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Repellent Effects of *Ocimum Suave* Extracts and Compounds against *Prostephanus Truncatus* Horn

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Abstract Insect pests cause significant loses to maize grains in storage. Larger grain borer (*Prostephanus truncatus* Horn) is one of the most important pests of stored maize. Synthetic pesticides are commercially available for managing the pests. However, some of the chemicals have been banned from use because of their adverse effects on environment and living organisms. Therefore, it is necessary the search for alternative control methods that are effective and environmentally friendly. This study determined the repellence activity of extracts and compounds from *Ocimum suave* for control of *P. truncatus*. Repellence activity was measured after exposing the insects to test materials for 2, 5, 24, 48 and 96 hours. Chromatographic analysis of extracts from *O. suave* yielded five compounds and were identified as β-sitosterol, stigmasterol, β-amyrin, lupeol and betulinic acid. The insects were repelled most by the essential oil (mean repellency = 4.61 cm) with repellence distances of 5.83 and 5.38 cm after 2-hours and 5-hours exposure durations respectively. Repellence activity of organic solvents extracts was in the order of *n*-hexane > ethyl acetate > methanol while that of isolated compounds was in the order of betulinic acid > lupeol > β-amyrin > β-sitosterol > stigmasterol. Findings from this study revealed that extracts of *O. suave* are effective against *P. truncatus* which destroy maize and other cereal grains both in the field and in storage.

Keywords Stored maize, Insect pest, *Prostephanus truncatus, Ocimum suave,* Repellence

1. Introduction

Food security is one of the major challenges for the 21st century especially in Africa. Maize (Zea mays L.) which is extensively grown in America, Asia and Africa, is the largest staple crop produced worldwide, and contributes over 20% of as food calories in parts of Africa and Mesoamerica [1]. The production of maize is seasonal but consumer needs extend throughout the year. Therefore maize grains must be properly stored to ensure a continuous food supply throughout the year. However, insect pests cause significant loss of maize in Africa Sub Saharan Africa [2]. Infestation by post-harvest pests commences in the field but most damage occurs during storage [3]. The beetle, larger grain borer (*Prostephanus truncatus* Horn) (Coleoptera; Bostrichidae), is one of the most important pests of maize. The pest causes stored maize-grain losses ranging from 30% to greater than 40% of total production in six months [4]. The insect reduces maize germination, increases moisture content and accelerates the storage contamination by fungi and bacteria [5]. These fungi, particularly Aspergillus flavus introduce aflatoxins which are carcinogenic.

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Synthetic pesticides are available for controlling the insect. However, some of chemicals have adverse effects on environment and humans since they persist and accumulate in the environment and are gradually absorbed into the food chain [6]. In addition, the available insecticides are expensive and mostly out of reach of most smallholder farmers [7]. There is a need to search for alternative pesticides that are effective and environmentally safe [8,9]. Plants are rich in secondary metabolites some of which are toxic to pests and pathogenic microorganism [10-15]. The use of botanical for pests and disease control is preferred over the conventional synthetic pesticides because they are safe and non-toxic to the environments [16-21]. In addition, chances of pests and pathogens developing resistance to botanical pesticides are highly unlikely [22]. Plants from the genus *Ocimum* have been reported to exhibit antimicrobial, adaptogenic, antidiabetic, hepato-protective, anti-inflammatory, anti-carcinogenic, cardio-protective and insect repellent activities [23-26]. *Ocimum suave* Willd (Lamiaceae) is traditionally used to treat ulcers, fever, stomach ache, and bronchopneumonic infections [27]. Essential oil from the plant showed insecticidal activity against the brown ear tick - *Rhipicephalus appendiculatus* [28], the lesser grain borer - *Rhyzopertha dominica* [29], housefly - *Musca domestica* [30] and *Sitophilus zeamais* [26] The present study was conducted to evaluate the grain protection activity of *O. suave* against *Prostephanus truncates* in stored maize.

2. Materials and Methods

2.1. 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℃ in brown glass bottles.

2.2. 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℃) for further tests.

2.3. 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) [13]. 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).

2.4. Mass Rearing of *P. truncatus*

Adult larger grain borer were obtained from infested maize grains purchased from local market and from this stock, new generation was reared on dry pest susceptible maize grains [31]. Two hundred insects of mixed sexes were introduced into a two liter glass jars containing 400 g weevil susceptible maize grains [32]. 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 [33].

2.5. Repellency Test

Repellency test was done according to Mwangangi and Mutisya [31] with some modifications. Transparent plastic tubing, 13 cm long x 1.3 cm diameter was used as test cylinders. Each test cylinder was plugged at one end with cotton ball containing the test material (leaf powder, essential oil, crude extracts or compounds) from the stem bark of *O. suave* while the other end was plugged with clean cotton ball which served as control. Actellic dust was used as a positive control. Ten-three-day old unsexed test insects were introduced at the middle of each test cylinder through a hole at the middle portion of the cylinder (0.0 cm) and let to move in any direction of their choice with scoring of distance moved measured in cm using a ruler. The score time was 2, 5, 24 and 96 hours after exposure. Data obtained from the experiments were subjected to analysis of variance (ANOVA) and means were separated by least significant difference (LCD) at five percent significant level.

3. Results and Discussion

3.1. Phytochemical Studies

Chromatographic fractionation of *n*-hexane and ethyl acetate extracts from *O. suave* leaves afforded five compounds which were identified as β-sitosterol, stigmasterol, β-amyrin, lupeol and betulinic acid. Structure determination of the compounds was earlier reported [34-36].

3.2. Repellent Activity

Repellence activity of leaf powder, essential oil, extracts and compounds from was measured against *P. truncatus* after exposing the insects to the test materials for 2, 5, 24, 48 and 96 hours and the results are presented in Table 1. The distance moved by the insects varied significantly ($P<0.05$) depending on the test material used and duration of exposure. For most test materials used, the repellence activity peaked within 5-hours of exposure. The insects were repelled most by the essential oil (mean repellency=4.61 cm) with repellence distances of 5.83 and 5.38 cm after 2-hours and 5-hours exposure durations respectively. Leaf powder of *O. suave* was the second best repellent after the essential oil (mean repellency=3.90 cm) and repelled the insects most after 5-hours of exposure. For the crude organic extracts, the repellence activity observed was in the following order: n-hexane > ethyl acetate > methanol. n-Hexane extract was the most repellent with a mean repellent of 3.84 cm and the highest repellence activity within 5-hour exposure period (repellence distance=4.81 cm). All the compounds isolated showed repellence activity which varied significantly ($P<0.05$) from one compound to another. However, the repellence activity of all the compounds was lower compared to the essential oil, leaf powder, *n*-hexane and ethyl acetate extracts. The repellence activity of the compounds was in the following order: betulinic acid > lupeol > β-amyrin > β-sitosterol > stigmasterol, with mean repellence of 2.95, 2.36, 2.28, 1.86 and 1.82 cm respectively.

These results are in agreement with previous studies which reported insect repellence activity of powdered plant parts, organic solvent extracts and compounds from *Ocimum* species [24,25,29,30]. Essential oil, leaf powder and extracts had been reported to repel maize weevil (*Sitophilus zeamais*) and the bioactive principles were found to be β-sitosterol, stigmasterol, β-amyrin, lupeol and betulinic acid [26]. Findings from this study revealed that extracts of O. suave have repellent, activity against *P. truncatus* which destroy maize and agricultural produce both in the field and storage. This proves that insect pests could be managed using herbal extracts as had also been observed in other studies [37-40].

Table 1. Repellent Activity of Extracts and Compounds from *Ocimum. suave* Against *P. truncatus*

Repellant Materials	Repellence*					
	Exposure Duration in Hours					
	2	5	24	48	96	Mean Repellency
Essential oil (0.2 ml)	5.83±0.15	5.38±0.21	4.48±0.12	3.83±0.32	3.53±0.12	4.61
Leaf powder (2 g)	3.84±0.06	4.41±0.15	4.25±0.15	3.64±0.12	3.37±0.23	3.90
n-Hexane extract (50 mg)	4.63±0.13	4.81±0.12	3.86±0.31	3.06±0.12	2.82±0.12	3.84
Ethyl acetate extract (50 mg)	3.27±0.15	3.83±0.06	3.44±0.14	2.78±0.21	2.35±0.11	3.13
Methanol extract (50 mg)	3.08±0.12	2.67±0.16	2.47±0.12	2.35±0.15	1.93±0.10	2.50
β-Sitosterol (2 mg)	1.85±0.10	2.29±0.10	1.93±10	1.67±0.16	1.56±0.15	1.86
Stigmasterol (2 mg)	2.03±0.10	1.88±0.21	2.13±0.20	1.57±0.13	1.47±0.10	1.82
β-Amyrin (2 mg)	2.27±0.12	2.36±0.16	2.64±0.21	2.25±0.16	1.89±0..12	2.28
Lupeol (2 mg)	2.67±0.12	2.91±0.12	2.48±0.15	2.09±0.14	1.67±0.15	2.36
Betulinic acid (2 mg)	3.17±0.25	3.47±0.18	3.23±0.06	2.54±0.12	2.33±0.13	2.95
Actellic dust (2 mg)	4.16±0.13	4.56±0.15	4.73±0.23	3.82±0.13	2.16±0.21	3.89
LSD, $P \leq 0.05$	0.13					

* Mean (±SD) distance (in cm) values of weevil away from the tube center (n=3)

4. Conclusions

Use of plant extracts as pesticides is environmentally safe compared to the chemicals. In addition, plant extracts are readily available, renewable and chances of insects developing resistance are negligible. Further studies aimed at investigating synergism and antagonism effects of the pure compounds are necessary to determine the combinations with best activities.

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