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Research Article

Efficacy of *Cymbopogon citratus* Stapf leaf extract as seed protectant against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) on stored maize (*Zea mays* L.)

DILIGENT OBOHO[1], JOSEPH EYO[2*], FELICIA EKEH[2] and SOLOMON OKWECHE[3]
[1]*Department of Animal and Environmental Biology, University of Uyo, NIGERIA*
[2]*Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, NIGERIA*
[3]*Department of Forestry and Wildlife Resources Management, University of Calabar, NIGERIA*
Corresponding author Email: joseph.eyo@unn.edu.ng

ABSTRACT: A laboratory experiment was conducted to evaluate the insecticidal property of *Cymbopogon citratus* leaf extract as seed protectant against maize weevil. One (1) gram each of acetone, chloroform, methanol and aqueous extracts were re-suspended in 5 ml of deionized water and used to impregnate filter papers set in four replicates. Varied concentrations of the aqueous extract per 50 g of *Zea mays* grains infested with 10 pairs of sexed *Sitophilus zeamais* for 28 days were tested for insecticidal properties and compared with experimental and synthetic conventional insecticide [Coopex (0.25 g)] controls. Parameters assessed were effect of *C. citratus* extracts on weevil mortality (toxicity test) and protection of maize against *S. zeamais*. Data obtained were analyzed using Analysis of Variance (ANOVA) and means were separated using New Duncan Multiple Range Test at 5% level of significance. Results showed significant ($P < 0.05$) concentration and duration dependent mortalities of *S. zeamais*. The aqueous extract gave the highest protection of the maize grains followed by chloroform, methanol and acetone extracts respectively. The insecticidal potency of *C. citratus* extracts and its availability places it as an attractive biopesticide in traditional post-harvest seed protection.

KEY WORDS: *Cymbopogon citratus* extract, Toxicity, *Zea mays* protectant, *Sitophilus zeamais,* mortality

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INTRODUCTION

Maize (*Zea mays L.*) is an essential component of global food security. It is the third most important cereal grown in Nigeria next only to sorghum and millet (Adegbola, 1990). Maize is one of the most important cereal crops grown in the world and it forms one of the major diets of millions of people. In Africa, maize is primarily grown by small-scale farmers for use as both human food and animal feed. Its cob is consumed in different ways. For example; it could be grilled, boiled, roasted or milled into various products (Polaszek and Khan, 1998). Industrially, maize is used to produce alcohol, starch, pulp abrasive, oil and bio-fuel (Morris, 2007; Acharya and Young, 2008; Sekoai and Yoro, 2016). In Zimbabwe, maize is used for beer brewing and as a medium of exchange for goods and services (Stanning, 1989). The principal producers of maize in sub-Saharan Africa are Kenya, South Africa, Tanzania, Ethiopia and Nigeria (Seshu-Reddy, 1998). Increased productivity in staple food, such as maize, is critical to raising rural incomes and stimulating broad-based economic growth (Eicher and Byerlee, 1997). The demand for maize in developing countries, unarguably, surpasses the demand for both wheat and rice. This is as a result of the growth in meat and poultry consumption, which consequently, have led to the rapid increase in the demand for maize as livestock feed. Thus, the exploding demand for maize presents an urgent challenge for most developing countries (Pingali and Pandey, 2000).

Despite the worldwide increase in the demand for maize, its production is constrained by various biological, physical and chemical factors. These include the problems of insect attack, weeds and pathogen infestation, soil fertility and climate (Sanchez *et al.,* 1997). In addition, the substitution of traditional cultivars by high-yielding varieties has raised the specter of massive maize failure because of increased susceptibility of the later to diseases and pests. Amidst other constraints of maize production, insects constitute a major threat. Insect pests destroy approximately 14% of all potential food production, including maize, despite the yearly application of more than 300 million kilogram of pesticides (Pimentel, 2007). Losing crops to insect pests constitutes a great constraint to the realization of food security for the ever increasing world population, it is necessary to address the issue of maize grain loss to insect pest damage (Berenbaum, 1995). The devastating loss of stored grains to insect attack has necessitated the use of various measures to control maize weevils. Maize grains treated with certain materials such as wood ash, plant oils and plant powders have proven to be effective in the control of *S. zeamais* infestation (Lale, 1992). However, the formulation of these plant products into dosage and the adoption of their use in large scale storage had not been adequately addressed. Synthetic chemical insecticides are commonly used by maize farmers to protect grains from infestation. However, the widespread use of insecticides for the control of stored product insect pests is of global concern with respect to environmental health hazards, insecticide resistance development, chemical residues in food, side effects on non-target organisms and its associated high costs (Cherry *et al.,* 2004; Adebe *et al.,* 2009).To this effect, the development of alternative control strategies such as the use of botanicals like *Cymbopogon citratus* (powder) in the control of *S. zeamais* is necessary. The essence of this work is therefore to provide environmentally friendly and safer means of controlling *S. zeamais* in stored maize where sophisticated pesticide and insecticide of grains are not affordable especially among peasant farmers.

MATERIALS AND METHODS

Collection of plant materials

The plant material (*C. citratus*) containing the leaves were harvested in Faculty of Pharmacy Medicinal Plants Farm (5.0333^{o}N, 79167^{o}E) University of Uyo, Akwa Ibom State Nigeria and identified by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State and the specimen kept in their herbarium with voucher no: UUH3276/ UYO. The plant is a perennial herb growing up to 50cm, short underground stems. Leaf simple and tapered at the end, linear 5.0 – 7.0 cm long, parallel venation, inflorescence spike, central vein appear more in lower epidermis. The plant leaves were washed and air-dried to a constant weight in an open laboratory until it became crispy and then grounded into very fine powder using an electric blender (Dike and Mbah, 1992). The powders were sieved using 0.5 mm size mesh. The powder was then stored in an airtight container to prevent active components from evaporating prior to used (Denloye *et al.,* 2010).

Preparation of extracts

Five hundred grams (500g) of the powdered leaves was soaked in 1000 ml of distilled water for 24 hours. Thereafter, the mixture was filtered using Whatman No.1 filter paper and the filtrate obtained evaporated to dryness in a vacuum using rotary evaporator and stored in sealed vials until used (Oloyede, 2009). One gram of powder was resuspended in 1 ml of distilled water before use. Also, three hundred grams (300g) powder of the leaves was also soaked in chloroform, acetone and methanol in different glass jars and left to stand for 96 hours. The filtrate obtained was evaporated to dryness in a vacuum using rotary evaporator. After the evaporation of the solvent, the different extract material was kept on water bath to remove the remaining solvent. The extract was stored in the sealed vials in refrigerator until used (Manzoor *et al*., 2011).

Contact toxicity on filter paper

The method described by Obeng-Ofori *et al.* (1998) was adopted. A Whatman No. 1 filter paper (10.9 cm diameter) was placed in a glass Petri dish (11.0 cm diameter). One gram of each extract was resuspended in 5ml of deionized water and used to impregnate the filter papers. Ten insects were introduced into the dish and laid in a complete randomized design. The filter papers in the control dishes were treated with deionized water and another with Coopex, a standard control pesticide only. Each treatment was replicated four times with each replicate having ten sexed insects. The insect mortality was recorded on day 7, 14, 21 and 28 post treatments. Insects were considered dead if they remain immobile and also failed to respond to three probing with a blunt dissecting probe after a 5 minute recovery period.

Contact toxicity by topical application

Ten insects of *S. zeamais* were placed in Petri dishes with moist filter paper (Obeng-Ofori *et al*., 1998). Insects were picked individually and with the aid of spatula, the extract was applied to the dorsal surface of the thorax of the insect. Deionized water was used in the control and the treatment was replicated four times. Insects were examined daily for mortality on day 7, 14, 21 and 28 post treatments. Any insect that does not move or respond to three probing with a blunt probe was considered dead.

Protection of maize by *Cymbopogon citratus* extract against damage

One kilogram of maize was kept in the deep freezer for two weeks to avoid hidden infestation. 50g of the grain was measured into plastic cups and the aqueous extract at concentration of 0, 1, 2, 3, 4, 5 and 6 g added. Ten pairs of *S. zeamais* were introduced into the cups and covered with white muslin cloth held in place with rubber bands and laid in a completely randomized design. Each treatment was replicated four times with each replicate having 10 pairs of insects and left to stand undisturbed for four weeks. Samples of 50 grains were taken from each cup (Obeng-Ofori *et al.*, 1998; Udo, 2005) and the number of damaged grains (grains with characteristic holes) and undamaged grains were counted and weighed. Percent weight loss was calculated following the method described by FAO (1988) thus:

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

where: U = weight of undamaged fraction in the sample, N = total number of grains in the sample, Ua = average weight of one undamaged grain and D = weight of damaged fraction in the sample

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS version 20.0). Means were separated using New Duncan Multiple Range Test (NDMRT) at 5% level of significance (Duncan, 1955).

RESULTS AND DISCUSSION

Contact toxicity of extracts

The effect of various extract of *C. citratus* against *S. zeamais* revealed different level of activity against the insect (Table 1). Contact mortality of less than 50% was recorded on *S. zeamais* by the extracts on 28 days after treatment. The aqueous showed a significant mortality of over 50% against the insect after 28 post treatment. The various extract also showed bioactivity over the experimental control for *S. zeamais* but not the same for Coopex, the standard control.

Table 1. Contact toxicity of extract of *Cymbopogon citratus* applied on filter paper against *Sitophilus zeamais*

Extracts (1g)	Mean mortality at different days after treatment 7	14	1	28
Acetone	1.25±0.50^{b1}	1.00±0.82^{b1}	1.25±0.50^{b1}	1.25±0.50^{b1}
Chloroform	1.00±0.82^{b1}	1.25±0.50^{b1}	1.00±0.82^{b1}	1.50±0.82^{b1}
Methanol	1.00±0.82^{b1}	1.00±0.58^{b1}	1.25±0.50^{b1}	1.50±0.50^{b1}
Aqueous	1.00±0.82^{b1}	0.50±0.58^{bc1}	1.00±0.82^{b1}	1.50±0.82^{b1}
Experimental control	3.00±0.00^{a2}	3.50±0.58^{a2}	4.50±0.58^{a1}	5.00±0.00^{a1}
Standard control	0.25±0.00^{b1}	0.00±0.00^{c1}	0.00±0.00^{c1}	0.25±0.00^{c1}

Mean values with different alphabets as superscript in a column are significant (P < 0.05)
Mean values with different numbers as superscript in a row are significant (P < 0.05)

Efficacy of extracts applied on filter paper against *Sitophilus zeamais*

Comparing the efficacy of the various extracts applied on filter paper against *S. zeamais* showed that aqueous and chloroform extracts gave the highest mean mortality and were more efficacious in causing mortality of the insect introduced on filter paper than methanol and acetone extracts when compared with experimental control in this order and the standard control gave mortality of 100% of the insects (Table 2).

Table 2. Efficacy of each extract applied on filter paper against *Sitophilus zeamais*

Extracts (1g)	Mean mortality at different days after treatment 7	14	21	28
Acetone	0.25±0.25^{b1}	0.25±0.25^{b1}	0.25±0.25^{b1}	0.25±0.25^{b1}
Chloroform	0.50±0.29^{b1}	0.50±0.29^{b1}	0.25±0.25^{b1}	0.50±0.29^{b1}
Methanol	0.25±0.25^{b1}	0.50±0.29^{b1}	0.50±0.29^{b1}	0.50±0.29^{b1}
Aqueous	0.50±0.29^{b1}	0.50±0.29^{b1}	0.50±0.29^{b1}	0.50±0.29^{b1}
Experimental control	0.00±0.00^{b2}	0.00±0.00^{b}	0.00±0.00^{b1}	0.00±0.00^{b1}
Standard control	2.00±0.82^{a2}	2.00±0.82^{a2}	2.00±0.00^{a2}	2.00±0.00^{a2}

Mean values with different alphabets as superscript in a column are significant (P < 0.05)
Mean values with different numbers as superscript in a row are significant (P < 0.05)

Effect of *Cymbopogon citratus* application on *Zea mays* grains

Grains treated with the extracts of *C. citratus* at different levels significantly reduced damage caused by *S. zeamais* to stored maize. The treatment significantly offered protection to maize grains in a dose-dependent manner. However, higher concentrations gave a highest significant (P < 0.05) reduction on damage caused by *S. zeamais* with a low percent weight loss when compared with experimental control. The Coopex insecticide significantly reduced weight loss as it offers about 100% protections (Table 3).

Table 3. Protection of maize by *Cymbopogon citratus* powder against damage by the stored product weevil

Treatment levels (g)	Percent weight loss (%)
I	13
2	11
3	9
4	7
5	5
6	4
Experimental control	50
Standard control	1

The mortality of S. zeamais on the filter paper was not very high and not statistically significant (P < 0.05). This is probably due to the possession of strong elytra that covers the entire abdomen of the insect. This confirmed earlier works by Inyang (2004), Akpabot *et al*.(2010) and Edelduok *et al*. (2012) who posited that coleopterans have exceptionally thick cuticles, epidermis and basement membrane as effective mechanisms of restricting toxicants absorption but disagreed with earlier work by Epidi *et al*. (2009), Denloye *et al*. (2010) and Udo *et al*.(2011) who observed significant contact action on *S. zeamais* since they are known to be poor fliers and so were always in contact with the treated filter paper. Again, the body size of *S. zeamais* might enhance its efficiency in detoxifying any toxic materials in the plant product applied. The ability of the extract to bring about significant ($P < 0.05$) insect mortality indicated that the powders have contact toxicity. The study also supports Ogban *et al*. (2015), who reported that *Acmella oleracea* was highly toxic and may act as antifeedant to insects thereby leading to starvation and subsequent death.

A very high potency was observed when the extracts were applied topically on the insect. Topical application facilitated direct contact of the toxicants or active ingredients in *C. citratus* with the insect body as this confirmed the study of Adedire and Ajayi (1996), Okonkwo and Okoye (1996) and Udo *et al.* (2011) who screened natural biopesticide for pest control. The toxicity of the plant extracts has been attributed to many chemical ingredients. This observation corroborated those made by Murugan *et al*. (1999), Onu and Baba (2003), Maina and Lale (2004) and Kabeh and Lale (2004) who found neem kernel powder and other plant extracts to have high toxic effect on insect physiological system. Abdullahi and Majeed (2010) used *Vitallaria paradoxa* seed powder on *C. maculatus* and found that the seed powder recorded 100% mortality after 24 hours of exposure at 10% w/w.

The extract tested at one gram was not significantly different in reducing the damage caused by the insect species but when the concentration was increased and up to 6 g, there was a significant reduction in the damage caused by the insect to the stored maize. The significant reduction in insects' damage suggested that the plants acted as an antifeedant and was in line with the finding of Nawrot *et al*. (1998) and Inyang and Emosirue (2005). This may also be due to lipid content of the plant. Schmutterer (1995) and Harborne and William (2000) linked the presence of esters in plants with antifeedant activities of insects. When the extract were screened against the insect, significant reduction in damage was observed coupled with high mortality which corresponds with the work of Okunji *et al*. (1996) and Momeni *et al*. (2005) that attributed this effect to the presence of secondary metabolites.

Considering the extract formulations used in this study, insect mortality may be due to their physical action since the particles may block spiracles of the test insects and cause death by asphyxiation, Hence, there is a direct relationship between particle size of the plant powders and insect mortality in treated grains as 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 contacts with the insects and killing them. In addition, the plant extracts cause abrasion of insect cuticle and lead to water loss which may cause stress and eventual death. The use of plant products in the form of extract in the management of stored product insect pests is perhaps the most convenient among resource-poor farmers because of ease of application, relative abundance and cheaper to procure, farmers could incorporate it into traditional storage systems. Furthermore, the ever increasing cost of agrochemicals in the market, lack of technical know-how in usage by non- literate local farmers and availability at critical period of needs emphasizes the needs to source for alternative means of preserving stored grains. Since the result indicated that *C. citratus* has potential for grain protection, it can be harnessed as an alternative to synthetic insecticides.

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