Journal of Asia-Pacific Entomology 11 (2008) 175–179

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Fumigant toxicity of essential oil from *Vitex pseudo-negundo* against *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* (L.)

Bibi Zahra Sahaf [a], Saeid Moharramipour [a,*], Mohammad Hadi Meshkatalsadat [b]

[a] Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, P. O. Box 14115-336, Tehran, Iran
[b] Department of Chemistry, Faculty of Basic Sciences, Lorestan University, Khoramabad, Iran

ARTICLE INFO

Article history:
Received 24 November 2007
Revised 27 May 2008
Accepted 17 September 2008

Keywords:
Botanical insecticides
Monoterpenoids
Stored-product beetles
Red flour beetle
Rice weevil

ABSTRACT

The objective of the current study was to determine the chemical constituents and fumigant toxicity of an essential oil that was isolated via hydrodistillation from dry leaves of *Vitex pseudo-negundo* (Hausskn.) Hand.-Mzz. The chemical composition of the essential oil was assessed via GC and GC-MS. 1, 8-Cineol (18.23%), α-Pinene (16.20%) and Sabinene (5.67%) were determined to be the major constituents of the oil. The fumigant toxicity of the essential oil was tested against 1–7 day-old adults of *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* (L.) at 27±1 °C and 60±5% r.h. in darkness. The mortality of adults was tested at different concentrations ranging from 37.0 to 925.9 μL/L air and different exposure times (1–30 h). The results demonstrated that the mortality increased with increases in concentration and exposure time. At concentrations higher than 185.2 μL/L air, the mortality was recorded at more than 50% after 10 h, and reached 100% after 12–16 h. Data probit analysis demonstrated that *S. oryzae* (LC_{50}=31.96 μL/L air) was more susceptible than *T. castaneum* (LC_{50}= 47.27 μL/L air). These results showed that the essential oil from *V. pseudo-negundo* could be applicable to the management of populations of stored-product insects.

Introduction

Tribolium and *Sitophilus* species are major pests of stored grains and grain products throughout the world (Howe, 1965; Sinha and Watters, 1985). Control of these insects relies heavily on the use of synthetic insecticides and fumigants, which has resulted in several problems, including environmental disturbances, increasing costs of application, pest resurgence, pest resistance to pesticides, and lethal effects on non-target organisms, in addition to direct toxicity to users (Champ and Dyte, 1976; Subramanyam and Hagstrum, 1995; White and Leesch, 1995; Jembere et al., 1995; Okonkwo and Okoye, 1996). Thus, repellents, fumigants, feeding deterrents, and insecticides of natural origin are all rational alternatives to synthetic insecticides. Botanical insecticides composed of essential oils may prove to be a reasonable alternative to the more persistent synthetic pesticides (Chiasson et al., 2004). Essential oils, obtained by the distillation of plant foliage, and even the foliage itself of certain aromatic plants have traditionally been utilized to protect stored grain and legumes (Isman, 2000). In recent years, essential oils have received a great deal of attention as pest control agents. They are volatile and can function as fumigants, and may also be applicable to the protection of stored products.

Iran is a country comprised largely of arid and semi-arid areas, and contains many indigenous aromatic plants from different families. *Vitex pseudo-negundo* is one of these medicinal herbs, and grows naturally in the vicinity of seasonal rivers in Iran. The medicinal properties of this species have been long recognized, and *V. pseudo-negundo* has become a familiar drug component (Filekesh et al., 2005). However, no studies regarding its insecticidal bioactivity have yet been conducted. The present study was, therefore, undertaken in order to assess the bioactivity of the essential oil of *V. pseudo-negundo* against adults of *Tribolium castaneum* and *Sitophilus oryzae*, two important stored-product beetles observed in grain storage facilities in different countries.

Materials and methods

Insect cultures

T. castaneum was reared in plastic containers (20 cm length×14 cm width×8 cm height) containing wheat flour mixed with yeast (10:1, w/w) and *S. oryzae* in plastic containers (3.7 cm height×7.5 cm diameter) containing whole polished grains of Alikazemi cultivar rice, which were covered by a fine mesh cloth for ventilation. The cultures were maintained in darkness in a growth chamber set at 27±1 °C and 60±5% r.h. Adult insects, 1–7 days old, were utilized for the fumigant toxicity test. All experimental procedures were conducted under environmental conditions identical to those of the cultures.

* Corresponding author. Fax: +98 2144196524.
E-mail address: moharami@modares.ac.ir (S. Moharramipour).

1226-8615/$ – see front matter
doi:10.1016/j.aspen.2008.09.001

Plant materials

Leaves of *V. pseudo-negundo* were collected on October 2005 from Sabzevar, Iran. The leaves were dried naturally on laboratory benches at room temperature (23–27 °C) for 6 days until they were crisp. The dried materials were stored at −24 °C until needed, then hydro-distilled to extract their essential oil.

Extraction and analysis of essential oil

Essential oil was extracted from leaves subjected to hydrodistillation using a modified Clevenger-type apparatus. The extraction condition was as follows: 50 g of leaves; 600 mL distilled water, and 4 h distillation. Anhydrous sodium sulphate was utilized to remove water after extraction. The oil yield (1% w/w) was calculated on a dry weight basis. The extracted oil was stored in a refrigerator at 4 °C.

GC analysis was conducted on a Shimadzu 17A gas chromatograph equipped with an FID and a BP-5 (non-polar) capillary column (30 m×0.32 mm×0.25 μm film thickness). The oven temperature was maintained at 60 °C for 3 min, and then programmed to increase at 5 °C/min to 260 °C. Other operating conditions were as follows: carrier gas He, at a flow rate of 5 mL/min; injector temperature 230 °C; detector temperature 245 °C; and split 40, column flow ratio, 1:8 mL/min. GC/MS analysis was conducted on a Shimadzu 17A GC coupled with a Shimadzu QP5050A. The operating conditions of the mass system were identical to those described above, but the carrier gas was He. The mass spectra were obtained at 70 eV. The mass range was from *m*/*z* 50-500 amu. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the oil were identified via comparison of their mass spectra and retention indices with those published in the literature (Adams, 1995), and presented in the MS computer library.

Fumigant toxicity bioassay

In an effort to determine the fumigant toxicity of the *V. pseudo-negundo* oil and the median effective time to cause mortality in 50% of test insects (LT_{50} values), filter papers (Whatman No. 1, cut into 2 cm diameter pieces) were impregnated with an appropriate concentration (37.0 to 925.9 μL/L air) of the oil without using any solvent. The impregnated filter paper was then attached to the undersurface of the screw cap of a 27 mL glass vial. The caps were screwed tightly onto the vials, each of which contained ten adults (1–7 days old with undefined sex) of one of the specified insect species. The combination of each concentration and exposure time (1–30 h) was replicated four times. The mortality was assessed via direct observation, and when no leg or antennal movements were observed, the insects were considered dead. Percentage insect mortality was calculated using the Abbott correction formula for natural mortality in untreated controls (Abbott, 1925). Time-mortality data for each experiment were analyzed via the method developed by Finney (1971), with time as the explanatory variable to derive the estimated hours for 50% mortality (LT_{50}). The estimates were compared using overlap of the 95% fiducial limits. Non-overlap at the 95% fiducial limits is equivalent to a test for significant differences.

Another experiment was designed in order to determine the 50% and 95% lethal doses. Different dilutions were prepared to evaluate insect mortality after an initial dose-setting experiment. 10 adults of *T. castaneum* and *S. oryzae* were examined in 280 mL glass bottles with screw lids. The concentrations of the oil tested on *T. castaneum* and *S. oryzae* were 0–71.43 μL/L air. The control insects were maintained under the same conditions without any essential oil. Each dose was replicated five times. The number of dead and live insects in each bottle was counted 24 h after initial exposure to the essential oil. The dead insects were then monitored for at least 48 h after the data were recorded, and none of the affected insects recovered. Probit analysis (Finney, 1971) was then conducted to estimate the LC_{50} and LC_{95} values with their fiducial limits by SAS 6.12 (SAS Institute, 1997). The samples for which the 95% fiducial limits did not overlap were considered to be significantly different.

Results

Chemical constituents of essential oil

The results of the chemical analysis are provided in Table 1. The major components of the oil from *V. pseudo-negundo* were 1, 8-Cineol (18.23%), α-Pinene (16.20%), Sabinene (5.67%), Bicyclogermacrene (5.38%) and β-Caryophyllene (4.43%) (Table 1).

Fumigant toxicity

These experiments were conducted in order to determine whether the insecticidal activity of *V. pseudo-negundo* oil was attributable to fumigant activity. In all cases, considerable differences in insect mortality were noted with different concentrations and exposure times. The graph in Fig. 1 shows that *V. pseudo-negundo* oil was toxic to *T. castaneum* and *S. oryzae*. The highest concentration (925.9 μL/L air) of the oil proved able to induce more than 50% mortality after 6 h, and achieved a level of 100% at 12 h after treatment. The lowest concentration proved able to kill 50% of the *T. castaneum* and *S. oryzae* within 24 h. At 740.7 μL/L air, the complete mortality of beetles was achieved after 15 h of exposure. At 370.4 μL/L air, the oil caused approximately 50% and 90% mortality at 12 and 24 h after exposure, respectively (Fig. 1). The LT_{50} values for *T. castaneum* ranged from 22.90 h for the lowest dose (37.0 μL/L air) to 5.80 h for the highest dose (925.9 μL/L air). With *S. oryzae*, the LT_{50} values ranged from 22.14 h to 5.83 h for the lowest and highest doses, respectively. On the basis of the fiducial limits shown in Table 2, the LT_{50} values for *S. oryzae* at 370.4–925.9 μL/L air do not appear to be applicable to *T. castaneum*. The LT_{95} estimates of *V. pseudo-negundo* at 925.9 μL/L air did not differ

Table 1
Chemical constituents of the essential oil from *Vitex pseudo-negundo*

Compound	Retention index	% Composition
α-Thujene	925	0.10
α-Pinene	930	16.20
Sabinene	967	5.67
Myrcene	985	0.55
α-Phellandrene	996	0.17
α-Terpinene	1013	0.14
ρ-Cymene	1020	0.16
1, 8-Cineol	1027	18.23
Trans-Ocimene	1044	0.12
γ-Terpinene	1054	0.29
Z-β-Terpineol	1059	0.11
Terpinolene	1083	0.10
Linalool	1096	0.17
Terpinen-4-ol	1171	0.90
α-Terpineol	1184	0.82
Thymol	1289	0.13
Citronellyl Acetate	1350	0.10
α-gurjunene	1411	0.39
β-caryophyllene	1415	4.43
α-Caryophyllene	1449	0.14
Trans-β-farnesene	1456	3.13
Germacrene D	1468	0.10
Bicyclogermacrene	1493	5.38
Dehydroaromadendrene	1503	0.14
Δ-Cadinene	1520	0.10
Ledol	1563	0.23
Spathulenol	1571	1.20
(-)-Caryophllene Oxide	1575	0.68
Viridiflorol	1594	1.15
α-Cadinol	1649	0.14
Other compounds		38.83