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Bioactivity of *Ocimum sanctum* Linn. Leaf Powder and Extracts against *Tribolium castaneum* Herbst

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ABSTRACT

Two formulations of *Ocimum sanctum* L. (powder and extract) were used to test for its toxic and repellent properties against *Tribolium castaneum* in the laboratory. A toxicity test consisted of exposing *T. castaneum* larvae to millet (*Pennisetum glaucum* (L.) R. Br.) seeds treated with three dosages (0, 0.15 and 0.3 ml/5 g seeds) and millet flour admixed with three dosages (0, 0.25 and 0.5 g/5 g flour). In the case of the leaf powder, mortality increased with increase in concentration, with highest percentage mortality (63.5%) observed with 0.5 g *O. sanctum.* However, when two extracting solvents were used for extraction, acetone evoked greater mortality than petroleum spirit. Although petroleum spirit had greater repellent effects, it was not dependent on dose. Percentage repellency followed a progressive increase when acetone was used for extraction with the significantly ($P < 0.05$) highest percentage repellency (86%) observed with 0.3 ml/30 cm^2.

Keywords: Holy basil, repellent properties, rust red flour beetle, toxicity

INTRODUCTION

Pearl millet (*Pennisetum glaucum*) is the most important millet grown in Nigeria and constitutes the major staple diet for the millions of people inhabiting the Sahelian parts of tropical Africa and Asia (Gupta and Singh 1996). The whole grain is the main part used, cracked or ground into flour or roasted whole and eaten directly. Also it can be ground into paste and fermented to produce pap. The grains may also be fed to livestock. Pearl millet is nutritionally better than most other cereals. It has high level of calcium, iron, zinc, lipids and high quality protein (Klopfenstein and Hoseney 1995). Despite the nutritional value and usefulness of millet, the cereal is attacked by many pests both on the field and in storage. Some of the major storage pests of millet are *Rhyzopertha dominica* Fab., *Tribolium castaneum* Herbst and *Corcyra cephalonica* Stainton (Ghelani *et al.* 2009). Both the larvae and the adult red rust flour beetle (*T. castaneum*), which is its major storage pest, feed on grain, which results in substantial losses in weight and quality of stored millet (Gupta and Singh 1996). Due to these losses there is need therefore, for the protection of these grains so that the resource-poor farmer can continue to have more food on his/her table and to better standard of living.

The protection of grains and seeds in storage are often possible by the use of synthetic insecticides, but their uses have been reported to contain antecedent health and environmental hazards (Sighamony *et al*. 1986). The application of botanical insecticides for the protection of cereals and pulses is therefore recently receiving attention in developing countries. First, botanical products are eco-friendly and economical (Adedire and Ajayi 1996; Singh and Saratchandra 2005; Gahukar 2008) and so are readily affordable to resource-poor farmers. Secondly, botanicals do not expose insect to the risk of resistance to insecticides as applicable to unguided use of synthetic pesticides (Pimentel *et al*. 2009).

Holy basil (*Ocimum sanctum* Linn.) is native to western and tropical Asia and is found throughout India and Nepal up to elevation 1800 m in the Himalayas and on the Andaman Nicobar Island. Today the plant is widespread in Africa and Europe and the western hemisphere as condiment, for medicinal purpose and as scent (Prakash and Gupta 2005; Kochhar *et al.* 2009; Khanna *et al*. 2010). The parts of the plant with pest controlling actions are the leaves and the stem. They have repellent, insecticidal, fungicidal and molluscidal properties (Gbolade *et al*. 2000). In order to establish the therapeutic uses of *O. sanctum* Linn. in modern medicine, in the last few decades several Indian scientists and researchers have studied the pharmacological effects of steam distilled, petroleum ether and benzene extracts of various parts of the plant and eugenol, its bioactive compound, on the immune system (Prakash and Gupta 2005; Dutta *et al*. 2007; Kochhar *et al*. 2009; Khanna *et al*. 2010; Lahon and Das 2011).

Although there are works on botanical control of *T. castaneum* (Negahban and Moharramipour 2007; Babarinde and Ogunkeyede 2008; Sahaf *et al*. 2008; Islam *et al.* 2009; Babarinde and Adeyemo 2010; Babarinde *et al*. 2011), evaluation of different extracting solvents has not received major attention. This is necessary because the disparity in the polarity of different solvents may have an interactive effect on the potency of a particular botanical. Therefore, the aim of this research is to evaluate the toxicity of *O. sanctum* powder to *T. castaneum* and the effect of *O. sanctum* extracted with different extracting solvents on the mortality and repellency of the insect species.

MATERIALS AND METHODS

Insect culture

T. castaneum was obtained from a culture maintained in the Storage Entomology Unit, Department of Crop and Environmental Protection, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria. They were reared on millet bought from Sabo Market, Ogbomoso according to Babarinde and Adeyemo (2010) in the laboratory under ambient temperature of 36 ±

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2°C and 67 ± 5% relative humidity. The bioassays were conducted under similar conditions of insect rearing.

Collection and extraction of *Ocimum sanctum*

The leaves of *O. sanctum* were obtained from a local garden in Adenike Area, Besides LAUTECH Campus, Ogbomoso, Nigeria. The leaves were air-dried until crisp and milled into a powder using a mortar and pestle. Plant powder (20 g) was soaked in 200 ml each of acetone and petroleum spirit to make (1:10 w/v) and left for 24 h in a separate 500 ml conical flask, after which the content was sieved using a Whatman No. 1 filter paper. The extracts were separately stored in glass jars at room temperature as stock solutions until use.

Toxicity test

Two bioassay methods were used to assess the toxicity of *O. sanctum* on *T. castaneum* larvae. In the first method, three dosages (0, 0.15 and 0.3 ml) of the extract were dissolved separately in 0.2 ml of either acetone or petroleum spirit. Each dosage was mixed with 5 g of millet seeds in 100-ml glass jars. The seeds were thoroughly stirred manually with a glass rod for 5 min as described by Lale and Alaga (2001) to ensure adequate coating and complete evaporation of acetone and petroleum spirit (Lale 1991). After 1 h of treatment of the seeds, 10 *T. castaneum* larvae were then introduced into each glass jar and covered with aerated lids which allowed respiration of test species and prevented the intrusion of other insects. The experiment was replicated six times. Data on percentage mortality was taken at 5 days post treatment.

In the second bioassay, three dosages (0, 0.25 and 0.5 g leaf powder) of *O. sanctum* were used. Each dosage was admixed with 5 g of millet flour in 100 ml glass jars. The content was thoroughly stirred for even distribution. Ten *T. castaneum* larvae similar in all respects to those used in the first bioassay were introduced into each glass jars and covered as done for the first toxicity bioassay. The experiment was replicated four times. Data on percentage mortality was taken at 5 days post treatment.

For both methods of bioassay, when mortality occurred in the control, observed mortality in the treated samples was corrected with Abbot's Formula (Abbot 1925).

Repellency test

The method used for testing repellency of *O. sanctum* extract against T. *castaneum* adults was based on the area preference test described by McDonald *et al*. (1970). The test arena consisted of 9 cm Whatman No. 1 filter papers cut in half. Three dosages (0.1, 0.2 and 0.3 ml) of the acetone and petroleum spirit extract were separately applied on a half paper disc of 30 cm^2 area as uniformly as possible by means of a syringe (Menojet®, China). The other half was treated with 0.1 ml acetone or petroleum spirit. The two halves were joined together with an adhesive tape (Pona Globe, China) and placed in a 9 cm diameter Petri dish. Ten *T. castaneum* adults were released at the centre of each repellency chamber and then covered. Each treatment and control was replicated six times. The number of insects present in the untreated (Nc) and the treated disc (Nt) were recorded 30 min after the experiment was initiated.

Percentage repellency (PR) values were computed as:

$$PR = [(Nc-Nt)/ (Nc+Nt)] \times 100$$

Repellency values were grouped into 5 repellency classes (RC) according to Dales (1996) where RC 0 = < 0.1%; RC I = 0.1–20%; RC II =20.1–40%; RC III = 40.1–60%; RC IV = 60.1–80%; RC V = 80.1–100%.

Experimental design and data analysis

Toxicity bioassay with extracts and repellency experiment were set up in a randomized complete block design (RCBD) with concentration as the treatment and extracting solvent as the block. The toxicity bioassay with leaf powder was set up in completely randomized design (CRD). Data were subjected to analysis of variance using SAS package (SAS Institute 2000). Standard errors of means were indicated for the RCBD experiment to show variations within the replicates. Where there were significant treatment effects, means were separated using least significant difference (LSD) at the 5% level of probability.

RESULTS AND DISCUSSION

In the first bioassay which evaluated the bioactivity of *O. sanctum* extract against *T. castaneum*, millet seeds were used, not millet flour. The justification for this bioassay was that *T. castaneum*, the experimental insect pest, is a pest of millet which can attack the seed even when it has not been milled into flour (Lale and Alaga 2001). In the second bioassay, *O. sanctum* powder was used because of two reasons. Firstly, *T. castaneum* is a secondary pest that thrives well in milled flour of cereals (Turaki *et al*. 2007). Secondly, in Africa, farmers admix botanical powder with stored produce for protection of the produce against post harvest insect pests. Gselase and Getu (2009) evaluated bioactivity of botanical plants powders against *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) in stored haricot beans under laboratory conditions in Ethiopia. In an early investigation, Rahman and Talukder (2006) reported the bioefficacies of extracts, powder, ash and oil from different plant/weed against *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) fed on black gram, *Vigna mungo*, seeds.

When *O. sanctum* leaf powder was mixed with millet flour, mortality was dose-dependent and the highest percentage mortality (68.50%) was recorded on 0.5 g/5 g millet flour (**Table 1**). However when extracts was used, the level of mortality was higher than what was observed in millet flour bioassay. Higher percentage mortality was recorded in acetone compared with the percentage mortality observed in petroleum spirit (**Table 2**).

Ocimum, which has several varieties and species, has been documented to have insecticidal properties against stored product insect pests (Obeng-Ofori and Reichmuth 1997; Ahmed and Din 2009), mosquito (Gbolade *et al.* 2000), termites (Manzoor *et al.* 2011). In this study, the result shows that *T. castaneum* larvae were susceptible to the residual deposit of *O. sanctum* leaf. Also, the result shows that the larvicidal property of powder was dependent on the rate of application. In previous studies, Abubarkar *et al.* (2000) evaluated the pesticidal properties of light petroleum and methanol extracts of *Cyperus articulatus* and reported that the methanol extract showed more antifeedant property than the light petroleum extract, while both the extracts were observed to have similar repellant actions. Lale and Yusuf (2001) reported the potential of integrating varietal resistance of millet with *Piper guineense* seed oil to control infestation of stored millet seeds and processed products by *T. castaneum*. Similarly, Bamaiyi and Bolanta (2006) used the seed and leaf powders of *Khaya senegalensis* for the control of *T. casteneum* on sorghum grains of variety SAMSORG-17 and discovered that *K. senegalensis* seed powder performed better than the leaf powder in en-

Table 1 Effect of *Ocimum sanctum* powdered leaf admixed with milled flour on the mortality of *Tribolium castaneum*.

Concentration (g/5 g millet flour)	% Mortality
Control (0)	0.00 a
0.25	54.25 b
0.50	68.50 b

n = 6. Means followed by the same small alphabets within the column are not significantly different at 5% probability level (LSD).

Table 2 Effect of *Ocimum sanctum* leaf extracted with different organic solvents on the mortality of *Tribolium castaneum* in millet seeds.

Solvent	Concentration (ml/30 cm^2)		
	0	3	6
Petroleum	0.00 ± 0.00 a	79.00 ± 21.02 a	47.33 ± 10.35 a
Acetone	0.00 ± 0.00 a	92.00 ± 8.01 a	88.00 ± 12.01 b

n = 6 ± SE (standard error). Means followed by the same small alphabets within the column are not significantly different at 5% probability level (LSD).

Table 3 Effect of *Ocimum sanctum* leaf extracted with different organic solvents on the repellency against *Tribolium castaneum*.

Solvent	Concentration (ml/30 cm^2)		
	0.1	**0.2**	**0.3**
Petroleum spirit	80.00 ± 0.00 a (IV)	80.00 ± 0.00 a (IV)	80.00 ± 0.00 a (IV)
Acetone	40.00 ± 11.56 b (II)	53.33 ± 13.3 b (III)	86.66 ± 11.55 a (IV)

n = 6 ± standard error. Data in parenthesis are repellency classes. Class I = 0.1 – 20.00%, Class II = 20.1 – 40.00%, Class III = 40.1 – 60.00%, Class IV = 60.1 – 80.00%. Means followed by the same small alphabets within the column and row are not significantly different at 5% probability level (LSD).

suring adult mortality of *T. castaneum*, suppression of F_1 progeny emergence and prevention of percentage grain damage. Babarinde and Ogunkeyede (2008) reported that *Tephrosia vogelli* and *Laggera aurita* significantly suppressed fecundity. Babarinde and Adeyemo (2010) reported that *X. aethiopica* caused a significant increase in *T. castaneum* adult mortality with an interaction effect of dosage of extract and exposure duration.

The level of mortality observed in leaf powder was lower than what was observed in extracts. This suggests that the use of extracting solvent to extract plants make their active principles more readily available for the insects to pick up lethal doses during the course of their feeding and movement within the food material. In the study with different extracting solvents, acetone appeared to be better in the extraction of the active principles of *O. sanctum* than petroleum spirit. The difference in this efficacy can be attributed to the disparity in their polarities. Asawalam *et al.* (2007) had earlier reported that when different extracting solvents were used to extract *Piper guineense* seed, acetone gave higher bioactivity than other extracting solvents used. Also, Owusu *et al.* (2007) reported that methanol gave the highest yield and efficacy of *Zanthoxylum xanthoxyloides* (Lam.) extracts compared with other extracting solvents against *Sitophilus zeamais* and *Callosobruchus maculatus.*

The result of the repellency bioassay is presented in **Table 3**. Although petroleum spirit extract evoked greater repellent effect than acetone extract, the performance was not dosage-dependent, since the same percentage repellency (80%) was observed in 0.1-0.3 ml/30 cm^2. For acetone extract, repellency was dose-dependent with 86.66% repellency which was significantly different from percentage repellency observed in lower dosages of the extract. The fact that the extracts repelled *T. castaneum* adult indicates that the product has the tendency to prevent cross infestation of millet by non-resident population of *T. castaneum.* The repellent potentials of various botanical formulations against *T. castaneum* have been reported by several workers. Lale and Alaga (2001) reported the repellency of *P. guineense* against *T. castaneum* while Garcia *et al*. (2005) reported the toxic and repellent effects of *Baccharis salicifolia* essential oil on *T. castaneum*. Chaubey (2007) reported the repellent properties of of *Trachysoermum ammi*, *Anethum graveolens* and *Nigella sativa* essential oils against *T. castaneum*. The repellency of *Artemisia vulgaris* against *T. castaneum* has been documented (Wang *et al*. 2006). Also, Babarinde *et al*. (2010) compared *Ricinus communis* extracted using two different methods and reported that extract obtained by hydraulic extract tagged *Ricinus* hydraulic seed extract was more repellent against *T. castaneum* than *Ricinus* ethanolic seed extract with adults more repelled than larvae.

Eugenol (l-hydroxy-2-methoxy-4-allylbenzene) has been reported to be the active constituent present in *O. sanctum* (Prakash and Gupta 2005). With its various listed medicinal and condimental uses (Khanna and Bhatia 2003; Prakash and Gupta 2005; Kochhar *et al.* 2009; Khanna *et al*. 2010), it is not likely to cause any health hazards if incorporated into millet grains meant for human consumption.

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