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Research Article

Insecticidal activity of *Xylopia aethiopica* (Family; Annonaceae) against *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae)

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Abstract

The efficacy of *Xylopia aethiopica* leaf dust and extract on *Callosobruchus maculatus* and *Sitophilus oryzae* was evaluated at different doses (1.0 – 3.0 g and 50.0-100 mg^{-ml}) with 10 unsexed adult weevils per 10 gram of substrate per replicate. All treatments were triplicated and mortality of the insects was recorded after every 24 hours (h) for 96 h exposure to powder and extract respectively. The parameter compared was the mortality rate of the adult pests. The negropepper was an active biopesticide against *C. maculatus* and *S. oryzae*. However, the plant products gave higher mortality on *S. oryzae* with a mean of mean mortality and LD_{50} of 82.2% and 1.06g respectively than on *C. maculatus* whose mean mortality and LD_{50} was 79.9% and 1.12g respectively over 96 hours exposure. Statistical analysis showed a significant difference ($P<0.05$) in pest mortality between treated and control samples. The results suggested that *X. aethiopica* is more promising botanical insecticides on *S. oryzae* than *C. maculatus*.

Key words: Pest Management, Insecticidal, toxicity, *Callosobruchus maculatus*, *Sitophilus oryzae*, *Xylopia aethiopica*.

Introduction

The unavailability of efficient storage facilities and control of stored grain pests has long been the aim of many entomologists throughout the world (Ashouri and Shayesteh, 2010; Ito and Utebor, 2018). Cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) and rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) infest and cause substantial qualitative and quantitative postharvest losses of stored food products particularly grains which represent an important component of the world food supply (Ito and Ighere, 2017a). Food commodities particularly cowpea (*Vigna unguiculata*) and rice (*Oryza sativa*) constitute the bulk of stored products (Ito and Ighere, 2017b & Helaly, 2018). They undergo qualitative and quantitative depreciation in storage due mainly to insect pest infestation and damage especially in tropical and subtropical regions of the world (Ngatanko *et al.,* 2017). In African countries, postharvest losses have been estimated to range between 20 – 40%, which is highly significant considering the low agricultural productivity in several regions of Africa (Abass *et al.,* 2014). Yearly loses of food grains in Sub-Sahara Africa (SSA) has been estimated to worth approximately USD 4 billion (Zorya et al., 2011). In Nigeria, 50% of cowpea seeds is loss traditionally in postharvest to bruchids, and 82% of the seeds have been documented to have perforations when stored as grains (Murdock et al., 1997). *C. maculatus* is responsible for up to 24% losses in stored pulses in Nigeria (Tapondjou et al., 2002). *S. oryzae* represent a vital component of the world food supply (Uwamose et al., 2017). FAO (1999) estimated that Nigeria, Cameroon, Sierre-Leone and Uganda lost 5.0%, 10-20%, 5-10% and 15-20% respectively of their stored rice due to insect pest infestation. Current estimation of rice losses in Nigeria has been reported as high as 24.9%, a loss equivalent to 56.7 billion Nigerian Naira (Kumar and Kalita, 2017). Grain storage forms part of the postharvest system through which food materials pass on their way from the field to consumers. It is at these grain storage facilities that these cereals are infested with weevils. *C. maculatus* and *S. oryzae* are two important pests of stored-grain products in tropical and subtropical regions of the world (Ito and Ighere, 2017a).

In view of the importance of stored food commodities like cereal grains as a revenue source and constituent articles of diet, their protection against insect damage all the year round is imperative especially in developing countries like Nigeria where the loss of these food materials is enormous. Over the years crops and food grains in storage were protected from insect pests with synthetic insecticides. The use of synthetic insecticides which came to the fore after the second world war later became unpopular (Jackai and Daoust, 1986) owing to their prohibitive cost to majority of African peasant farmers and serious attendant environmental problems which include toxic residues, pest resistance and negative impact on non-target beneficial species (Cherry et al., 2005). The shortcoming of synthetic insecticides rekindled the interest of researchers worldwide to search for alternative means which are biodegradable and ecofriendly for the control of insect pests (Ito and Utebor, 2018; Ntonifor 2011). In this context, botanical pesticides and natural plant products fulfil these criteria besides being available, sustainable and inexpensive.

Opinion has shifted from the reliance on synthetic insecticides to the use of plant materials for the protection of stored food products because of their environmental safety (Omotoso, 2008; Pérez et al., 2010). The pool of insecticidal plants in the tropics is enormous and this has, in recent years, greatly motivated further investigation of the efficacy of plants and their exploitation as potential sources of natural control agents of insect pests. Plant extracts, and their derivatives such as derris, rotenone, pyrethrum, nicotine have been variously tested and used as protectants of postharvest food grains in storage for a decade (Sarfraz and Keddie, 2005). The protection and preservation of cowpea and rice in storage should be encouraged and sustained because they are important common articles of diet in many parts of the world. The focus of this study is to evaluate the insecticidal potentials of *X. aethiopica* (negropepper) as a protectant of cowpea (*Vigna unguiculata*) and rice (*Oryza sativum*) against two common storage pests: *Callosobruchus maculatus* (cowpea weevil) and *Sitophilus oryzae* (rice weevil).

Materials and Methods

Preparation of plant powders

The fruits of *Xylopia aethiopica* used in this study were purchased locally, and air dried indoors for ten days (Ouko et al., 2017). The dry fruits were milled to a fine powder with the aid of a Binatone electric blender (Model BLG-400) and the powder was stored in airtight jars.

Preparation of crude extract

95% ethanol was used as a solvent for the extraction. The extract was prepared by dissolving 25.0, 37.5 and 50.0g milled powder severally in 500ml ethanol in three glass jars of 1000ml capacity. The solution was allowed to extract for three days with repeated stirring using a glass rod. The extract was filtered with Whatman No 1 filter paper and the solvent evaporated by applying very slight heat to the filtrate through a water bath system. Thus a syrupy *X. aethiopica* crude extract of different concentrations (50.0, 75.0 and 100.0 mg^{-ml}) corresponding to the weights of powder dissolved was prepared. The extract was stored in a refrigerator maintained at 5-10 ^{o}C and were removed only when needed for assay.

Culture of insect pests

The insect pests for this study were the cowpea seed bruchid (CSB), *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) and rice weevil, *Sitophilus oryzae* (Linn.) (Coleoptera: Curculionidae) which are among the insects most often encountered in stored food materials. The insects were cultured by the method described by Dimetry *et al.*, (2015), and Ito and Ighere (2017b). The uninfested substrate (cowpea and rice) were placed severally in seven plastic containers to which heavily infested cowpea and rice were added. The containers were then covered with polythene nets fastened with rubber bands and kept in the laboratory for 25-30 days for oviposition and development to occur. Thus the insect pest cultures were established from which unsexed first filial generation (F_1) adults were used for the study.

Insecticidal bioassay

X. aethiopica powders and crude extract were assayed for contact toxicity against the insect pests following the methods of Gbaye *et al.* (2015), and Mailafiya *et al*., 2014).

Invitro Toxicity Test of Powder

Twenty (20) grams disinfected substrates (cowpea and rice) were weighed and placed in three separate sterilized Petri dishes. Powder doses of 1.0, 2.0 and 3.0g (1.0:20, 2.0:20, and 3.0:20g w/w powder: substrate) were added to each petri dish and replicated thrice. The dishes were then covered and manually shaken thoroughly to ensure uniform smearing of the substrate with the powder after which ten (10) unsexed adult insects were introduced into the dishes and covered. A control consisting of a 20g substrate and ten weevils but devoid of *X. aethiopica* powders was set up and replicated thrice. Mortality of the insects was recorded after every 24 hours for 96 hours exposure period according to the duration in FAO bulletin (1999). The insects were considered dead if they failed to move or respond to gentle touch with a pin at the abdomen. The dead weevils were removed and counted.

Invitro Toxicity Test of Extract

Ten gram (10g) disinfected cowpea seeds were placed severally in three test tubes and treated with 50.0, 75.0 and 100.0 mg^{-ml} extract concentrations. A control consisting of 10g substrate untreated with extract and ten adult weevils was set up. Both treatment and control were replicated thrice.

The tube was shaken gently for one minute to enable the substrate to get coated with the extract after which they were removed and placed on filter papers for 24 hours for the solvent to evaporate. The treated substrates were transferred to separate fresh test tubes and 10 adult unsexed weevils were added. The test tubes were closed with plastic stopper bearing gauze window for ventilation. Mortality count of the insect was taken every 24 hours for 96 hours exposure period. Any of the insects was considered dead and removed if it failed to move or respond to gentle touch.

Statistical Analysis

The data were subjected to analysis of variance (ANOVA) to check for significant differences in mortality between treatment and time post-exposure. Mortality data at 48 – and 96h post exposure time points were analyzed with probit (Finney, 1971) to determine the LC_{50} and LD_{50} for each pest.

Results

Toxicity of Powder

The results of the insecticidal activity of *X. aethiopica* powder at the various application doses of 1.0, 2.0 and 3.0g against the test insect pests: *C. maculatus* and *S. oryzae* over 96 hours exposure are shown in Table 1. *X. aethiopica* showed greater insecticidal efficacy against *S. oryzae* than *C. maculatus*. The mean of mean mortality of *S. oryzae* was 82.2% while *C. maculatus* recorded 79.9% mortality over the 96 hours exposure period. The LD_{50} for *S. oryzae* was 1.06g compared to 1.12g for *C. maculatus*.

Table1. Cumulative mean percentage mortality of *C. maculatus* and *S. oryzae* treated with different doses of *X. aethiopica*

	% mean kill posttreatment (Hrs)							
Doses (g)	***C. maculatus***				***S. oryzae***			
	24	**48**	**72**	**96 (Hrs)**	**24**	**48**	**72**	**96 (Hrs)**
1.0	33.3	53.3	66.6	76.6	36.6	63.3	70.0	76.6
2.0	33.3	53.3	70.0	76.6	40.0	66.6	76.6	83.3
3.0	46.6	60.0	73.3	86.6	46.6	66.6	76.6	86.6
Control	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3

C. maculatus: mean of mean mortality = 79.9%
S. oryzae: mean of mean mortality = 82.2%

The insecticidal efficacy of *X. aethiopica* powder at various doses over 96 hours exposure is compared in Figure 1.

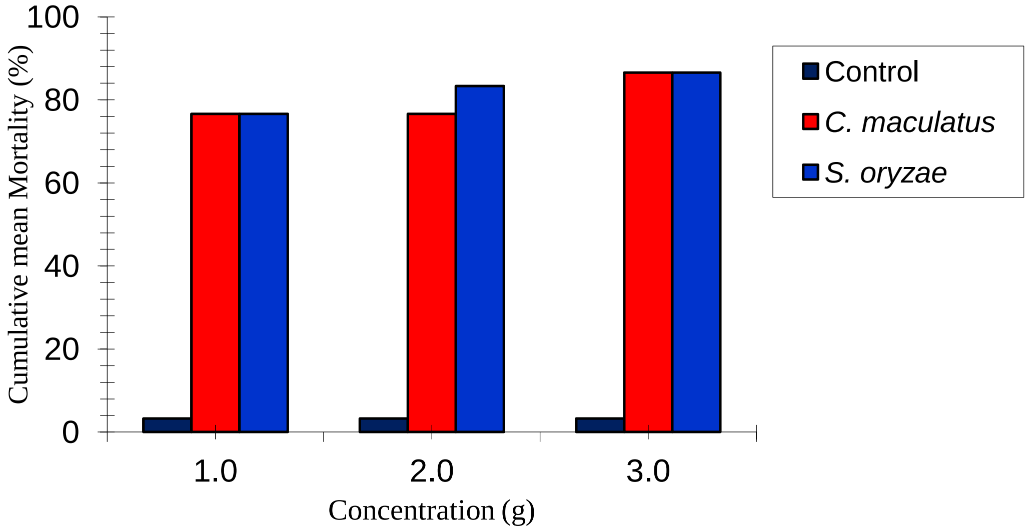

Figure 1. Cumulative mean of mean mortality of *C. maculatus* and *S. oryzae* exposed to *X. aethiopica* powder over 96 hours.

Toxicity of Extract

The extract was insecticidally potent against the test insect pests. The cumulative mean kills by the extract at 50.0 mg $^{-ml}$ concentration over 96 hours was 63.3% and 56.6% for *C. maculatus* and *S. oryzae* respectively. *C. maculatus* recorded 73.3% cumulative mean mortality compared to 56.6% kill for S. oryzae at 75.0 mg $^{-ml}$ extract concentration. The concentration 100.0mg^{-ml} generated mean mortality of 73.3% and 63.3% for *C. maculatus* and *S. oryzae* respectively. The respective LC_{50} for *C. maculatus* and *S. oryzae* over 96 hours exposure was 577.7 and 600.0 mg. The results indicated that, unlike the powder, *X. aethiopica* extract was more active as an insecticide against *C. maculatus* than *S. oryzae* and the toxicity was dependent more on concentration than the time of exposure.

Table 2. Effect of *X. aethiopica* Extract of Different Concentrations on *C. maculatus* and *S. oryzae* over 96 hours.

	Mean Percentage kill	
Conc. (mg^{-ml})	***C. maculatus***	***S. oryzae***
50.0	63.6	56.6
75.0	73.3	56.6
100.0	73.3	63.3

The insecticidal strength of *X. aethiopica* extract against *C. maculatus* and *S. oryzae* is compared in Figure 2.

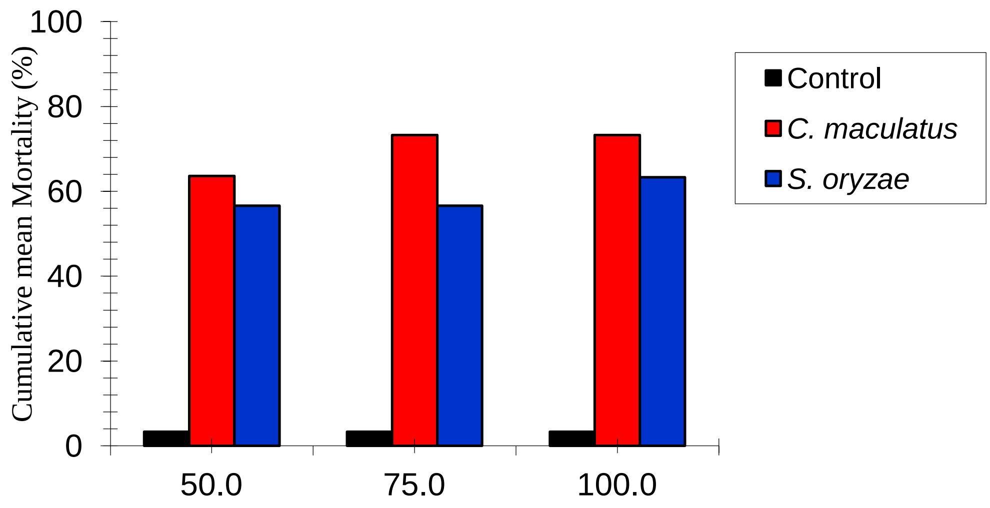

Figure 2. Cumulative Mean Mortality of *C. maculatus* and *S. oryzae* Exposed to Various Concentrations of *X. aethiopica* Extract over 96 Hours

Discussion

The use of botanicals as entomocide is an age-long practice in tropical African (Ito and Ukpohwo, 2018). Consequently, plant parts of several floras have been investigated for their insecticidal properties against stored product pest. It is important to note that these botanicals have helped in reducing losses associated with most of these insect pests. The current study revealed that *X. aethiopica*, fruit powder and extract were active biopesticides against the test insect pest: *C. maculatus* and *S. oryzae*. The results of this study conform to some degree with the earlier reports (Opareke and Dike, 2005; Babarinde et al., 2008; Mukanga et al., 2010; Adedire et al., 2011; Ileke and Oni, 2011; Onekutu et al., 2015; Ito and Ighere, 2017b; Louise et al., 2018) that certain botanicals are effectively toxic against storage insect pests including *C. maculatus*. Negropepper (*X. aethiopica*) has been reported lethal to a variety of living organism including bacteria (Asekun and Adeniyi, 2004; Okigbo et al., 2005).

X. aethiopica exhibits insecticidal potency because it contains various toxic complex compounds (Fleischer, 2003). The principal chemical constituents in the extracted oils from *X. aethiopica* were various terpenes and their derivatives (Pérez et al., 2010). Other active ingredient in *X. aethiopica* are β-pinene, terpinen-4-ol, sabinene, ά-terpineol, 1,8-cineole, myrtenol and kaurane derivatives. The toxicity exhibited by *X. aethiopica* may be due to these hydrocarbon compounds which have active ingredients of diverse chemical nature (Ito and Ighere, 2017b). Terpenes have been reported toxic against the rice weevil, *S. oryzae* (Byung-Ho et al., 2001) and *C. maculatus* (Agrawal et al., 1998). Keane and Ryan (1999) had demonstrated that terpenes affect the nervous system by inhibiting the enzyme acetylcholinesterase activity as shown in the wax moth *Galleria melonella*. It may also be postulated that the insect pests in this study died from suffocation caused by the blockade of the external openings of the tracheal system by the plant powders.

In Cameroon, the insecticidal efficacy of whole and fruits of *X. aethiopica* essential oils have been tested on adults *S. zeamais* (Jirovetz et al., 2005), also one of the main pest in granaries in Nigeria. Jirovetz *et al* (2005), documented as high as 93.3% mortality after 96h exposure of *S. zemais* to *X. aethiopica* oil. This is in agreement with 86.6% mortality observed in this current study after 96h exposure of *C. maculatus* and *S. oryzae* to 3.0g of *X. aethiopica* dust. The mortality difference in this present study might be attributed to the difference in pest species, the concentration of the treatment, methodology of research, laboratory and environmental differences.

In this study, weevil mortality increase with an increase in the concentration of *X. aethiopica* powders and extracts. The study showed that *X. aethiopica* powder gave higher kill on *S. oryzae* than *C. maculatus* while the extract was more insecticidal against *C. maculatus* than *S. oryzae*. The sensitivity of adult *C. maculatus* and *S. oryzae* to various concentration of powder and extracts of *X. aethiopica* revealed considerable variation in effectiveness. In this study, the LD_{50} for *S. oryzae* was 1.06g compared to 1.12g for *C. maculatus*. Similarly, the extract gave an LC_{50} of 577.7 and 600.0 mg for *C. maculatus* and *S. oryzae* respectively over 96 hours exposure. This indicates that *X. aethiopica* possess more entomotoxic effect of powder on *S. oryzae* and *C. maculatus* than the extracts which gave less percentage (%) kill of the weevils. It is, therefore suggested that the extract rather than powder should be applied on *C. maculatus* infested stored food products. Conversely *S. oryzae* could be better controlled with *X. aethiopica* powder than the extract.

Conclusion

Negropepper is a spice and edible fruit. Its use as a protectant of stored food commodities will be acceptable to local farmers, housewives and traders. The plant material does not affect the appearance, flavour and overall acceptability of treated stored products. Negropepper could serve as a better alternative to synthetic insecticides because it is readily available and within reach of peasant farmers.

Conflict of interest statement

We declare that no conflict of interests exist regards to this research publication.

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