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Insecticidal activities and chemical composition of the essential oil from *Tarchonanthus camphoratus* (L.), leaves against *Sitophilus zeamais* Motschulsky, and *Sitophilus oryzae* (L.)

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The essential oil of *Tarchonanthus camphoratus* dry leaves growing in Kwa-Zulu Natal, South Africa was obtained by hydrodistillation and evaluated for its repellent effect, contact and fumigation toxicity against both *Sitophilus zeamais* and *Sarocladium oryzae.* Chemical composition of the essential oil was analysed by gas chromatography mass spectrometry (GC/MS). The study revealed that the essential oil of *T. camphoratus* had no contact and fumigation toxicity against stored insect pests, *S. zeamais* and *S. oryzae*. The oil, however, showed good repellent activity of over 50% after 24 h for all the concentrations used on both *S. zeamais* and *S. oryzae*. A total of 27 compounds accounting for 73% of the total oil composition were identified of which sesquiterpene hydrocarbons, (59.18%), were the most dominant. These results suggest that the essential oil of *T. camphoratus* could be considered a potential control agent of stored grain pests as a repellent.

Key words: Essential oil, chemical composition, *Tarchonanthus camphoratus, Sitophilus zeamais, Sitophilus oryzae,* fumigation, repellency, toxicity.

INTRODUCTION

Sitophilus zeamais Motschulsky and *Sitophilus oryzae* (L.) are pests of stored grains capable of surviving in extreme cold and hot temperatures and hence found all over the world (Walgenbach and Burkholder, 1986). Although they are primarily associated with maize and rice, *S. zeamais* and *S. oryzae* are capable of

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developing on all cereal grains and cereal products (Walgenbach and Burkholder, 1986). They are internal feeders that not only cause damage to the grains but also promote secondary insect pest and fungal infestations that further affect the quality and quantity of grains (Gupta et al., 1999). Currently, the control of stored product insects relies heavily on the use of non-natural insecticides and fumigants, which has led to problems such as environmental pollution, pest resurgence, resistance to pesticides and lethal effects on non-target organisms in addition to direct toxicity to the users (Zettler and Arthur, 2000). Plant derived natural products are considered a potential alternative to these toxic and environmentally unsustainable compounds. Because they represent a rich source of bioactive chemicals it was hypothesized that plants native to South Africa could provide alternatives to currently used insect control agents. More than 2,000 plant species have been found to possess insecticidal activity with the most well known botanical pesticides being pyrethrum, neem, rotenone, nicotine and plant essential oils (Philogene et al., 2005; Isman, 2006). Essential oils have been shown to control stored product pests by fumigant activity, contact insecticides and as repellents. Additionally, these bioactive plant secondary metabolites do affect insect growth rate and oviposition (Denloye et al., 2011; Chen et al., 2011; Stefanazzi et al., 2011).

The camphor bush, *Tarchonanathus campharatus* (L.), (family Asteraceae) is a shrub of reaching six meters in height and occurs in a wide range of habitats (van Wyk et al., 1997). The strongly scented tree of *T. campharatus* has many medicinal applications in traditional healing in South Africa, such as smoking the leaves or drinking infusions or decoctions. The infusions and tinctures of the leaves are used for abdominal pain, headache, toothache, asthma, bronchitis and inflammation and smoke from the fresh or dried plant is inhaled for rheumatism (Hutchings and Van Staden, 1994). In East Africa, the dry leaf infusion is drunk for tapeworm, the leaves are put underarm as perfume and to prevent tiredness and they are used for the control of bedbugs (Anonymous, 2005). The plant shows powerful insect repellent action (Omolo et al., 2004) and wild animals living in the areas where *T. camphoratus* grows, particularly Cape buffaloes and black rhinoceri, rub themselves against the leaves to deter mosquitoes and flies. The plant also seems to drive away tse-tse fly, a pathogenic agent of trypanosomiasis (Anonymous, 2005).

The purpose of this study was to determine the insecticidal activities of the essential oil of the dry leaves of *T. camphoratus* under laboratory conditions against *S. zeamais* and *S. oryzae*. *T. camphoratus* was selected as a model for this study based on its broad spectrum use in traditional medicine and preliminary reports of its insecticidal activity (Omolo et al., 2004; Anonymous, 2005).

MATERIALS AND METHODS

Plant material

Fresh materials of *T. camphoratus* were collected from Sangoyana in the northern part of Kwa-Zulu Natal province, South Africa in March, 2010. The plant was identified by the local people during the time of collection and further identified by Mrs N.R Ntuli in the Department of Botany, University of Zululand. A voucher specimen, (NSKN 1), was deposited at the University of Zululand herbarium.

Extraction of the essential oil

Air-dried leaves were subjected to hydro-distillation using a Clevenger-type apparatus (British Pharmacopia, 1980). The essential oil was collected 4 h after boiling, weighed and kept at 4°C in sealed glass vials before analysis and bioassay.

Determination of the insecticidal activity

Rearing of test insects

Adults of *S. zeamais* and *S. oryzae* were obtained from a colony maintained by the Plant Protection Research Institute, Pretoria. South Africa. These were mass reared on whole maize grains in 5 L glass jars in a controlled chamber, at 28 ± 20°C and 56 to 65% Relative humidity in the Department of Agriculture, University of Zululand. Newly emerged, one week old insects were used in the bioassay (Odeyemi et al., 2008).

Fumigant toxicity of the essential oil

The fumigation chambers consisted of 500 ml glass jars with screw-on lids. For the bioassay, solutions of 0, 5, 10, 20, 30 and 40µl of the oil were each diluted with 1ml hexane to correspond to concentrations of 0, 10, 20, 40, 60 and 80 µl/L air. One ml of each concentration was then separately applied to 7 mm discs of WhatmanNo.1 filter paper, air-dried for 10 min and placed at the bottom of the jars. Twenty, one-week old, adult insects were placed on muslin cloths (21 x 29 mm) each with 40 g whole maize grains. The cloths were tied closed with rubber bands and hung at the centre of the jars, which were then sealed with air-tight lids. There were four replicates for each concentration. Fumigation was carried out for 24 h after which the insects were transferred from the fumigation chambers onto clean maize, and mortality was checked daily for 28 days (Tapondjou et al., 2005).

Contact toxicity of the essential oil

The contact effect of the essential oil of *T. camphoratus* on the adults of *S. zeamais* and *S. oryzae* was investigated (Tapondjou et al., 2005). Maize grains were treated with concentrations of 0, 25, 50, 100, 200 and 300 µl of essential oil in 1 ml hexane. The different concentrations of the oil were mixed with 40 g of maize in 500 ml glass jars, corresponding to concentrations of 0, 0.625, 1.25, 2.5, 5.0 and 7.5 µl/g of maize grain respectively. These were thoroughly stirred to allow for homogeneity of the oil on the treated grains. Treated samples were air dried for an hour in order to get rid of the solvent. The grains were then infested with twenty, one-week old, *S. zeamais* or *S. oryzae* adults per jar and each jar was covered with a cotton mesh held in place by cover rims. There were four replicates per treatment. Insect mortality was checked daily for 28 days.

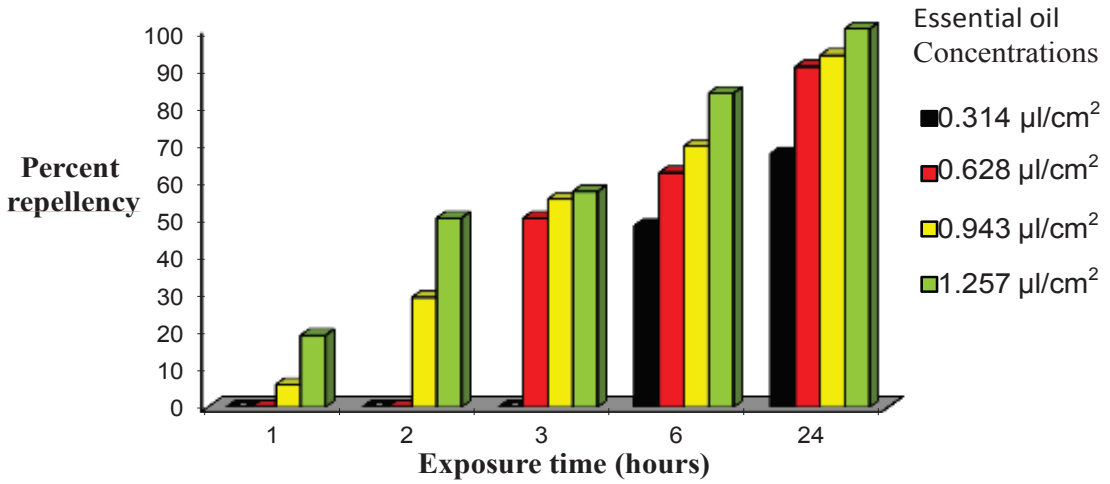

Figure 1. Plot of percent repellency against time of exposure, *S. zeamais*.

Repellency tests

The repellent effect of *T. camphoratus* essential oil against *S. zeamais* and *S. oryzae* was studied using a modified area preference method (Tapondjou et al., 2005). The test area consisted of a 9 cm Whatman No.1 filter paper cut into two halves. Different oil concentrations were prepared by diluting 10, 20, 30 and 40 µl of the oil in 1ml hexane and these corresponded to concentrations of 0.314, 0.628, 0.943 and 1.257 µl of oil/cm^2 of the filter paper respectively. The other half was treated with 0.5 ml hexane alone and this served as a control. Both essential oil treated and hexane treated filter paper halves were air-dried under a fan to evaporate the solvent completely. With the aid of a clear adhesive tape, both halves were later joined together into full discs and placed in 9 cm glass Petri dishes. Twenty one-week old, unsexed adult insects were released at the centre of the rejoined filter paper disc and the Petri dish was covered. Each treatment was replicated four times for each *S. zeamais* and *S. oryzae*. The number of insects present on the control and on the treated areas of the filter paper was recorded after 1, 2, 6, 4 and 24 h. Percentage repellency (PR) was calculated as follows (Nerio et al., 2009):

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

Nc was the number of insects on the untreated area after the exposure interval and Nt was the number of insects on the treated area after the exposure interval. The mean number of insects on the treated portion of the filter paper was compared with the number on the untreated portion. Results were presented as the mean of percentage repellency ± the standard error.

Statistical analysis

Data was analysed using the QED statistics software. Means for percentage repellency for both insects at the four concentrations at a particular time interval were compared using one way ANOVA. The median repellent dose (RD_{50}) was determined from the linear regression equation through regression analysis.

Gas chromatography-mass spectrometry analysis

The GC-MS analysis was carried out using an Agilent 6890 GC with an Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 45-400 amu, and scan rate = 3.99 scans/s], and an Agilent ChemStation data system. The GC was equipped with a fused silica capillary HP-5 MS column of an internal diameter of 0.25 mm, film thickness 0.25 µm and a length of 30 m. The initial temperature of the column was 70°C and was heated to 240°C at a rate of 5°C/min. Helium was used as the carrier gas at a flow rate of 1 ml/min. The split ratio was 1:25. Scan time was 50 min with a scanning range of 35 to 450 amu. A 1%, w/v, solution of the sample in hexane was prepared and 1 µl was injected using a splitless injection technique.

Identification of components

The identification of the oil constituents was based on their retention indices determined by reference to a homologous series of *n*-alkanes (C_8-C_{30}), and by comparison of their mass spectral fragmentation patterns with those reported by Joulain and Koening (1998) and Adams (2007) and stored in the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA version C.00.01.080)]. The percentages of each component are reported as raw percentages based on the total ion current without standardization.

RESULTS

Insecticidal activities

The essential oil of *T. camphoratus* did not show fumigation and contact toxicity against both *S. zeamais* and *S. oryzae* at the concentrations used. All the *S. zeamais* and *S. oryzae* tested remained alive after the 28 days of exposure. However, the essential oil showed repellent activity against *S. zeamais* and *S. oryzae* at the concentrations used. A percent repellence (PR) value of greater than 50% from the four replicates was noted at all concentrations for both *S. zeamais* and *S. oryzae* 24 h after treatment (Figures 1 and 2). Repellent action was highly dependent upon oil concentration and exposure

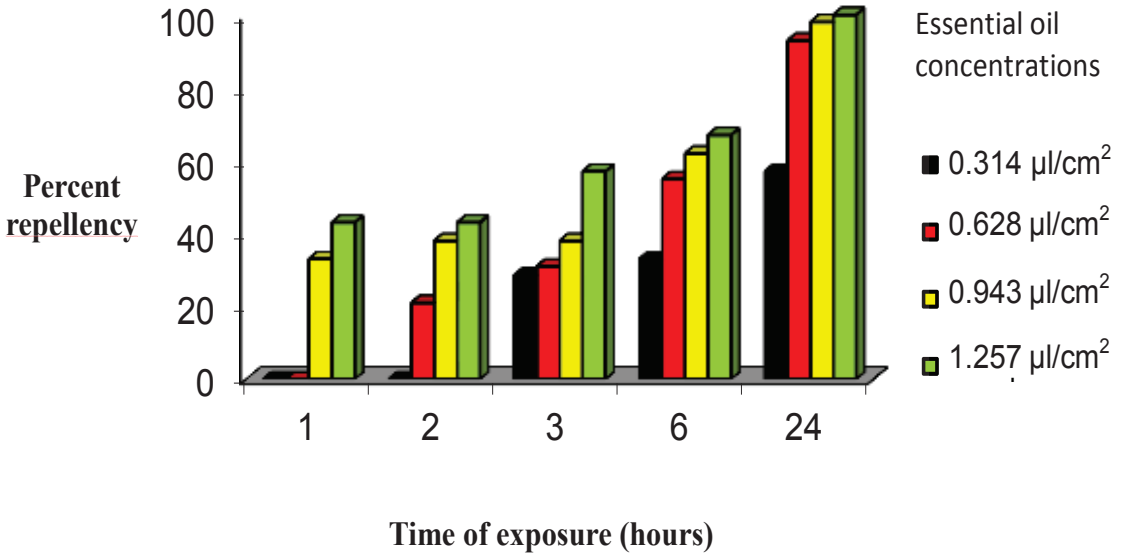

Figure 2. Plot of percent repellency against time of exposure, *S. oryzae*.

time. There were no significant differences between the means of percentage repellence for both insects at the four concentrations at a particular time interval at $p< 0.05$. The median repellent doses were 0.945 and 0.910 µl/cm^2 for *S. zeamais* and *S. oryzae* respectively. Percent repellence increased with both increasing concentration and time of exposure.

Chemical composition

The dry leaves of *T. camphoratus* yielded 0.23% (w/w) of yellowish green oil with a strong camphor aroma. Twenty seven compounds were identified in the oil accounting for 73.01% of the total oil composition. The oil was dominated by sesquiterpene hydrocarbons (59.18%) of which allo-Aaromadendrene, β-Guaiene, γ-Cadinene, δ-Cadinene, aromadendrene, Beta-caryophyllene and γ-Muurolene were the major components (Table 1). Monoterpene hydrocarbons formed 1.61% of the oil and the percentage composition of the oxygenated monoterpenes and oxygenated sesquiterpenes were 6.26 and 3.19% respectively.

DISCUSSION

The essential oil of the dry leaves of *T. camphoratus* showed no contact and fumigation toxicity on both *S. zeamais* and *S. oryzae* at the concentrations used. Previous studies have shown that the toxicity of essential oils obtained from aromatic plants against storage pests is related to the oil's main components (Lee et al., 2003). Among the essential oil components monoterpenes have drawn the greatest attention for insecticidal activity against stored product pests (Asgar, 2011). Various monoterpenes like 1,8 cineole, linalool, α-pinene, terpinen-4-ol, and α-terpinene have been reported to show contact and fumigation toxicity to stored product pests (Papachristos et al., 2004; Stamopoulos et al., 2007). These monoterpenes, although present in the essential oil under study were in trace amounts and lack of toxicity of the essential oil may be attributed to the low total concentration of monoterpenes in the oil (Table 1). However, the oil showed good repellent activity against both *S. zeamais* and *S. oryzae*. One of the major compounds in the oil, δ-cadinene, has been reported to have repellent activity against some anthropods (Yatagai et al., 2002), and may be responsible for the observed repellent activity of the oil. However, there is a possibility of synergetic action between major and minor components to effect the repellent action of the oil. Biological activity of essential oils has been reported to be affected by interactions among the structural components of the oil where even the minor compounds can have critical function due to coupled effects and additive action between the different chemical classes (Tapondjou et al., 2005).

Conclusion

The essential oil of the dry leaves of *T. camphoratus* from Kwa-Zulu Natal, South Africa is mainly dominated by sesquiterpene hydrocarbons. The oil is not toxic to *S. zeamais* and *S. oryzae* but could be considered a potential in the control of stored product pests as a repellent.

Table 1. Percent chemical composition of the essential oil of the dry leaves of *T. camphoratus* from Kwa-Zulu Natal, South Africa.

Compound	Kovat Index	Percent composition
Monoterpene hydrocarbons		**1.61**
α- Pinene	938	0.45
Camphene	952	0.33
α-Terpinene	1017	0.65
p-Cymene	1026	0.18
Sesquiterpene hydrocarbons		**59.18**
α-Copaene	1378	2.33
α-Elemene	1393	2.98
Calarene	1403	3.60
(-)-Isoledene	1419	2.72
Beta-caryophyllene	1427	**5.48**
α-Guaiene	1439	2.73
α-humulene	1461	0.97
γ-gurjunene	1472	0.43
Aromandrene	1475	**6.12**
γ-Muurolene	1480	**5.13**
Eremophilene	1486	0.10
β-Guaiene	1500	**10.70**
γ-Cadinene	1513	**9.09**
δ-Cadinene	1526	**6.80**
Oxygenated monoterpenes		**6.26**
1,8-Cineole	1033	1.94
Linalool	1098	1.77
Camphor	1145	0.62
Terpinene-4-ol	1180	0.43
(-)-α-Terpineol	1190	0.82
Carvacrol	1299	0.68
Oxygenated sesquiterpenes		**3.19**
Elemol	1549	2.76
Spathulenol	1578	0.43
Others		**2.77**
Butanal	620	2.77

Conflict of Interest

The authors have not declared any conflict of interest.

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