CAF2020 Paper No. P-8-4-65

Kumar R, Pandey PS, Tiwari SN (2021) Chemical constituents and insecticidal activity of essential oils against three stored product beetles in stored wheat. Pp. 253-259. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Chemical constituents and insecticidal activity of essential oils against three stored product beetles in stored wheat

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Abstract

Laboratory experiments were conducted to determine the chemical constituents and insecticidal activity of *Chenopodium botrys*, *Citrus reticulata*, and *Callistimone citrinus* essential oils against *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), and *Tribolium castaneum* (Herbst) in stored wheat. In the *Chenopodium botrys* essential oil, Ascaridol (39.89%), Terpinyl acetate (17.85%) and Para cymene (11.78 %) were major compounds. The *Citrus reticulata* essential oil contained Limonene (69.32%), Linolool (7.83%) and Myrcen (5.38%) as major compounds. The essential oil of *Callistimon citrinus* contained 1-8 Cineole (42.03%), Alpha-terpineol (15.65%), Limonene (11.23%) as major compounds. The toxicity test found that selected essential oils of *C. botrys*, *C. reticulata*, and *C. citrinus* completely suppressed feeding and breeding as they caused full inhibition in progeny development of *S. oryzae*, *R. dominica* and *T. castaneum* after one year of treatment at 0.2 and 0.4% concentration. The essential oils of *C. botrys*, *C. reticulata*, and *C. citrinus* resulted in 100% mortality of *S. oryzae*, *R. dominica* and *T. castaneum* after 24 h treatment. The essential oils of *C. botrys*, *C. reticulata*, and *C. citrinus* had highly repellent activity against *S. oryzae*, *R. dominica* and *T. castaneum*. All tested essential oils were highly effective in terms of fumigant toxicity and mortality against *S. oryzae*, *R. dominica* and *T. castaneum* with strong repellent activity, as they contained potent insecticidal constituents.

Keywords: *Chenopodium botrys, Citrus reticulata, Callistimon citrinus,* Chemical constituents, *Sitophilus oryzae, Rhyzopertha dominica, Tribolium castaneum ,* Fumigant toxicity, Mortality, Repellent activity

Introduction

The *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) are notorious

insects of stored wheat in India. Each year, enormous amounts of globally-stored human food resources are spoiled due to the infestation of several insect pests. Human food security depends not only on primary agricultural production, but also on sufficient post-harvest storage and distribution of agricultural commodities and food products. In order to search for alternatives to traditional chemical fumigants, several essential oils from the plant kingdom were evaluated for their fumigant toxicity, contact toxicity, ovicidal activity, mortality and repellent activity against insect pests of stored commodities (Kumar, 2017; Kumar et al., 2020; Rajendran et al., 2008; Tripathi et al., 2002). The objective of the present experiment was to determine the chemical constituents and insecticidal activity of essential oils against three stored product beetles in stored wheat.

Material and methods

Culture of insects

The experiments were conducted in the Postharvest Entomology Laboratory, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. Pure cultures of *S. oryzae*, *R. dominica* and *T. castaneum* were reared in an incubator maintained at 27 ±1°C and 70±5% relative humidity (RH). Plastic jars of 1.0 kg capacity were used for rearing purposes. At the center of the lid a hole of 1.8 cm diameter was made and covered with 30 mesh copper wire net to facilitate air exchange in the jar. *Sitophilus oryzae* and *R. dominica* were reared on wheat kernels (variety DBW 14), while *T. castaneum* was reared on wheat flour (95%) and yeast powder (5%). The appropriate feed was filled in plastic jars and 100 adults (mixed sex) were released in each jar and maintained in the incubator. First generation adults (0-7 d old) were used for all tests.

Extraction and analysis of essential oil

Fresh leaves of *C. botrys, C. citrinus* and peels of *C. reticulata* were collected from fields during winter, and then their essential oils were extracted by steam distillation using a Clevenger apparatus (model-475/4, JSGW, Ambala Cant, India).

The analysis of chemical constituents in these essential oils was carried out using a gas chromatograph (HP-5890 series II, Rtx-1-MS-889068, USA), equipped with a flam ionization detector (FID) and an electronic pressure control (EPC) injector. A polar HP-innowax (PEG) column (30 m × 0.25 mm × 0.25 µm film thickness) and a polar HP-5 column (30 m × 0.25 mm coated with 5% phenyl methyl silicone and 95% di methyl polysiloxane, 0.25µm film thickness) from Agilent were used. Carrier gas (N_2) flow was 30.0 mL/min and the split ratio was 80:20. Analysis was performed using the following temperature program: oven kept isothermally at 70°C for 2 min, increased from 70 to 180°C at the rate of 4°C/min for 8 min, and from 180 to 230°C at the rate of 6°C/min for 12 min. Injector and detector temperature were held at 270 and 280°C, respectively. The GC-MS analysis was made using the HP-5972 Mass Spectrometer with electron impact ionization (70 eV) coupled with the HP-5890 series 2nd Gas Chromatograph. Helium was used as the carrier gas with a flow rate of 1.21 mL/min and split ratio of 80:20. Scan time and mass range were 0.50 s and 40-850 m/z, respectively. The essential oil volatile compounds were identified by calculating their retention time relative to (C9-C18) n-alkenes, from data for authentic compounds available in the literature, and also by matching their mass spectrum fragmentation patterns with corresponding data stored in the mass spectra library of the GC-MS data system (NIST) and other published mass spectra. The percentage compositions of these essential oils were calculated according to the area of chromatographic peaks.

Fumigant toxicity test

The fumigant toxicity of these essential oils against *S. oryzae*, *R. dominica,* and *T. castaneum,* were evaluated on untreated and graded wheat seed, variety DBW-14. The experiment was conducted in plastic jars with 1 kg capacity in which 500 g of wheat with a moisture content of 13.5% (wb) were filled. Twenty adults of each test insect were released into each jar. One week after the insect introduction, the filter paper soaked with essential oil was inserted into the jars and jars were sealed for 12 mo. There were three replicates for each treatment. After 12 mo, the grains were analyzed to record percent inhibition and the number of adults that had emerged. The data were analyzed by STPR 3 software with log (X+1) transformation.

Mortality test

The experiment was conducted on *S. oryzae*, *R. dominica,* and *T. castaneum* to find out the actual time required for the essential oils to cause 100% insect mortality. The experiment was performed under controlled conditions at 27±1°C and 70±5% RH. Fifty grams of wheat grain with 13.5% moisture content was filled in a 100 mL plastic vial. Four sets of these vials were prepared for test insects to record their mortality after 6, 12, 18 and 24 h. Ten adult insects (0-7 d old) of *S. oryzae*, *R. dominica*, and *T. castaneum* were released in each vial. After 24 h of releasing the adults, the required quantity of oil soaked on absorbent mats was inserted in each vial, after which each one was closed and sealed with paraffin wax. These treatments were replicated three times. Observation was recorded after every 6 h of treatment up to 24 h.

Repellency test

A repellency test was conducted as per the method of Talukdar and Howse (1993). Petri dishes, 9 cm in diameter, were used to confine insects during the experiment. The essential oils were diluted in ethanol to 1.0, 2.0, 3.0 and 4.0% concentrations and pure ethanol was used as a control. Filter paper of 9 cm diameter was cut into two halves and 1 mL of each concentration was applied separately to one half with a micropipette. The other half was treated with 1mL of pure ethanol. Both the treated halves were then air dried to evaporate the solvent completely. A full disc was carefully recreated by attaching each treated half with transparent tape. Care was taken so that the attachment did not prevent free movement of insects from one half to the other, and then this filter paper was placed in a petri dish. Twenty insects were released in the centre of each filter paper disc, and then covered with the petri dish lid. Three replications were used and the experiment was repeated twice for each test insect. The calculation of % repellency was done as per Abbott (1925) and the repellent class was categorized as per the scale of Roy et al. (2005).

Result and discussion

Chemical constituents of C. botrys essential oil

There were 31 compounds identified in *C. botrys* essential oil (Fig.1). These included Ascaridole (33.89%), Terpinyl acetate (17.85%), Para cymene (11.78%), Kemitracin (5.42%), Cyclo octanone (4.31%), Linalool acetate (2.92%), 1-Octadecene (2.58%), Doecenol (2.21%), Phytol acetate (2.12%), Thymol (2.06%), 1-Propanol (1.90%), p Cumic aldehyde (1.41%), Heptadecane (1.35%), Phthalic acid (1.30%), Precocene (1.16%), Hexonic acid (1.14%), Cyclobutane ethanol (1.02%), and other remaining compounds in trace amounts.

The pinene major compound in essential oil of *M. koenigii*, has been reported to be toxic to several stored grain insects (Kordali, 2006). These oxygenated monoterpenes have strong fumigant action against *S. oryzae* (Lee et al., 2001).

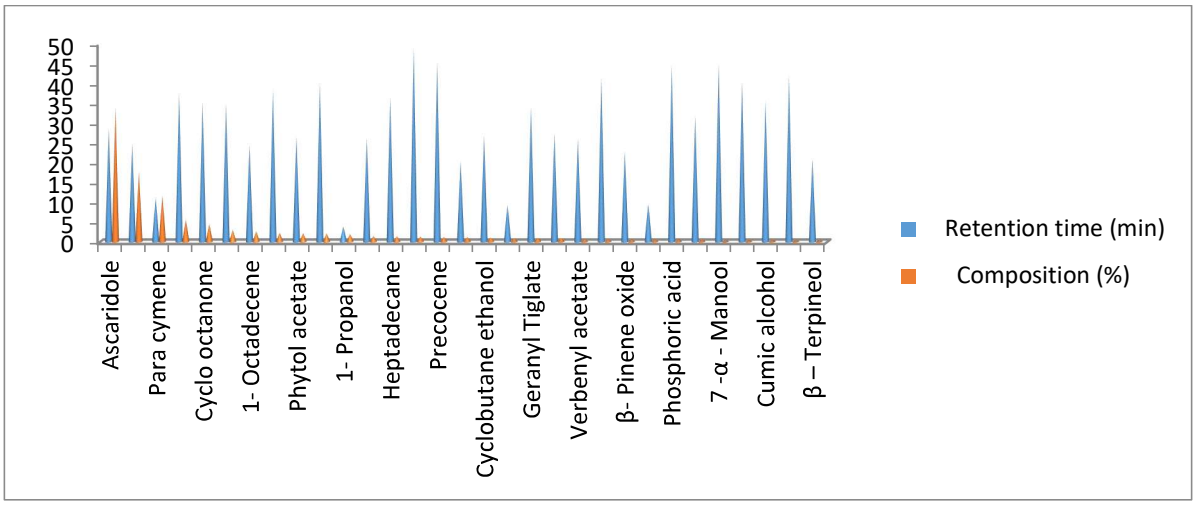

Fig. 1. Chemical composition of *C. botrys* essential oil.

Chemical constituents of C. reticulata **essential oil**

There were 18 compounds identified in *C. reticulata* essential oil (Fig. 2). These included Limonene (69.32%), Linalool (7.83%), Myrcene (5.38%), α-Terpineol (3.35%), Sabinene (2.16%), α-Pinene (2.11%), Octanol (1.74%), Geranial (1.13%), Carene (1.05%), and other remaining compounds in trace quantities. Tripathi et al. (2002) reported the insecticidal activity of Limonene (a major constituent of citrus oil) against *T. castaneum* to be effective in inhibiting progeny production.

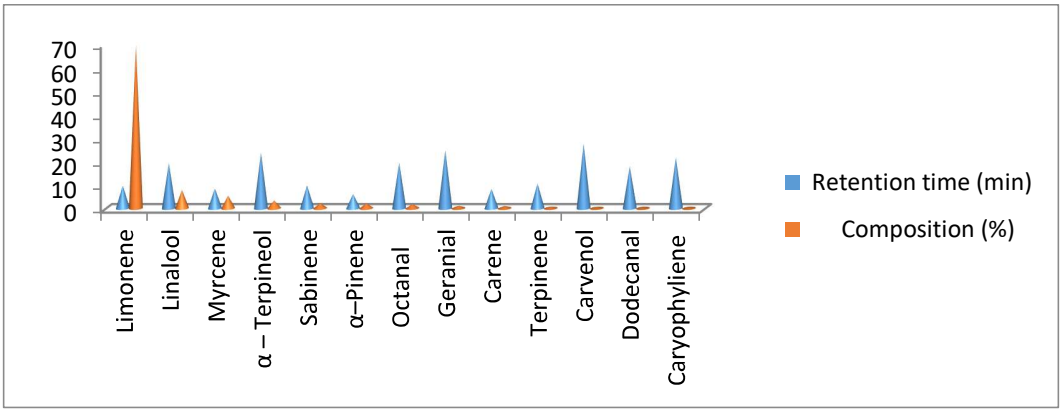

Fig. 2. Chemical composition of *C. reticulata* essential oil.

Chemical constituents of C. citrinus essential oil

There were 15 compounds identified in the essential oil of *C. citrinus* (Fig. 3). These included 1,8-Cineol (42.03%), α-Terpineol (15.65%) and Limonene (11.23%) as major compounds, followed by α-Pinene (10.47%), Cymene (5.93%), α-Phellandrene (3.21%), Linalool (3.05%), Myrcene (1.5%), and other remaining compounds in trace quantities.

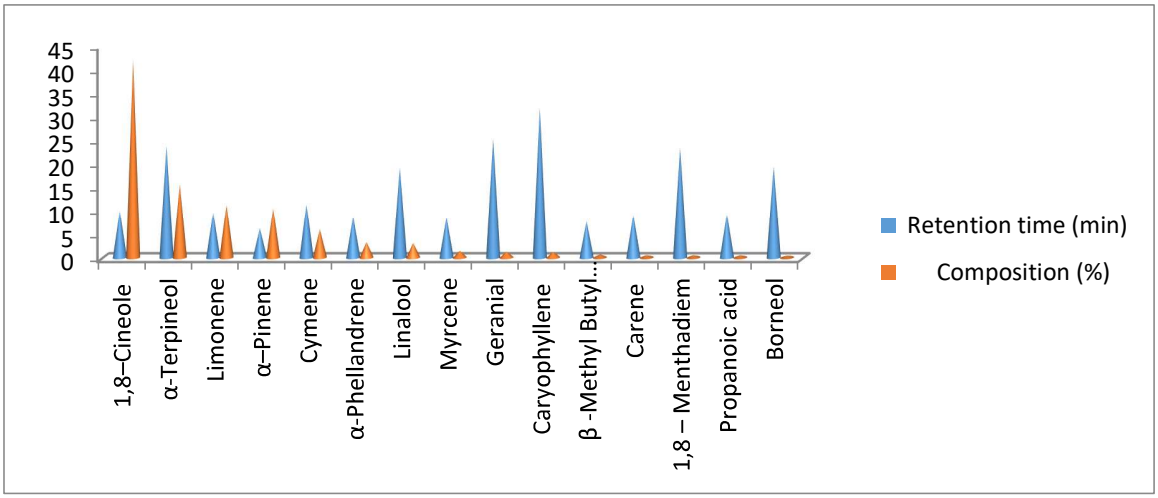

Fig. 3. Chemical composition of *C. citrinus* essential oil.

Fumigant toxicity of essential oils against test insects

The essential oils of *C. botrys*, *C. reticulata*, and *C. citrinus* caused full inhibition (zero emergence) in progeny development of *S. oryzae*, *R. dominica*, and *T. castaneum* after one year of treatment at 0.2 and 0.4% concentration as compared to untreated controls with emergence of 14.5, 65.0 and 43.0%, respectively. The essential oils of *M. koenigii*, *C. reticulata*, *C. citrinus* either alone at 0.2% or two component combinations were highly effective against *S. oryzae* and *R. dominica* (Kumar et al., 2018). The essential oils of *C. botrys, C. reticulata, L. camara, P. roxburghii* at 0.1, 0.2, 0.3, and 0.4% were highly effective against *T. castaneum* seeing as full inhibitions were caused compared to the untreated control (Kumar et al., 2021).

Mortality test against test insects

The average mortality caused by the essential oils of *C. botrys*, *C. reticulata*, and *C. citrinus* against *S. oryzae*, *R. dominica*, and *T. castaneum* after 24 h of treatment was 100% as compared to the untreated control. Tunc et al. (2000), observed fumigant toxicity with 100% mortality caused by the essential oil of Cumin (*C. cyminum*) against the eggs of *T. confusum* and *Ephestia kuehniella* (Z.).

Repellency test against test insects

The essential oils of *C. botrys*, *C. reticulata* and *C. citrinus* at 1.0, 2.0, 3.0, and 4.0% showed strong repellency against *S. Oryzae*, *R. dominica*, and *T. castaneum* (Table 1.) because of their potent insecticidal constituents. Joshi and Tiwari (2019) reported that *C. limetta, M. koenigii, C. citrinus, C. longa* and *P. roxburghii* at 3% concentration exhibited 92.51, 83.48, 92.75, 98.20 and 89.78% mean repellency against *R. dominica*, respectively.

Conclusions

All tested essential oils were highly effective in terms of fumigant toxicity and mortality against *S. oryzae*, *R. dominica* and *T. castaneum* with strong repellent activity, as they contained potent insecticidal constituents.

Table 1. Mean repellency of essential oils against tested insects

Essential oil	Conc. %	Mean repellency after 24 h of treatment		
		S. oryzae	*R. dominica*	*T. castaneum*
C. botrys	1.0	96.01	95.32	98.39
	2.0	97.10	96.19	100.00
	3.0	98.66	97.83	100.00
	4.0	99.53	99.10	100.00
C. reticulata	1.0	94.82	93.16	97.84
	2.0	97.31	94.50	100.00
	3.0	98.56	95.87	100.00
	4.0	99.95	97.54	100.00
C. citrinus	1.0	96.02	94.47	98.65
	2.0	96.53	95.31	100.00
	3.0	97.81	96.99	100.00
	4.0	98.89	98.64	100.00

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