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EntomoToxicity of *Xylopia aethiopica* and *Aframomum melegueta* in Suppressing Oviposition and Adult Emergence of *Callasobruchus maculatus* (Fabricus) (Coleoptera: Chrysomelidae) Infesting Stored Cowpea Seeds

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Abstract

The cowpea beetle, *Callosobruchus maculatus* (Fabricus) (Coleoptera: Chrysomelidae), is a major pest of stored cowpea militating against food security in developing nations. The comparative study of *Xylopia aethiopica* and *Aframomum melegueta* powder in respect to their phytochemical and insecticidal properties against *C. maculatus* was carried out using a Complete Randomized Design (CRD) with five treatments (0, 1.0, 1.5, 2.0 and 2.5g/20g cowpea seeds corresponding to 0.0, 0.05, 0.075, 0.1 and 0.13% v/w) replicated thrice under ambient laboratory condition (28±2°C temperature and 75±5% relative humidity). The phytochemical screening showed the presence of flavonoids, saponins, tannins, cardiac glycoside in both plants, while alkaloids was present in *A. melegueta* and absent in *X. aethiopica*. The mortality of *C. maculatus* increased gradually with exposure time and dosage of the plant powders. *X. aethiopica* caused 75.15% adult mortality and *A. meleguta* exerted 85% mortality at 120 hrs post infestation. Maximum oviposition deterrent activity was observed with *X. aethiopica* (54.26%) compared to *A. melegueta* (51.32%). Conclusively, both plants showed highly useful bioactivity against *C. maculatus* in suppressing oviposition and adult emergence and, therefore, can be used in formulating ecofriendly herbal insecticides.

Keywords: Adult emergence, bioactivity, herbal insecticides, insecticidal properties, oviposition deterrent, phytochemical.

1. Introduction

Cowpea (*Vigna unguiculata* L.) is an important food crop that accounts for about 60% of human dietary protein intake and can provide a comparatively cheaper alternative to animal proteins in Nigeria. The high protein, amino acid and lysine contents of the seeds make them a natural supplement to staple diet cereals, roots, tubers and fruits (Somta *et al*., 2008). Stored cowpea grains are heavily infested by the cowpea beetle, *Callosobruchus maculatus* (Fabricus) (Coleoptera: Chrysomelidae) and caused over 90% of the insect damage to cowpea seeds. This is the cause of the main reduction in cowpea production (Radha and Susheela, 2014). Damaged cowpea seeds are unsuitable for human consumption and cannot be effectively used for agricultural and commercial purposes as result of the substantial reduction in both quantity and quality.

Management of *C. maculatus* on stored cowpea in Nigeria and developing nations has been primarily through the use of synthetic fumigants over the years. While these synthetic fumigants control are popular and effective, their improper application has resulted in environmental, human health problems and insect resistance. These serious limitation posed by the use of synthetic fumigants as preservatives during storage called for the search for new alternative methods of controlling the stored product insect pests, such as the use of promoting plant products (Ileke *et al*., 2014). Certain

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plants possess secondary metabolites which act as antifeedants, oviposition deterrents, larvicidal and insect growth regulators (Pugazhvendan *et al.*, 2009). They hold a promise as alternatives to chemical insecticides to reduce pesticide load in the environment and food chain.

Xylopia aethiopica (Dunal) A. Rich. (Family: Annonaceae), popularly known as African pepper, Ethiopian pepper or spice (Ndukwu and Ben-Nwadibia, 2005), is an important, deciduous evergreen, aromatic/medicinal plant, growing up to 20 m high and widely distributed in low land rain forest and moist fringe forests in the Savanna zones and coastal regions of Africa (Kieta *et al*., 2003). Concoctions prepared from its morphological parts are used in traditional medicine for the treatment of skin infection, candidiasis, cough, fever, dysentery and stomach ache (Okigbo *et al*., 2005). Extracts from *X. aethiopica* have been reported to exhibit mosquito repellent (Adewoyin *et al.*, 2006) and termite antifeedant (Lajide *et al.,* 1995) activities. The fruit powder and its essential oil were found effective to control maize weevil (*Sitophillus zeamais* Motsch) (Kouninkil *et al.*, 2005).

Aframomum melegueta K. Schum., known as Alligator pepper or Grains of Paradise (Family *Zingberaceae*), is a herbaceous tropical perennial West Africa spice plant with a short stem growing up to 5m tall, highly branched with lanceolate leaves and adventitious roots (Okujagu, 2008). *A. melegueta* is a very popular spice which imparts a pungent peppery flavor with hints of citrus and used mainly as food, in brewing, and in both veterinary and traditional medicine (Igwe *et al.,* 1999). It is believed to have purgative and hemostatic properties and also to be very effective against schistomiasis (Alaje *et al.,* 2014). Various researchers reported the potential of *A. melegueta* for the management of stored products' insect pests (Ofuya, 1990; Adedire and Lajide, 1999; Onekutu *et al*., 2015).

However, the ovipositional deterrents activity of the plants documentation is scare and in light of the foregoing, the present study assesses the oviposition deterrent and progeny suppression potential of *X. aethiopica* and *A. melegueta* on *C. maculatus* infesting stored cowpea.

2. Materials and Methods

The experiment was conducted at the Entomology Laboratory, Department of Crop, Soil and Pest Management Technology, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria (7° 11' N and 5° 35' E) under ambient temperature of 28±2°C, 75±5% relative humidity and 12hrs photoperiod. The initial *C. maculatus* culture was obtained from an infested stock of cowpea bought from Ulede Market, Owo, Ondo State, Nigeria and sub-cultured on Sokoto white (a susceptible cultivar) in a 2 l kilner jar, covered with muslin cloth to allow for proper ventilation. The jar was kept for the insect to breed and multiply in order to supply insects for the study.

Fruits of *X. aethiopica* and *A. melegueta* were obtained from traditional herbal trader at Ulede Market, Owo, Ondo State, Nigeria and were authenticated at the Forestry and Wood Technology Department, Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria. The fruits were rinsed in clean water and air dried. The seeds were made into powder using mortar and pestle. The powder was further sieved and packed into plastic containers with tight lids and stored in a laboratory cupboard until use.

Cowpea (cv. Oloyin), used for the experiment, was bought from the food grain stall at Ulede market, Owo, Ondo State, Nigeria. Damaged seeds were sorted out by handpicking leaving healthy seeds; these were nevertheless kept in a deep freezer at -15°C for 48 hrs to eliminate any stages of the insect. Powder of *X. aethiopica* and *A. melegueta* were evaluated at different concentrations of 0, 1.0, 1.5, 2.0 and 2.5g corresponding to 0.0, 0.05, 0.075, 0.1 and 0.13% v/w mixed thoroughly and separately with 20g of uninfested cowpea seeds in 125 ml plastic container. Ten (2-3 days old) unsexed *C. maculatus* adults were introduced into each plastic containers; these were covered to prevent entry and exist of insects. There was also a control treatment that did not involve the addition of any plant powder onto the seeds. Adult mortality was monitored at 24-h-interval (24, 48, 72, 96 and 120 hrs) post infestation. Insects were considered dead when they did not respond to gentle pressure using a fingertip. To avoid the possibility of death mimicry, the insects were watched for 2 min and again subjected to gentle pressure. Adult mortality was corrected using abbot's formula (Abbot, 1925).

The oviposition deterrent activity was assessed by admixing 0, 1.0, 1.5, 2.0 and 2.5g corresponding to 0.0, 0.05, 0.075, 0.1 and 0.13% v/w of *X. aethiopica* and *A. melegueta* powder with 20g of uninfested cowpea seeds in 125 ml plastic containers. Ten (2-3 days old) unsexed *C. maculatus* adults were introduced into each plastic container covered with lid. The insects were allowed to remain in the container for 7 days to allow the insect oviposit. The number of eggs laid on treated and control containers were counted using a hand lens and the percentage of oviposition deterrent activity was calculated using the formula adopted by Arivoli and Tennyson (2013).

% oviposition deterrent activity =

$$\frac{\text{no of eggs laid in control dish} - \text{no of eggs laid in treated dish}}{\text{no of eggs laid in control dish} + \text{no of eggs laid in treated dish}} \times \frac{100}{1}$$

To determine the F1 progeny deterrent efficacy of the plant powder, 20g of cowpea seeds were placed in 125 ml plastic container and admixed with different dosage rates of *X. aethiopica* and *A. melegueta* powder as stated above. After the egg count, the experimental set up was kept undisturbed until the emergence of F1 adults. The number of F1 adults that emerged from each replicate in the control and treated seeds were counted with the aid of aspirator and recorded at 30 days post infestation and was used to calculate percentage reduction in adult emergence.

% reduction in adult emergence =

$$\frac{\text{no of emerged adult from control dish} - \text{no of emerged adult from treated dish}}{\text{no of emerged adult from control dish}} \times \frac{100}{1}$$

The phytochemical screening of the plant powders for tannins, saponins, alkaloids and cardiac glycosides were carried out by the methods described by Harborne (1973), Sofowora (1993) and Trease and Evans (1998). For tannin, 5 g of each portion of plant powder was stirred with 10 ml of distilled water and filtered as described by

Trease and Evans (1998). Blue black, green, or blue-green precipitate formed following the addition of few drops of 5% ferric chloride confirmed the presence of tannins.

Salkowski's test, as described by Sofowora (1993), was used to test cardiac glycosides. Plant powder (0.5 g) was dissolved in 2 ml of chloroform prior to the careful addition of 1% (v/v) H_2SO_4 to form a lower layer. A reddish-brown colour at the interface confirmed that cardiac glycoside was present.

In Alkaloids determination, 0.5g of each powder was stirred with 5ml of 5% aqueous HCl on water bath and filtered; 1ml of the filtrate was treated with a few drops of Dragendorff's reagent. Precipitation or turbidity was taken as preliminary evidence for the presence of alkaloids in the plant being evaluated (Harborne, 1973).

The method described by Ekpo *et al*. (2012) was used to determine Saponins. About 0.5g of each plant extract was shaken with 10ml of distilled water in a test tube. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins.

For flavonoids, about 0.5g of each extract was stirred with few drops of Mg strips and conc. HCl was then added. A reddish coloration indicates a positive test for flavonoids (Sofowora, 1993).

Prior to analysis, egg count and adult emergence were subjected to square root transformation and percentages were arc sine transformed to normalize data. All data collected were subjected to Analysis Of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) for windows version (SPSS, 1999). Treatment means were separated using Least Significant Difference (LSD) at 5% probability level (Gomez and Gomez, 1984). The graph was designed using MS Excel 2010 version.

3. Results and Discussion

The contact toxicity on the survival of adult beetles after treatment with the plant powders is presented in Figure 1. The mean percent mortality of *C. maculatus* was observed to be directly proportional to the exposure period and concentration. Although none of the tested plant powder was able to exert 100% adult mortality, *A. melegueta* 0.13% v/w (2.5g/20g cowpea) caused 81.14% adult mortality and *X. aethiopica* exerted 70% adult mortality at 120 hrs post infestation. The striking effects of plant powders could be attributed to the presence of their toxic components and irritating smell which prevented physical contact of adult weevils with grains and caused suffocation or starvation of the pest (Sarwar *et al*., 2012) or to the induction of some unknown physiological changes (Mathur *et al*., 1985), which significantly (P<0.05) reduced the number of the tested insect. This shows that both powders contained toxic ingredients for *C. maculatus*. The insect mortality may equally be due to blocking of spiracles of the insect by dust particles and death caused by asphyxia (Adedire *et al.*, 2011; Fernando and Karunaratne, 2012). Further, it was revealed that the plant powder may cause abrasion of insect cuticle, which led to water loss. The water loss in the insect ultimately results in its death (Sousa *et al.*, 2005). The significantly high mortality rate indicates the probable presence of insecticidal properties in the plants. This confirms the findings of Ajayi and Wintoba (2006) and Onekutu *et al.* (2015) that reported the insecticidal bioactivity of the tested plant against *C. maculatus* infesting stored cowpea.

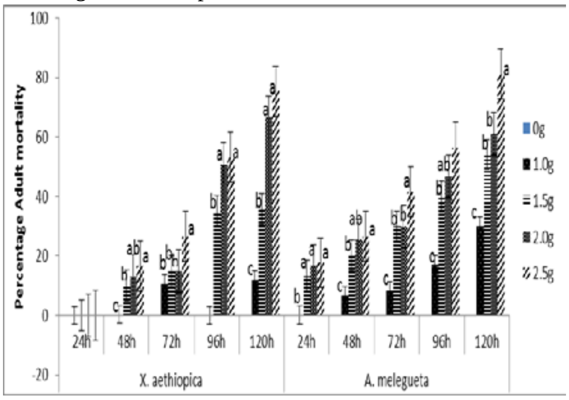

Figure 1. Corrected Mean Percentage Adult Mortality of *C. maculatus* treated with powder of *Xylopia aethiopica* and *Aframomum meleguta.* Means with the same letter for each plant powder are not significantly different using LSD at 95 % confidence level.

The data shown in Figure 2 revealed the effect of the evaluated plant powders on oviposition deterrent of *C. maculatus*. The result shows that the reduction in oviposition increases with the dosage increase. The higher dosage shows that the insects treated with 2.5g/20g cowpea seeds were found to be effective in suppressing egg laying as compared to lower dosage rate; although no concentration of the plant powders could completely prevent the females from oviposition. Maximum oviposition deterrent activity was observed with *A. melegueta*. This corroborates with the findings of Olaifa and Erhun (1998) and Adesina and Ofuya (2015) who found out that higher concentration of plant powder of *Piper guineense* and *Secamone afzelii* extracts, respectively, significantly reduced *C. maculatus* oviposition. It is noteworthy that all these plant powders showed more than 50% deterrent activity and significantly suppressed oviposition, even at lower concentration compared to unprotected cowpea seeds. Though, Ofuya (1990) reported that both seed powder and extract of *A. melegueta* did not significantly affect oviposition and egg hatchability of *C. maculatus* but, from the present study, it appears that these plant powders might possess oviposition deterrent principles; the survival and egg laying of *C. maculatus* was significantly affected by the treatment suggesting the presence of insecticidal and ovipositional active compound in the plants which delayed and completely inhibited the oviposition of the insects. This is in agreement with the findings of Abdullah and Muhammad (2004) who reported that the powder of *Piper guineense* adversely affected survival and egg laying capacity, higher ovicidal effects, reduced oviposition rates. The deterrent activity of the insect might be attributed to the change in the behavior and physiology of the insect after the treatment with the plant powders due to the chemical nature of the powder which adversely affects the egg laying capacity (Shifa Vanmathi, 2010). Besides, the plant powder can reduce insect movement, sexual communication and disrupts mating activities and as well as deterring females from laying eggs (Ileke *et al.*, 2012). The present study is in agreement with the findings of Dolui *et al.* (2010) and Dolui *et al.* (2012) who reported a considerable reduction in the number of eggs laid per female *Helopeltis theivora* after treatment with *X. aethiopica*. In a related development, Gehlot and Singhvi (2006) and Ravinder (2011) reported that oviposition of *C. maculatus* was also significantly reduced by the treatment of Eucalyptus leaf extract, turmeric powder, black pepper powder and garlic clove powder.

It is pertinent to note that shortened adult life-span by the treatments must have also been responsible for reduced oviposition. More so, since female beetles deposit most of their eggs in the first 3 days of adult life (Wasserman, 1985), any reduction in adult lifespan, as a result of the plant powder would be expected to have, contributed to the reduced oviposition

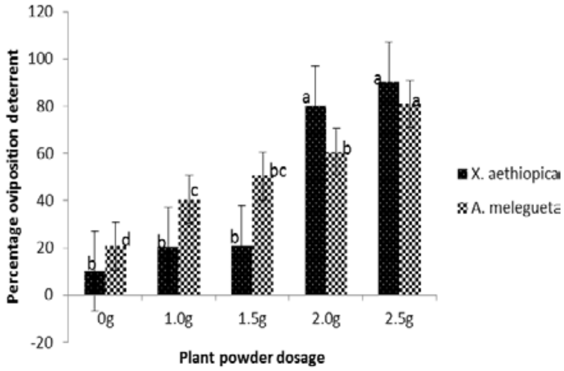

Figure 2. Mean percentage oviposition deterrent of *C. maculatus* on treated cowpea seeds. Means with the same letter for each plant powder are not significantly different using LSD at 95 % confidence level.

A significant reduction in adult emergence was recorded among the various treatments (Figure 3). In the present study, adult emergence decreased significantly (P<0.05) as the concentration of the powders increased. The result indicated that the adult emergence reduction is dosage rate dependent. The reduction in adult emergence could either be due to egg mortality or larval mortality or even reduction in the egg hatching. It has been reported that the larvae hatching from the eggs of *Callosobruchus* species must penetrate the seeds to survive (FAO, 1999). The plant powders might have inhibited the larval penetration into the seed and thus showed maximum adult emergence reduction (Khalequzzman and Goni, 2009). Jayakumar *et al.* (2003) reported that plant products have obvious effects on postembryonic survival of insects and resulting to reduction in adult emergence in all the concentrations of different plants. Annie Bright (2001) and Raja *et al.* (2001) reported that botanicals inhibit adult emergence of C. *maculatus* in cowpea. They further stated that when the eggs were laid on treated seeds, the toxic substance present in the plant products may enter into the egg through chorion and suppressed their embryonic development, thus, reduced adult emergence. The ability of the evaluated plants to significantly (P<0.05) suppress adult emergence suggested that the plants might possess ovicidal and larvicidal properties and this confirms the findings of Ofuya (1990).

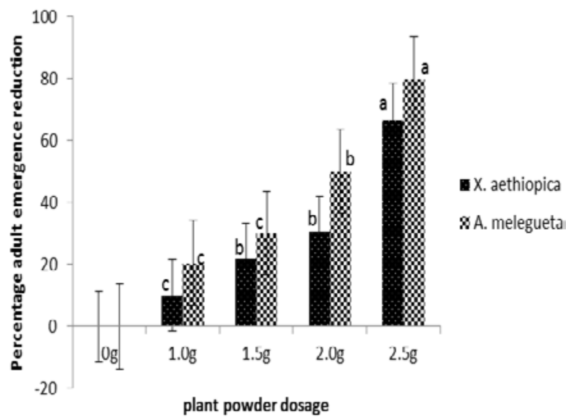

Figure 3. Mean percentage adult emergence reduction of *C. maculatus* protected with *Xylopia aethiopica* and *A*framomum *melegueta.* Means with the same letter for each plant powder are not significantly different using LSD at 95 % confidence level.

The results of the phytochemical constituents of both plant powders, as shown in Table 1, revealed the presence of tannins, cardiac glycosides and saponins in both plants, while alkaloids is absent in *X. aethiopica* and present in *A. melegueta.* Secondary metabolites such as phenolic compounds, saponins alkaloids, flavonoids and terpenoids have been identified to exhibit strong activities against several pathogens and insect pests (De Geyter *et al.*, 2007).

Table 1. Phtyochemical constituents of *Xylopia aethiopica* and *Aframomum melegueta*

Phtyochemical	X. aethiopica	A. melegueta
Alkaloids	-ve	+ve
Flavonoids	+ve	+ve
Cardiac glycosides	+ve	+ve
Tannins	+ve	+ve
Saponins	+ve	+ve

Karamanoli *et al.* (2011) reported that tannins exert their action by combination of mechanism that includes iron chelation and enzyme inhibition. Though the exact mechanism behind the observed action of both plant powders is not yet known. Chaieb (2010) extensively reviewed insecticidal effects of saponins, linking their insecticidal activity with cholesterol which results in impaired ecdysteroid synthesis. Some of the reported observed effects of saponins are increased mortality, lowered food intake, weight reduction, retardation in development and decreased reproduction (Chaieb, 2010). Dolui *et al.* (2012), on the other hand, reported that tannin combined with protein to inhibit enzyme activity and reduce the availability of protein in haeolymph in insects. The ability of the plant powders to reduce the egg laying capability by the female beetles may be attributed to the presence of flavonoids in the plant. Righi-Assia *et al.* (2010) stated that flavonoids significantly reduced the egg laying and fertility in *C. Chinensis.* The insecticidal activity exhibited by the evaluated plants in this study correlates with the findings of Dolui *et al.* (2012) and Kannahi and Vinotha (2013) who previously reported the pesticidal potentials of the plants. In fact, complex mixtures of secondary compounds in plant extracts were reported to contribute to a great deal for synergism, which enhances the joint action of active compounds against insect and reduces the rate of resistance development (Feng and Isman, 1995).

Adedire and Lajide (1999) and Sugita *et al.* (2013) 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. While Olonisakin *et al.* (2007) reported the presence of β-pinene, β-phellandrene, γ-terpinene, eucalyptol and α-pinene as the predominant bioactive compounds responsible for the insecticidal activity of *X. aethiopica*, *A. melegueta* was reported to contain the following bioactive molecules: α-caryophyllene, β-caryophyllene, E-nerolidol, linalool, gingerdione, paradol, shagaol and humulene (Owokotomo *et al.*, 2014)

Findings from the present study confirm the plant bioactivity in suppressing oviposition and adult emergence of *C. maculatus* and thus are efficacious in protecting cowpea seeds from the insect infestation and damage at limited resource farmers' level and low volume seed storage. Since adult *C. maculatus* do not feed on stored cowpea seeds but only deposit their eggs, admixing the plant powders is recommended as ecofriendly and non-toxic methods in the management of *C. maculatus* for short duration storage. A more extensive study is necessary to determine the relative amounts of these materials quantitatively required for pest control strategy.

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