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Stored Grains Protection Activity of *Ocimum Suave* Extracts and Compounds on Larger Grain Borer

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Abstract
Post-harvest pests cause serious losses to agricultural products in Africa. The pests consist mostly of beetles or moths and infest the grains at the crop stage or during the storage. Larger grain borer, Prostephanus truncatus (Horn), causes maize-grain losses during storage ranging from 30% to greater 40% of total production in 6 months. It reduces germination potential of maize grain and increases the grain's moisture content thus accelerating contamination by fungi and bacteria. The aim of this study was to determine the insecticidal extracts and compounds from Ocimum suave. Percentage mortality of the insects were recorded 1, 2, 14 and 21 days after treatment while adult emergence inhibition and weight loss prevention activities were recorded 42 days after treatment. The essential oil of the plant gave 75.4, 77.1 and 78.5% mortality after 7, 14 and 21 days respectively, while leaf powder gave 50.3, 63.7 and 66.6% mortality after 7, 14 and 21 days respectively. Betulinic acid gave 56.9 and 58.1% mortality 14 and 21 days after treatment respectively. The essential oil completely prevented the emergence of the insects and also completely prevented weight loss of maize grains. The findings from this study have provided scientific data to support the use of Ocimum suave to control insect pests. Further studies aimed at identification on plant materials which can be used in pest management are highly recommended.

***Keywords**: Post-harvest loss; insect pests, mortality; plant extracts; larger grain borer*

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I. Introduction

Postharvest loss leads to direct physical losses and quality losses that reduce the economic value of crop, or make it unsuitable for human consumption. The losses can be up to 80% of total production in severe cases [1]. These losses range between 20 to 40% in African countries, which is very significant considering the low the agricultural productivity in most regions of Africa [2]. Pests cause serious post-harvest losses to agricultural products and almost 9% in developed countries to almost 20% or more in developing countries [3]. Storage pests mostly consist of beetles or moths and infest the grains at the crop stage or during the storage. The majority of stored grain pests belong to the order of Coleoptera and Lepidoptera that accounting for almost 60 and 10% respectively [4]. The larger grain borer, *Prostephanus truncatus* (Horn), is a serious pest of stored maize and dried cassava roots, and attacks maize on the cob, both before and after harvest. Adults bore into the cassava or maize husks, cobs or grain, making holes and tunneling extensively converting the farm products in to dust as they tunnel [5]. In developing countries, the larger grain borer causes maize-grain losses during storage ranging from 30% to greater 40% of total production in 6 months [6, 7]. Apart from reducing germination potential of maize grain, the insects increases the grain's moisture content hence expediting contamination by fungi and bacteria. The fungi, particularly *Aspergillus flavus*, introduce aflatoxins in food products which are carcinogenic.

Pesticides such as organochlorines, organophosphates, carbamates, organoarsenicals and organothiocynates are available in the market for control of the beetles. However, some of the synthetic pesticides are detrimental to the environment and humans due to their persistence, accumulation and gradual absorption into the food chain [8]. Furthermore, insecticides are expensive and mostly out of reach of most smallholder farmers. There is an urgent need to develop safe alternatives that are of readily available, convenient to use and environmentally friendly. Previous studies have shown that plants synthesis important compounds that are toxic to pests and pathogenic microorganism [9-19]. Bioassay guided fractionation of plants extracts has led to identification of bioactive secondary metabolites including alkaloids, terpenoids, flavonoids, steroids and quinones [20-27]. Such compounds represent an important source of drugs in the process of developing new pharmacologically active compounds. The use of botanical for pests and disease control is preferred because they are environmentally friendly and non-toxic to non-targeted organisms [28-39]. In addition, chances of pests and pathogens developing resistance to botanical pesticides are highly unlikely [40]. Reports from previous studies show that *Ocimum* species possess antimicrobial, antidiabetic, hepato-protective, anti-inflammatory, anti-carcinogenic, cardio-protective and insecticidal activities [23, 25, 41, 42]. *Ocimum suave* Willd (Lamiaceae) is traditionally used to treat ulcers, fever, stomach ache, and bronchopneumonic infections [43]. Essential oil from the plant showed insecticidal activity against the brown ear tick - *Rhipicephalus appendiculatus* [44], housefly - *Musca domestica* [45] (Ojianwuna et al 2011) and *Sitophilus zeamais* [46]. The plant extracts also showed repellents activity against the larger grain borer [27, 47]. The present study reports the toxicity, adult emergence and weight loss prevention activities of *O. suave* against the larger grain borer.

II. Materials and Methods

Plant Materials

Ocimum suave leaves were collected from Kitambo region in Kenya. Sample identified was done at Maseno University Herbarium by comparison with authentic samples. The plant materials were chopped into small pieces, air dried and ground into fine powder using a mill. Powdered plant material (2 kg) was extracted sequentially with *n*-hexane, ethyl acetate and methanol by soaking the material in the solvent for seven days with occasional shaking. The mixture was filtered and the solvent evaporated using rotary evaporator to yield 49.5, 70.8 and 120 g of *n*-hexane, ethyl acetate and methanol extracts, respectively. The extracts were stored at 4°C in brown glass bottles.

Extraction of Essential Oil

Fresh leaves of *O. suave* (2 kg) were cut into pieces and distilled using Clevenger-type apparatus for six hours. The superior phase was collected from the condenser, dried over anhydrous sodium sulfate and kept in a refrigerator (4°C) for further tests.

Isolation of Compounds

Hexane extract (40 g) was dissolved in small amount of n-hexane and adsorbed onto silica gel for column chromatography. Fractionation of the extract using gradient of n-hexane-ethyl acetate afforded 200 fractions (20 ml each) whose composition were monitored by TLC using solvent systems n-hexane-ethyl acetate 9:1, 4:1 and 2:1. Fractions with similar TLC profiles were combined resulting into four pools (I-IV). Pool II (fractions 24-76, 18 g) contained two major spots and was further purified using medium pressure chromatography (pressure ≈ 1 bar), eluting with n-hexane-ethyl acetate (9:1 and 4:1) to give β-sitosterol (124 mg) and stigmasterol (88 mg). Pool III (fractions 77-143, 12 g) on subjected to repeated fractionation using n-hexane-ethyl acetate (4:1 and 3:1) yielded stigmasterol (78 mg), β-amyrin (84 mg) and lupeol (65 mg). Pool IV (fractions 144-200, 7.2 g) gave stigmasterol (24 mg) and lupeol (34 mg).

Ethyl acetate extract (40 g) was pre-adsorbed onto silica gel and chromatographed with n-hexane-ethyl acetate gradient to pure ethyl acetate to afford 133 fractions of 20 ml each. The composition of the fractions was monitored by TLC using hexane-ethyl acetate mixtures 4:1, 3:2 and 1:1. Fractions that exhibited similar TLC profiles were combined to constitute two major pools (V and VI). Pool V (fractions 33-79, 17 g) was further purified by chromatography using n-hexane- ethyl acetate (4:1) followed by the same solvent system in the ratio 3:2 to give β-amyrin (53 mg), lupeol (42 mg) and betulinic acid (96 mg). The remaining fractions (pool VI, 6 g) contained one major compound as shown by its TLC profile. The fraction was further purified by chromatography using n-hexane-ethyl acetate (3:2) followed by the same solvent system in the ratio 1:1 to yield betulinic acid (26 mg).

Mass Rearing of insects

Adult insects were obtained from infested maize grains purchased from local market and from this stock, new generation was reared on dry pest susceptible maize grains [48]. Two hundred insects of mixed sexes were introduced into a two liter glass jars containing 400 g weevil susceptible maize grains [49]. The mouths of the jars were then covered with nylon mesh held in place with rubber bands and the jars left undisturbed for 35 days for oviposition. Thereafter, all adult insects were removed through sieving and each jar was left undisturbed for another 35 days. Emerging adult insects were collected and kept in separate jars according to their age. Adults that emerged on same day were considered of the same age [50].

Adult Mortality Test

Toxicity of leaf powder, essential oil, crude extracts and compounds from the plant were tested against adult weevils as described by Ileke and Oni [51] with some modifications. The test samples were mixed with talc thoroughly and the dust was admixed with 20 g of maize held in 12 cm high x 6.5 cm diameter glass jars covered with ventilated lids. To ensure a thorough admixture, the grain was put in 12 cm high x 6.5 cm diameter glass jars, dust applied and top lid replaced. The grain was then swirled within the jar until a proper admixture was realized [52]. Twenty-three-day old unsexed insect pairs were then introduced into each dish and exposed to

treatments. Actellic dust was used as a positive control and all tests were done in three replicates. Maize weevils were considered dead when probed with sharp objects and there were no responses [51]. The number of dead insects in each vial was counted after 1, 2, 14 and 21 days after treatment to estimate maize weevil mortality as follows:
% Mortality = 100* (Number of dead insects) / (Total number of insect).

Data on percentage adult weevil mortality were corrected using Abbott's formula [53].
PT = (Po – Pc) / (100 - Pc)
Where PT = Corrected mortality (%); Po = Observed mortality (%); PC = Control mortality (%).

Growth Inhibition Assay

The test was done according to Ileke and Oni [51] with some modifications. Twenty (20) g of clean undamaged and uninfected corn grains were placed in 12 cm high x 6.5 cm diameter glass jars glass jars. Test materials were thoroughly mixed with the grains in each jar. Crude extracts and pure compounds were mixed with talc thoroughly before being applied to the grains [52]. A mixture of twenty-seven-day old unsexed maize weevils was introduced in each jar and covered with filter paper [50]. The female adults were allowed to oviposit on the seeds for 4 days. On day 5, all insects were removed from each container and the seeds returned to their respective containers. Progeny emergence (F1) was recorded at six weeks (42 days). The containers were sieved out and newly emerged adult weevils were counted [51]. At week six, the grains were reweighed and the percentage loss in weight was determined as follow:
% Weight loss = 100* (Initial weight - Final weight)/ Initial weight

III. Results and Discussion

Repeated chromatographic separation of *Ocimum suave n*-hexane and ethyl acetate extracts from leaves gave five namely lupeol, stigmasterol, β-sitosterol, β-amyrin, and betulinic acid. The structures of the compounds were determined using spectral analysis [54]. Leaf powder, essential oil, crude extracts and pure compounds isolated from *O. suave* were tested against adult weevils and the percentage mortality was recorded at 1, 2, 14 and 21 days after treatment. The essential oil which was the most toxic to the insects gave 75.4, 77.1 and 78.5% mortality after 7, 14 and 21 days respectively (Figure 1). The leaf powder gave 50.3, 63.7 and 66.6% mortality after 7, 14 and 21 days respectively. Among the crude extracts, ethyl acetate was the most toxic to the insects followed by hexane and methanol extract. The percentage mortality was 61.7, 55.4 and 43.2% for ethyl acetate, hexane and methanol extracts respectively at 21 days after treatment. For the pure compounds, betulinic acid was the most toxic the insects and it gave 56.9 and 58.1% mortality 14 and 21 days after treatment respectively. Lupeol and β-sitosterol gave 43.1 and 41.3% mortality respectively at 21 days.

The essential oil completely prevented the emergence of the insects after 42 days of exposure and the activity was comparable to that of actellic dust which was used as a positive control (Figure 2). The leaf powder, n-hexane, ethyl acetate and methanol extracts exhibited moderate growth emergence activity with 6.2, 8.1 and 5.7 as the number of the emerging insects respectively. For the pure compounds, betulinic acid and lupeol were the most promising and with the numbers of emerging insects being 7.3 and 13.7 respectively. In grain weight loss prevention test, the essential oil was the most promising extract and it completely protected the grains. The leaf powder, n-hexane, ethyl acetate and methanol extracts and betulinic acid also gave promising results.

The results were in agreement with findings from previous studies which reported insecticidal activity of extracts from Ocimum species [23, 45, 49]. Essential oil, leaf powder and extracts had been reported to exhibit growth inhibition and grain loss prevention activities against grain storage insect pests [46]. Findings from this study showed that extracts of *O. suave* have insecticidal activity against the lager grain borer which destroys maize and other agricultural produce both in the field and storage. This proves that insect pests could be managed using herbal extracts.

IV. Conclusions

The use of plant extracts for pest management is more acceptable since it is it has no adverse effects to humans and the environment compared to synthetic pesticides. Furthermore, plant extracts are renewable, readily available and chances of insects developing resistance are negligible. More studies aimed at testing and identification on plant materials which can be used in pest management is highly recommended.

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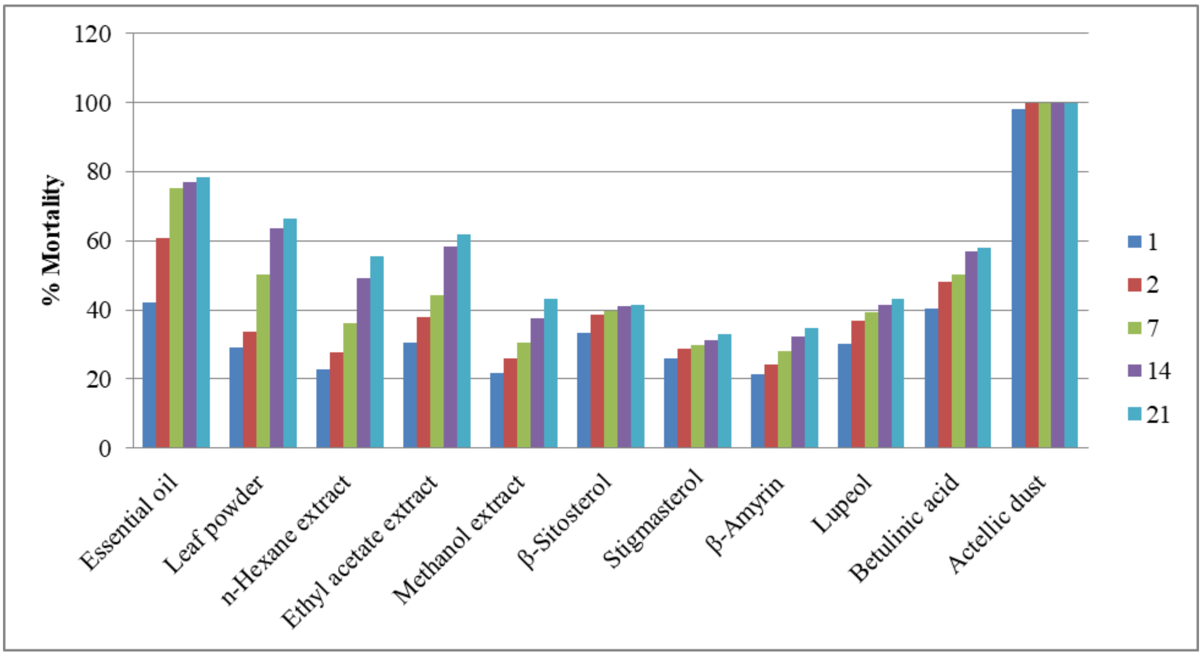

Figure no 1: Mortality of *Ocimum suave* extracts and compounds against the larger grain borer

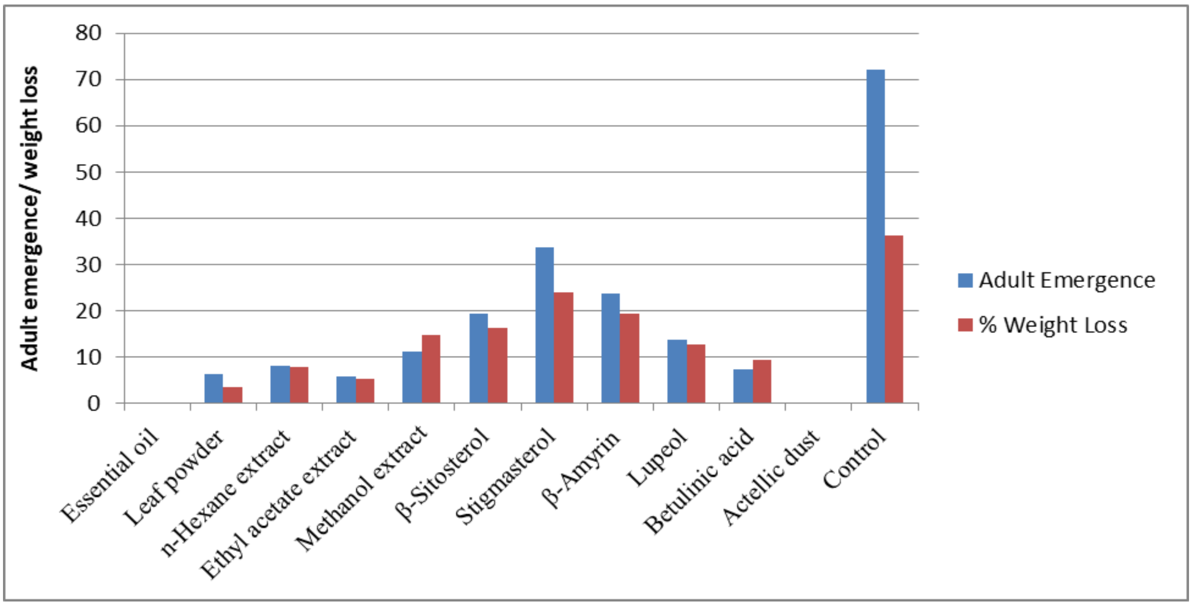

Figure no 2. Growth inhibition weight loss prevention activities of *Ocimum suave* extracts and compounds against the larger grain borer

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