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Full Length Research Paper

Essential oil of *Ocimum grattissimum* (Labiatae) as *Sitophilus zeamais* (Coleoptera: Curculionidae) protectant

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Ocimum grattissimum **L. (Labiatae) leaves are widely eaten as a vegetable in Nigeria, and in the eastern parts, are traditionally used in post-harvest protection and relieving stomach aches. The effect of the essential oil of *O. grattissimum* leaves on *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) was assessed for repellency, mortality, progeny emergence and maize damage in the laboratory. The oil was found to be moderately repellent to the maize weevil and induced high mortality in the weevils. In addition, grains treated with the essential oil showed significant reduction in the number of progeny derived from surviving *S. zeamais*. There was no observable feeding damage on grains treated with the highest dosage of the essential oil extract. Gas chromatography-linked mass spectrometry (GC-MS) and GC co-injections with authentic samples showed the presence of the following major constituents: thymol (32.7%), paracymene (25.4%), γ-terpinene (10.8%), *β*-selinene (4.5%), phellandrene (3.9%) and *β*-myrcene (3.1%). The results provide a scientific rationale for the use of the plant in post-harvest protection.**

Key words: *Ocimum grattissimum*, essential oil, *Sitophilus zeamais,* maize, repellency.

INTRODUCTION

Maize (*Zea mays* L.) is one of the foremost cereals cultivated in the world today (Purseglove, 1975; Rouanet, 1992) and it is a major source of dietary carbohydrate in the tropics (Wudiri and Fatobi, 1992). The maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera:Curculionidae), is a serious pest of maize and it is capable of developing on all other cereal grains and cereal products (Walgenbach and Burkholder, 1986; Tipping et al., 1987). Initial infestation of maize grain occurs in the field just before harvest and insects are carried into the store where the population builds up rapidly (Appert, 1987; Adedire and Lajide, 2003). The huge post-harvest losses and quality deterioration caused by this pest is a major obstacle to achieving food security in developing countries (Rouanet, 1992).

The efficient and effective control of storage insects like *S. zeamais* has centered mainly on the use of synthetic insecticides (Menn, 1983; Redlinger et al., 1988). However, many problems are associated with these chemicals, such as the development of insect resistance, toxic residues in food, workers' safety, and high cost of procurement (Sighamony et al., 1990). These problems have necessitated search for alternative eco-friendly insect pest control methods amongst which are the use of botanical pesticides (Cobbinah and Appiah-Kwarteng, 1989; Hassanali et al., 1990; Niber, 1994; Jembere et al., 1995; Bekele et al., 1996; Lajide et al, 1998; Asawalam and Adesiyan, 2001; Asawalam and Adesiyan, 2002; Bekele, 2002; Asawalam and Arukwe,

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2004).

Ocimum grattissimum L. (African Curry plant) is a low growing shrubby species in the family Labiatae. All the species in the genus contain strongly scented essential oils. It is grown as pot herbs for local medicines and exists in diversity of forms and cultivars (Schippers, 2000). The powders and essential oils of *Ocimum* species have been widely used for control of insect pests especially storage insect pests (Oparaeke et al., 2002). It is a slightly hairy annual with much branched angular stems carrying opposite, ovate leaves which are usually less than 1 cm long and borne on fairly long petioles. In eastern Nigeria, it serves as a source of stimulant and condiment in soup. Medicinally, it is used for the treatment of stomach ache, sores and in management of babies cord after delivery among the Igbos of Nigeria (Ijeh et al., 2004).

The aim of the present study was to document the biological effects of the essential oil on the maize weevil and to analyse its composition in order to assess its relative safety.

MATERIALS AND METHODS

Sitophilus zeamais culture

S. zeamais was cultured in the laboratory at 27 ± 2ºC, 60 - 65% R.H and 12 h : 12 h light : dark regime. The food media used was insecticide-free whole maize grains purchased from a local market (Dikomba) outside Nairobi in Kenya. Fifty pairs of *S. zeamais* were placed in 1 L glass jar containing 400 g of maize grains. The jars were then covered with nylon mesh held in place with rubber bands. Grains were disinfested in the oven at 40°C for 4 h (Jembere et al., 1995) and kept in the laboratory before use.

Plant collection and isolation of their essential oil

O. grattissimum leaves were collected from Umudike, Nigeria in January 2006. The identity of the plant was confirmed at the herbarium of Michael Okpara University of Agriculture, Umudike, Nigeria, before using them for the present study. Plants were air-dried in a well-ventilated area for five days before extraction. Voucher specimens are kept at the University herbarium.

The essential oil was extracted by steam distillation using Clavenger apparatus (Guenter, 1949). The condensing oils were collected in n-hexane (Aldrich HPLC grade) and the solution was filtered and exposed to anhydrous sodium sulphate to remove any traces of water. Hexane was then removed by distillation at 60°C from 'Contes' Short Path distillation apparatus and the oil collected and weighed, and stored in small amber-coloured vials.

Analysis of essential oil

Gas chromatographic (GC) analyses were performed on a capillary gas-chromatograph Hewlett Packard (HP) 5890 Series II equipped with a split-less capillary injector system, 50 m x 0.20 mm (i.d.) cross-linked with HP-ultra 1methylsilicone 0.33 µm (film thickness) capillary column, and Flame Ionization Detector (FID) coupled to HP 3393A Series II integrator. The integrator was used to calculate the peak areas. The carrier gas was nitrogen at a flow rate of 0.84 ml/min. The temperature programme comprised of an initial temperature of 40°C (0 min) to 90°C at 7°C/min, a hold at this temperature for 5 min, then to 115 at 3°C/min followed by another hold for 5 min, and finally to 280°C at 4°C/min where it was maintained for 20 min.

Gas chromatography–linked mass Spectrometry (GC-MS) analysis was carried out on a HP 8060 Series II gas chromatograph coupled to VG Platform II Mass Spectrometer (manufactured by Micromass, UK, formerly known as VG Biotech). The MS was operated in the Electron impact mode (EI) at 70 eV and an emission current of 200 µA. The temperature of source was held at 180°C and the multiplier voltage at 300 V. The pressure of the ion source and MS detector were held at 9.4×10^{-6} and 9.4×10^{-6} mbar, respectively. The MS had a scan cycle of 1.5 s (scan duration of 1 s and inter-scan delay, 0.5 s). The mass and scan range was set at m/z 1 - 1400 and 38 - 650, respectively. The instrument was calibrated using heptacosafluorotributyl amine, $[CF_3(CF_2)_3]_3N$, (Apollo Scientific Ltd., UK). Column (film thickness 0.5 µm) temperature was programmed as in the case of GC analysis. All GC-MS analyses were made in the splitless mode with helium as the carrier gas. Preliminary identification of constituents was based on computer matching components of mass spectral data against the standard Wiley and NIST library spectra, constituted from spectra of pure substances and components of the known essential oils, and literature MS data. They were confirmed by their GC retention time comparison with those of reference compounds, peak enhancement as well as co-injection /co-elution with authentic samples. The samples used were obtained from Aldrich Chemicals UK. Relative proportion of the essential oil was computed in each case from GC-MS peak areas.

Repellency

Repellent action of the essential oil against *S. zeamais* was assessed in a choice bioassay system at 27 ± 2°C and 60 - 65% R.H. previously reported by Bekele et al. (1996). It consisted of two 1 L glass jars connected together at their rims by means of a 30 x 10 cm nylon mesh tube. A 5.0 cm diameter circular hole was cut at the middle of the mesh for the introduction of test insects. 250 g of maize were put into each glass jars. Grains in one jar were treated with the essential oil at the rate of 0.012, 0.06 and 0.3% (30, 150 and 750 mg/250 g) while untreated grains in the other jar acted as control. Twenty-five adults of *S. zeamais* were introduced into the nylon mesh tube through the circular hole by means of a 5 cm diameter funnel. The number of insects present at the control (N_C) and treated (N_T) jars were recorded after 1-hour exposure. All repellency assays were replicated four times. Percent repellency (PR) values were computed from the formula:

$$PR = [N_C - N_T / N_C + N_T] \times 100.$$

PR data were analyzed using ANOVA after arcsine transformation.

Effect of essential oil on mortality

Essential oil was applied to the grains at the rate of 0.012, 0.06 and 0.3% dissolved in 10 ml of 95% n-hexane and shaken thoroughly to ensure uniform distribution over grain surface. Treated grains were kept for 24 h to allow the hexane to evaporate completely before bioassays were conducted. Two blank controls were run concurrently consisting of hexane treated grain and untreated grain. Ten pairs of 5/7-day-old *S. zeamais* adults

Table 1. Biological activity of different doses of *O. grattissimum* essential oil against *S. zeamais*.

Treatments	Mean repellency (%)	Mean no of F1 progeny	Mean weight loss (%)
30 mg (0.012%)	36.5 ± 1.6^{c}	119.25 ± 2.6^{c}	14.8 ± 1.6^{c}
150 mg (0.06%)	58.75 ± 2.1^{b}	82.75 ± 3.5^{b}	8.35 ± 2.1^{b}
750 mg 0.6%)	77.5 ± 1.7^{a}	0 ± 0^{a}	0 ± 0^{a}
Hexane	0 ± 0^{d}	152.75 ± 3.2^{d}	22.1 ± 1.9^{d}
Untreated	0 ± 0^{d}	156.28 ± 2.6^{d}	23.15 ± 1.8^{d}

Values are mean ± SE (Standard error).
Treatment means (average of four replicates) within each column followed by the same letter are not significantly different from each other at 5% level of probability according to Student –Newman – Keuls (SNK) test.

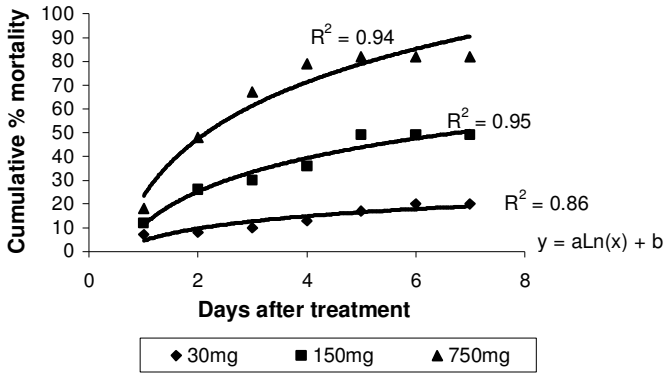

Figure 1. Cumulative mean (%) mortality of *S. zeamais* in maize grains treated with different concentrations of *O. grattissimum* essential oil.

were introduced into each of the treated and untreated jars. The jars were covered with nylon mesh held with rubber bands. Each treatment was replicated four times. The experiment was arranged in completely randomized design in the laboratory.

Number of dead insects in each vial was counted daily for 7 days to estimate maize weevil mortality. The percentage mortality was corrected using Abbott's (1925) formula

$$PT = (P_o - P_c) / (100 - P_c)$$

Where P_T = corrected mortality (%), P_o= observed mortality (%), P_c = control mortality (%).

Effect of essential oil on progeny production

10 pairs of *S. zeamais* adults were introduced into each treated (at the same doses as above) and untreated grains. Insects subsequently emerging were counted to estimate F_I progeny production. Counting was stopped after 33 days to avoid overlapping of generation.

Effect of essential oil on maize damage by the weevil

Damage assessment was carried out on treated (at the 3 doses as above) and untreated grains. Samples of 100 grains were taken from each jar and the number of undamaged and damaged (grains with characteristic holes) grains were counted and weighed. Percentage weight loss was calculated using the formula:

$$\text{Weight loss (\%)} = [UaNv - (U+D)] / UaN \times 100$$

Where U = weight of undamaged fraction in the sample, N = total number of grains in the sample, Ua = average weight of one undamaged grain, D = weight of damaged fraction in the sample (FAO, 1985).

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) using a general linear model procedure (SAS, 2002) and means were separated by Student Newman-Keuls (SNK) test at $P < 0.05$.

RESULTS

Repellency effect of the essential oil on *S. zeamais*

The mean repellency values of the essential oil at the three dose levels against *S. zeamais* are provided in Table 1. All the dosages were repellent to *S. zeamais* in a dose-response manner. Analysis of variance indicated significant differences ($P < 0.05$) between weevil responses to the three dosages tested.

Adult mortality in grains

Figure 1 also shows the cumulative mean percentage mortality of *S. zeamais* in maize grains treated with different concentrations of *O. grattissimum* essential oil. All treatments with essential oil showed significant level of mortality with the highest dose (corresponding to 0.3%) inducing 82% mortality after 7 days treatment. There was no significant mortality in the untreated controls.

Effect of essential oil on progeny production

The number of progeny produced by *S. zeamais* in un-

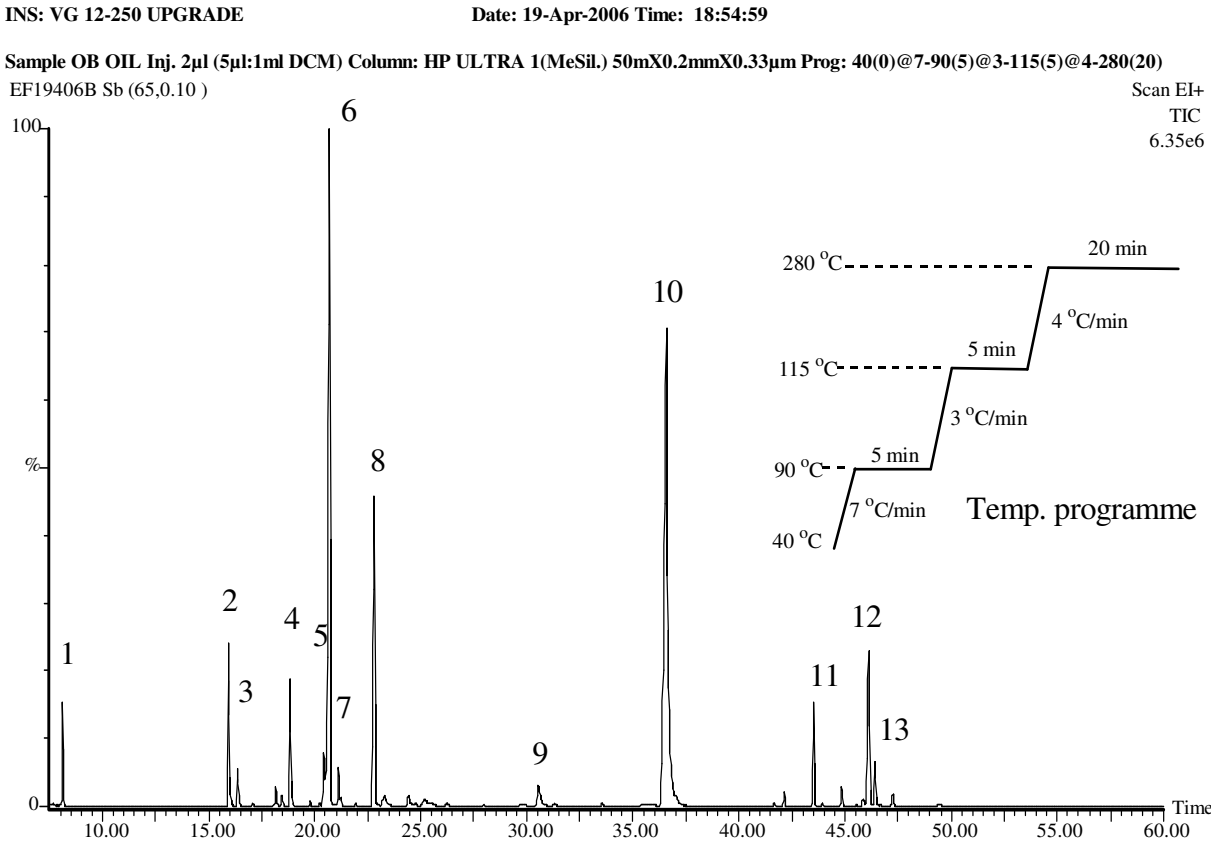

Figure 2. GC-MS chromatogram for *O. grattissimum* essential oil

untreated grains and grains treated with different concentrations of *O. grattissimum* essential oil is shown in Table 1. Significantly lower number of F_1 progenies was produced by *S. zeamais* in the treated grains. No progeny was produced in grains treated with the highest dose.

Effect of the essential oil on weight loss of grains

Weight loss caused by *S. zeamais* to treated and untreated grains are shown in Table 1. Weight loss caused by *S. zeamais* was significantly ($P < 0.05$) higher in the control compared with grains treated with essential oil. There was no significant weight loss in the grains treated with the highest dose of the oil.

Essential oil composition

The analysis of the oil revealed a complex mixture of constituents (Figure 2). Components present in >1% were identified by comparison of the mass spectral fragmentation pattern with those in Wiley and NIST library and by GC-co injection with authentic samples (Table 2).

DISCUSSION

The essential oil of the leaves of *O. grattissimum* was significantly ($P < 0.05$) repellent and toxic to the maize weevil. It joins a series of other essential oils with similar effects on this post-harvest pest and provides a scientific rationale for their use in traditional post-harvest practices (Hassanali et al., 1990; Weaver et al., 1991; Jembere et al., 1995; Bekele et al., 1996; Bekele et al., 1997; Bekele and Hassanali, 2001; Renault-Roger et al., 1993; Bouda et al., 2001). In the present study, the toxic action of the oil is also reflected in the growth and development of eggs, which corresponds to decreased numbers of progeny that emerged in treated grains. Similar results were obtained in experiments that involved treating maize with the essential oil of *Ocimum kenyense* (Bekele et al., 1997). These multiple effects of the essential oils and potential for their local availability make them attractive

Table 2. Major identified constituents of *O. grattissimum* essential oil and their relative proportion in the oil

GC peak number	Component	Formula	Peak area (% Composition)	Retention time
1	Heptane	C_7H_{16}	1.7	8.1
2	Phellandrene	$C_{10}H_{16}$	3.9	15.9
3	α-Pinene	$C_{10}H_{16}$	0.9	16.4
4	β-Myrcene	$C_{10}H_{16}$	3.1	18.9
5	(+) -2- carene	$C_{10}H_{16}$	1.7	20.4
6	Paracymene	$C_{10}H_{14}$	25.4	20.7
7	Limonene	$C_{10}H_{16}$	1.2	21.1
8	γ-Terpinene	$C_{10}H_{16}$	10.8	22.6
9	Terpinene-4-ol	$C_{10}H_{16}O$	1.3	30.5
10	Thymol	$C_{10}H_{14}O$	32.7	36.6
11	Isocaryophyllene	$C_{15}H_{24}$	3.4	43.6
12	β-Selinene	$C_{15}H_{21}$	4.5	46.1
13	α-Selinene	$C_{15}H_{21}$	1.2	46.4

candidates in upgrading traditional post-harvest protection practices.

The repellent effect associated with these relatively volatile oils (in addition to their toxic action) may have an important implication in traditional post-harvest storage systems. The insect's response to the terpenoid blend is released immediately, and if the storage system allows the pests to depart from treated grain, many could survive and return to infect the grain when the effects of the oil have subsided. Thus, the design of these systems may need to take this into account in the optimum deployment of the oils in post-harvest protection.

The major constituent of the essential oil was found to be thymol, but the major constituent of *O. kenyense* essential was found to be 1,8-cineole (Obeng-Ofori et al., 1997) and that of *O. basilicum* oil (Ruberto et al., 1991), was found to be dihydrotagetone. Although pure 1,8-cineole at appropriate doses has been shown to be intrinsically toxic to post-harvest pests, in a study with different blends of *O. kenyense* constituents in proportion they occur in the essential oil, the terpene ether was found to act in synergism with other constituents of the oil (Bekele and Hassanali, 2001). Thus, it would be interesting to see if the major hydrocarbon and alcohol constituents of *O. grattissimum* essential oil also make similar contribu-tion to the natural blend in conferring its repellent and toxic properties to the maize weevil.

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