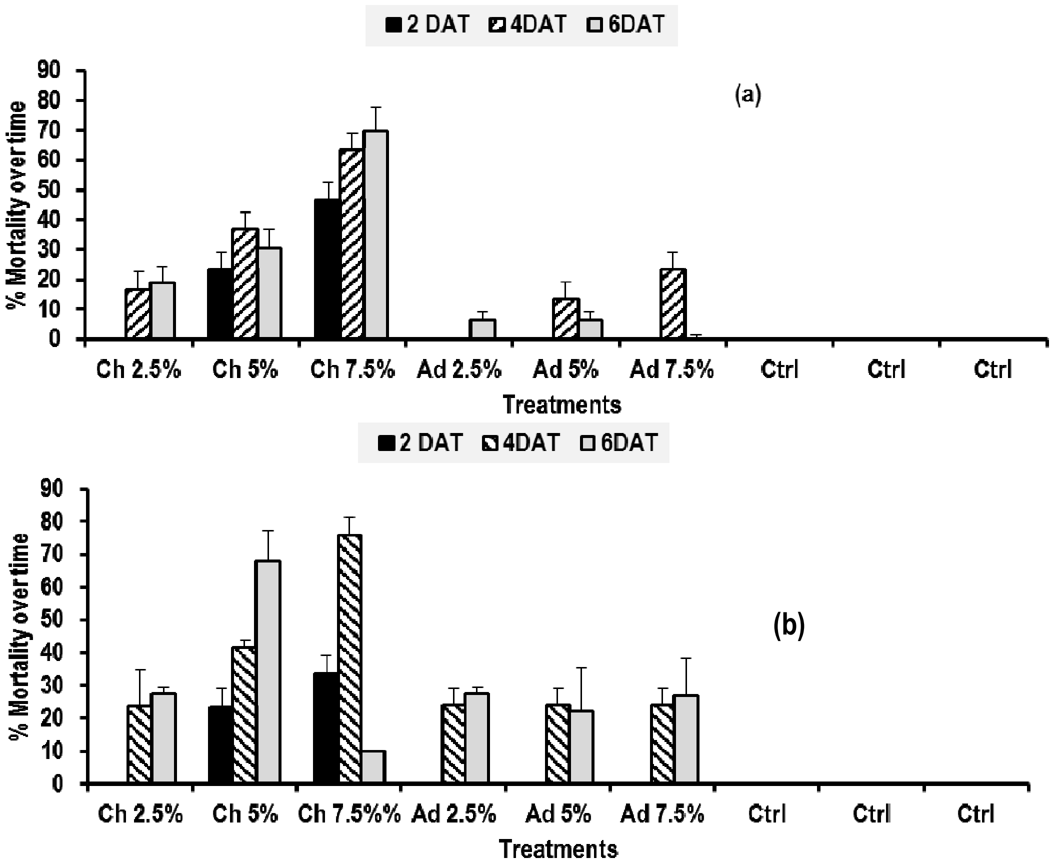

Figure 1: *C. maculatus* adult mortality due to botanical powders in bambara grains (a) and kersting's grains (b) at 2, 4 and 6 days after treatments. Ch: *C. ambrosioides*; Ad: *A. cissampeloides*; Ctrl: Control.

Exposure of adults showed that the different vegetable powders had a significant effect on the mortality of the beetles ($p < 0.05$; Figure 1). *C. ambrosioides* powder caused 100% mortality in adults fed on bambara groundnuts at 7.5%, 6 days after treatment (Figure 1b) and 69.64% mortality in adults fed on Kersting's groundnuts for the same duration (Figure 1a). The probit statistics, estimate of LC_{50} and their 95% fiducial limits for 2, 4 and 6 days after treatment are presented in tables 5 and 6. From this analysis, it was found that *C. ambrosioides* powder is the most toxic product (Tables 5 and 6). *A. cissampeloides* powder had the lowest toxic effect on *C. maculatus* (LC_{50}= 24.10/20g of kersting's groundnuts 2 days after treatment). The *C. ambrosioides* powder had the highest toxic effect against pulse beetle and lowest LC_{50} values (3.57/20g of kersting's groundnuts 2 days after treatment) (Table 6).

Table 3: Concentration (g/20g) of plant's extracts and fiducial limits (FL, with 95% confidence interval) required obtaining 50%, mortality (LC_{50}) from probit analysis of mortality data of *Callosobruchus Maculatus* fed on bambara groundnuts.

Name of plant powder	Number of insects used	LC_{50} (g/20g)	95%fiducial limits	
2 days post treatment :				
C. ambrosioides	90	3.00890	2.84988	3.21771
A. cissampeloides	90	6.13017	5.89665	6.67842
4 days post treatment :				
C. ambrosioides	90	2.40874	2.23279	2.62929
A. cissampeloides	90	4.31705	3.79682	5.30878
6 days post treatment :				
C. ambrosioides	90	2.75730	2.41339	3.33599
A. cissampeloides	90	2.350513	2.294264	2.410141

Table 4: Concentration (g/20g) of plant's extracts and fiducial limits (FL, with 95% confidence interval) required obtaining 50% mortality (LC_{50}) from probit analysis of mortality data of *Callosobruchus maculatus* fed on kersting's groundnuts.

Name of plant powder	Number of insects used	LC_{50} (g/20g)	95%fiducial limits	
2 days post treatment :				
C. ambrosioides	90	3.57430	3.26985	4.04985
A. cissampeloides	90	24.10830	24.08506	24.19742
4 days post treatment :				
C. ambrosioides	90	1.96949	1.83371	2.11877
A. cissampeloides	90	17.10830	17.0425	17.20146
6 days post treatment :				
C. ambrosioides	90	1.39594	1.32240	1.46863
A. cissampeloides	90	11.42164	11.03565	12.5687

Results showed that the high concentrations of plant extracts lead to the high mortality of *C. maculatus*.

Seed weight loss: After 45 days, low percentages weight losses were observed at 7.5% for both *C. ambrosioides* and *A. cissampeloides* powders (Figure 2).

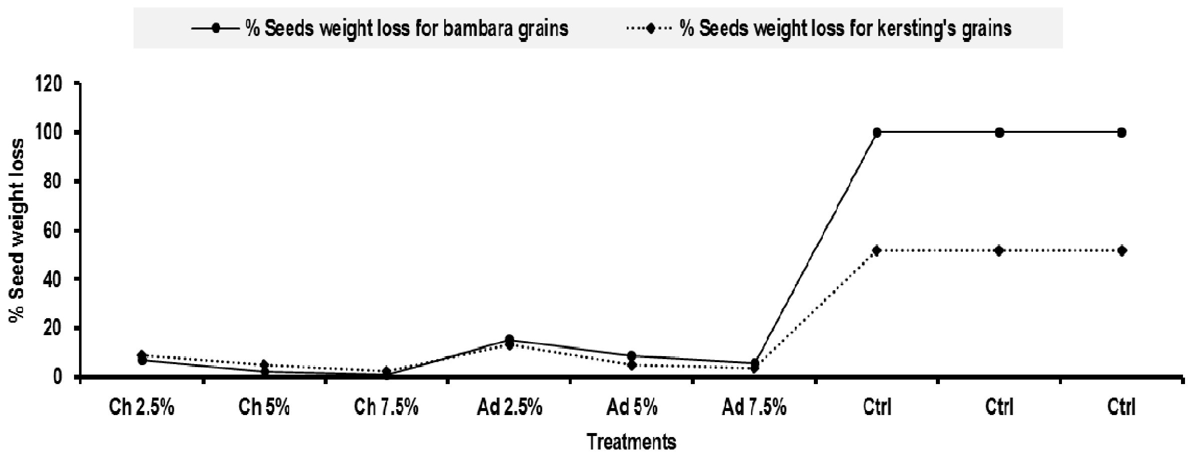

Figure 2: Seed weight loss percentage of *V. subterranea* and *K. geocarpa* grains with different dosages of botanical powders. Ch: *C. ambrosioides*; Ad: *A. cissampeloides*; Ctrl: Control.

Plants powders ensured significant protection of seeds against *C. maculatus*. The lowest percentage weigh loss (0.84±0.38%) was obtained with *C. ambrosioides* at 7.5% (Figure 2). Higher doses of vegetable powders generated high reduction of seed weight loss.

DISCUSSION

In this study, leaf powders of *C. ambrosioides* and *A. cissampeloides*, applied at different rates caused high mortality of *C. maculatus* compared to untreated controls but the *C. ambrosioides* leaf powder showed the best seed protection. Although the mode of action of *C. ambrosioides* powder is not clearly understood, its effectiveness was reported by Tapondjou *et al.* (2002) against *C. maculatus*. Moreover, Schoohoven (1978) demonstrated that insect death caused by *C. ambrosioides* leaves powder is due to anoxia or interference in normal respiration resulting in suffocation. This powder could also act as antifeedant or can modify the storage micro-environment thereby discouraging insect penetration and feeding (Obeng-ofori, 1995). In this study experiments, the *C. ambrosioides* powder was more effective (100% at 7.5%) than *A. cissampeloides* (26.78% at 7.5%) 6 days after treatment. Oviposition reduction reached 75% after applying each powder at the dose of 7.5% either in kersting's groundnut or in Bambara groundnut. The highest reduction rate (91.65%) was observed with *C. ambrosiodes* powder on kersting's groundnut. Malik and Mujtaba Naqvi (1984) reported that the powder of dried leaves of *C. ambrosiodes* had anti-feedant effect on *Rhyzopertha dominica*, and that could be the case with *C. maculatus*. Similarly, Delobel and Malonga (1987) found that the dose from the dried powder of *C. ambrosioides*' leaves led to 90% mortality of *Caryedon serratus* adults 13 days after treatment. Moreover, insecticidal activity of botanical extracts of *C. ambrosioides* was reported on a big range of insect pests, especially those attacking stored products (Leach et Johnson, 1925; Hartzell and Wilcoxon, 1941; Su, 1991). According to Credland (1992), ovicidal effect of plant powders on bruchid is mainly through asphyxia because the powders obstruct the respiratory tract and prevent the normal exchange of gas between the chorion and the external environment. The effectiveness of these botanical insecticides could be due to the nature of their active components. As for *P. guineene*, for instance, it contains the piperine, the chavicine and the alkaloids (Lale, 1995). *C. ambrosioides* contains the ascaridiol known to have insecticidal activities on bruchids (Malloy, 1923; Pollack *et al.*, 1990).

CONCLUSION

The presence of potential reduction of oviposition and insecticidal effects has been shown by the plant extracts in this study. Accurate identification and isolation of bio-active ingredients of these plant extracts should be explored as key issue for further study. In addition, the synergic effect from the mixture of these plant extracts should be tested for their effective use against field and storage insect pests.

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