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Efficacy of two plant powders as cowpea grain protectants against *Callosobruchus maculatus* Fabricius (Coleoptera, Chrysomelideae: Bruchinae)

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ABSTRACT

Objective: This study was carried out to study insecticidal effect of powders from *Chenopodium ambrosioides* leaves and *Aframomum melegueta* seeds on cowpea weevil *Callosobruchus maculatus*.

Methodology and Results: 5g of both powders of *A. melegueta* and *C. ambrosioides* were used in the proportions of *C. ambrosioides* 100%:*A. melegueta* 0%, *C. ambrosioides* 0%: *A. melegueta* 100%, *C. ambrosioides* 30%: *A. melegueta* 70%, *C. ambrosioides* 70%: *A. melegueta* 30% and *C. ambrosioides* 50%: *A. melegueta* 50%. Plant powders were added to 20g of cowpea grains. Bioassays were conducted at 26.64±0.74°C and 72.55±4.38% relative humidity. Insect mortality was evaluated from 24 to 144 hours after treatment. Results obtained indicated that plant powders had significant effect on *C. maculatus* mortality. The highest mortality rate (70.00±26.45%) was recorded in jar treated with *C. ambrosioides*.

Conclusion and application of findings: Either plant powders, alone or mixed had high insecticidal effect on *C. maculatus*. Because of their effectiveness, these plant powders could be recommended as grain protectants against *C. maculatus*.

Key words: *Chenopodium ambrosioides*, *Aframomum melegueta*, *Callosobruchus maculatus,* Insecticidal activity

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp., is an important component in diet of southern Benin population. Postharvest losses due to cowpea beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae, Bruchinae) are well documented (Emeasor, 2005; Udo and Epidi, 2009; Gusmão *et al*., 2013). *C. maculatus* is a cosmopolitan pest of cowpea. It is both field and stored pest. Infestation often begins in the field when pods are mature (Sathyaseelan *et al*., 2008) and when seeds are harvested and stored, the pest population increases rapidly and results in total destruction during short period of 3-4 months (Rahman and Talukder, 2006). Synthetic insecticides employed in the control of cowpea beetle proved effective, very expensive and unavailable at critical periods and they sometimes constitute health hazards to consumers (Lale, 2002). Due to the accumulation of residues of chemicals in grains, the selection of resistant insect population and other side effects, alternative approaches in Integrated Pest Management (IPM) have been considered. There is now renewed interest in the use of pesticides of plant origin in order to obviate the problems of environmental pollution, killing of non-target species and humans as well as reducing cost of purchasing synthetic chemical pesticides. In this context, several plants and constituent bioactive substances, also called insecticides of plant origin or botanical insecticides, have been tested and considered promising in the control of cowpea beetle (Denloye *et al*., 2010, Kheradmand *et al*., 2010, Udo, 2011). *Chenopodium ambrosioides* Linn and *Aframomum melegueta* K. Schum were chosen because they have shown promise of medicinal and insecticidal activity in Malawi, Central Africa countries such as Cameroon and Gabon and Southern Africa (Gadano, 2002, Tapondjou *et al*., 2002, Konning *et al*., 2004; Okwu, 2005, Odugbemi, 2006, Denloye *et al*., 2009). This study aimed to assess insecticide effect of *C. ambrosioides* leaf and *A. melegueta* seed powders against *C. maculatus* during in stored cowpea.

MATERIALS AND METHODS

Insects rearing: Adults of *C. maculatus* were reared in the laboratory under 26.64±0.74°C, 72.55±4.38% r.h. and 12/12 hours photoperiod. The original stock was obtained from stock cultures of Laboratory of Research and Applied Biology of University of Abomey-Calavi (Benin). All grains used for this study were purchased at the market (the international market of Dantokpa, Cotonou, Benin).

Collection and preparation of plant materials: *A. melegueta* seeds were purchased at the market of Zobe (6°27'33.768''N, 2°11'13.394''E) and *C. ambrosioides* leaves were collected at Kpomasse (6°25'44.868''N, 2°0'48.096''E). Both markets were in Ouidah district (Southern Benin). The National Herbarium of Benin identified plant materials. They were air dried on laboratory benches for 22 days at room temperature (25°C–27°C) before grinding in an electric mill and sieved through a mesh of 250-µm size.

Experimental set up: Plant powders were thoroughly mixed with 20g of cowpea grains in 250 ml glass jars. 5g of both powders of *A. melegueta* and *C. ambrosioides* were used in the proportions of *C. ambrosioides* 100%:*A. melegueta* 0% (T1), *C. ambrosioides* 0%: *A. melegueta* 100% (T2), *C. ambrosioides* 30%: *A. melegueta* 70% (T3), *C. ambrosioides* 70%: *A. melegueta* 30% (T4) and *C. ambrosioides* 50%: *A. melegueta* 50% (T5). For each set of treatments, a non-treated seeds was considered as control. Ten unsexed *C. maculatus* (1-5 days old) adults were randomly selected and introduced to each of the containers. Three replicates of the treatments and untreated controls were laid out in a completely randomized design.

Data collection: The number of dead insects in each jar was counted 24, 48, 72, 96, 120 and 144 hours after treatment and the Mortality Rate (MR) was calculated with following formula:

$$MR\,(\%) = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

Dead *C. maculatus* were removed and discarded after every count. Control serves as comparison to treated jars.

Data analysis: Data were analyzed using statistical program R 3.3.1 (R Core Team, 2016). Analysis of variance (ANOVA) was used to compare treatments using aov function of agricolae package (de Mendiburu, 2015). Significant differences between means were determined by the Least Significant Differences (LSD) ($P \leq 0.05$) using LSD. test function of agricolae package (de Mendiburu, 2015).

RESULTS

Analysis of variance indicated highly significant differences of mortality rates among various treatments ($p < 0.001$), exposure duration ($p < 0.001$) and combined effect of treatment and exposure duration ($p < 0.001$). Mortality rates of *C. maculatus* with exposure time are given in Table 1. All treatments exhibited varying degrees of insecticidal activities killing *C. maculatus* adults more than control at the 5% level of probability. Mortality rates increased proportionally with duration of exposure time. At 72 hours after treatments application, *C. ambrosioides* caused the highest mortality of 83.33±20.81% followed by mixture of *C. ambrosioides* (70%) and *A. melegueta* (30%) with 70.00±10.00%.

Table 1. Mortality of *C. maculatus* with treatments exposure duration

	Mean mortality rate (%) of *C. maculatus* at:					
Treatment	**24 HAT**	**48 HAT**	**72 HAT**	**96 HAT**	**120 HAT**	**144 HAT**
T1	26.66±20.81a*	70.00±26.45a	83.33±20.81a	96.66±5.77a	100±0.00a	100±0.00a
T2	10.00±10.00ab	26.66±15.27b	50.00±0.00b	66.66±11.54b	83.33±5.77bc	93.33±5.77ab
T3	6.66±5.77b	23.33±11.54bc	50.00±10.00b	70.00±0.00b	76.66±5.77cd	86.66±5.77b
T4	3.33±5.77b	36.66±5.77b	70.00±10.00a	86.66±5.77a	93.33±11.54ab	96.66±5.77a
T5	6.66±5.77b	23.33±5.77bc	50.00±0.00b	63.33±5.77b	66.66±5.77d	73.33±5.77c
Control	0.00±0.00b	0.00±0.00c	6.66±5.77c	20.00±10.00c	23.33±5.77e	26.66±5.77d
LSD Value	18.27	24.44	18.75	13.25	11.85	9.37

*Means in each column bearing the same letter (s) are not significantly different at the 5% level of probability by Least Significant Difference test. T1: *C. ambrosioides*100%:*A. melegueta*0%, T2: *C. ambrosioides*0%: *A. melegueta*100%, T3: *C. ambrosioides*30%: *A. melegueta*70%, T4: *C. ambrosioides* 70%: *A. melegueta*30% and T5: *C. ambrosioides* 50%: *A. melegueta* 50%; HAT: Hour after treatment. LSD: Least Significant Difference

Mean mortality rate of *C. maculatus* adults is slightly highest in cowpea grains treated with powder mixture of *C. ambrosioides* (70%) and *A. melegueta* (30%) (93.33±11.54%) followed by *C. ambrosioides* (0%) and *A. melegueta* (100%) (83.33±5.77%) and *C. ambrosioides* (30%) and *A. melegueta* (70%) mixture (76.66±5.77%). The lowest mortality rate (23.33±5.77%) was recorded in control jar at 120 hours after treatment. Mortality rate recorded with all treatments were significantly different from each other ($p<0.05$) at 120 hours after treatment. The mortality effect by *C. ambrosioides* (T1) was significantly higher than that of mixture T3 (*C. ambrosioides* 30%: *A. melegueta* 70%) and mixture T5 (*C. ambrosioides* 50%: *A. melegueta* 50%) but did not differ significantly from that of T4 (*C. ambrosioides* 70%: *A. melegueta* 30%), 144 hours after treatment ($p<0.05$).

DISCUSSION

The present study revealed that *C. ambrosioides* and *A. Melegueta,* alone or in mixture had insecticidal activity against *C. maculatus* and can be used for the control of the bruchid. Some studies proved efficacy of *C. ambrosioides* for controlling *C. maculatus* including toxicity to adults, reduction of oviposition, ovicidal activity and toxicity to immature stages prior to or immediately following penetration of plant tissue (Tapondjou *et al*., 2002; Emeasor, 2005; Denloye *et al*., 2010). High mortality rates obtained in this study are in accordance with the results reported by Chougourou *et al*., 2015, which had 100% of mortality of *C. maculatus* with 1.5g of *C. ambrosioides* per 20g of *Vigna subterranea,* 120 hours after treatment. Results showed significant insecticidal effect of *A. Melegueta.* Adesina *et al*., 2015 demonstrated in their work insecticidal effect, oviposition deterrent effect and adult emergence reduction of *A. Melegueta* on *C. maculatus. A. melegueta* seed powder caused 81.14% adult mortality at dose of 2.5g for 20g cowpea 120 hours after treatment. Results also revealed mixture of two botanicals at ratio 70:30, 30:70 and 50:50 had significant insecticidal effect (more than 60%, 96 hours after treatment). This shows that both powders contained toxic ingredients for *C. maculatus.* High insecticidal effect of both botanicals may be due to its physical action since the particles may block spiracles of *C. maculatus* and cause death by asphyxiation. Although there is no direct evidence of this in the present work, earlier works such as those of Ofuya and Dawodu (2002) showed that there is a direct relationship between particle size of plant powders and insect mortality in treated grains. Fine particle size aids even distribution of powders on the surface of seeds and the walls of the storage container thus increasing their possibility of making contact with the insects and killing them. In addition, plant powders cause abrasion of insect cuticle and lead to water loss (Sousa *et al*., 2005), which may cause stress and eventual death. Insecticidal effect of both botanicals may also due to its active components. Phytochemical screening of *C. ambrosioides* revealed the presence of alkaloids, tannins, saponins, flavonoïds, terpenes, sterols, cardenolide aglycone, volatile oils and carbohyfrates (Okhale *et al*., 2012). Adesina *et al*. (2015) revealed after phytochemical analysis, the presence of tannins, cardiac glycosides, saponins, alkaloids and flavonoids in *A. Melegueta*. Karamanoli *et al*. (2011) reported that tannins exert their action by combination of mechanism that includes iron chelation and enzyme inhibition. However, the exact mechanism behind the observed action of both plant powders is not yet known. Dolui *et al*. (2012) reported that tannin combined with protein to inhibit enzyme activity and reduce the availability of protein in haeolymph in insects. Some of the reported observed effects of saponins are increased mortality, lowered food intake, weight reduction, retardation in development and decreased reproduction (Chaieb, 2010). Adedire and Lajide (1999) reported 6-paradol, 6-gingerol and 6-shagaol (an alkyl phenol aromatic ketones) as the major insecticidal constituent of *A. melegueta*, which is responsible for sharp and peppery taste of the seeds. Owokotomo *et al*. (2014) reported that *A. melegueta* contains the following bioactive molecules: α- caryophyllene, β-caryophyllene, E-nerolidol, linalool, gingerdione, paradol, shagaol and humulene.

CONCLUSION

The findings of this research have shown insecticidal effects of plant powders. These results indicate that both plant powders have potential for cowpea grain protection. They can be used as an alternative to synthetic insecticides. Accurate identification and isolation of bioactive ingredients of these plant extracts should be explored as key issue for further study. Further research activities should be carried out against other storage insect pests and in the field against pests of field crops.

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