Journal of Stored Products Research 46 (2010) 48–51

Contents lists available at ScienceDirect

Journal of Stored Products Research

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

Variation in the susceptibility of two *Callosobruchus* species to essential oils

Wolali S. Nyamador [a], Guillaume K. Ketoh [a,*], Komina Amévoin [a], Yaovi Nuto [a], Honoré K. Koumaglo [b], Isabelle A. Glitho [a]

[a] Laboratoire d'Entomologie Appliquée, Faculté des Sciences, Université de Lomé, B.P. 1515, Lome, Togo
[b] Laboratoire des Extraits Végétaux et Arômes Naturels, Faculté des Sciences, Université de Lomé, B.P. 1515, Lome, Togo

ARTICLE INFO

Article history:
Accepted 30 September 2009

Keywords:
Callosobruchus maculatus
Callosobruchus subinnotatus
Cymbopogon giganteus
Cymbopogon nardus
Essential oils

ABSTRACT

GC/MS analysis of essential oils extracted from two *Cymbopogon* species revealed that limonene (23%) and *p*-mentha-2,8-dien-1-ol in *cis* (14.3%) and *trans* (5.6%) forms were the main compounds in *Cymbopogon giganteus* oil whereas citronellal (31%) and geraniol (24%) were identified in *Cymbopogon nardus* oil. The toxicity tests performed by fumigation on eggs and adults of *Callosobruchus maculatus* and *Callosobruchus subinnotatus* using both essential oils showed a variation in bruchid susceptibility. Essential oil of *C. giganteus* was more toxic to adults of both bruchid species while essential oil of *C. nardus* showed the better ovicidal activity. Comparative susceptibility analysis suggested that eggs and adults of *C. subinnotatus* were two-fold more tolerant to essential oils than *C. maculatus* in both stages. Oviposition of treated females was strongly reduced in the presence of essential oils. *Callosobruchus subinnotatus* was more affected than *C. maculatus* by the essential oil of *C. giganteus* (oviposition reduction by at least 91% v.s 81% in *C. maculatus* at 5 µL/L) but the two species were affected similarly by the essential oil of *C. nardus*.

Published by Elsevier Ltd.

1. Introduction

Bruchid pest populations annually cause heavy damage to bean stores in Togo, which significantly affects grain legume production figures. From 1997 to 2003, the annual production of bambarra groundnut (*Vigna subterranea* (L.) Verdc.) decreased from 10 200t to 4104 t while the production of cowpea (*Vigna unguiculata* (L.) Walp.) stagnated at 40 000 t. Four bruchid species are found in grain legume stores in Togo. *Callosobruchus maculatus* (F.), *C. rhodesianus* (Pic) and *Bruchidius atrolineatus* (Pic) develop on cowpea and *Callosobruchus subinnotatus* (Pic) is observed in bambarra groundnut (Ketoh et al., 2001). Among bruchids, *C. maculatus* is the main pest because its populations persist and cause stock destruction over a period of five to six months (Ketoh and Glitho, 2006) whereas *C. subinnotatus* causes most of the damage to bambarra groundnut (Ketoh et al., 2001; Appleby and Credland, 2001; Mbata, 1991). The presence of both *C. maculatus* and *C. subinnotatus* has been reported on cowpea (Mbata, 1993) and on bambarra groundnut (Ajayi and Lale, 2000). The simultaneous presence of *C. maculatus* and *C. subinnotatus* in bambarra groundnut stores leads to severe damage under storage conditions.

Under these circumstances, farmers resort to risky strategies such as chemical use, as traditional practices are not suited to protecting products from insect damage especially during severe attacks. Low-income farmers use prohibited, nonappropriated or non-recommended insecticides and stock up with them through illicit distribution networks. The lack of operational regulation services aggravates the issue and leads to an escalation of chemical application frequency and dosage. This results in environmental health risks and pest resistance may increase the tendency for vector borne disease outbreaks. An alternative to this situation could be the use of plant derived-products as low-risk botanical insecticides. The insecticidal activity of many plant-products has been reported extensively against stored-product pests (Lale and Mustapha, 2000; Tripathi et al., 2000; Kéita et al., 2001; Cox, 2004; Han et al., 2006; Rozman et al., 2007).

Among plant-derived products, essential oils provide botanicals capable of replacing conventional fumigants such as methyl bromide, the phase-out of which was decided after the discovery of its role in ozone depletion (WMO, 1995). Some products extracted from aromatic plants show insecticidal activity against bruchid species (Ketoh et al., 2000, 2006; Raja et al., 2001; Pascual-Villalobos and Ballesta-Acosta, 2003). However, their activities are influenced by chemical composition and the bruchid developmental stage (Ketoh et al., 2002). The effectiveness of their use to control the entire development of bruchids is complicated by 1) the

* Corresponding author. Tel.: +228 225 50 94; fax: +228 225 87 84.
E-mail addresses: gketoh@univ-lome.tg, gketoh@tg.refer.org (G.K. Ketoh).

0022-474X/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.jspr.2009.09.002

Table 1
Main compounds in *C. giganteus* essential oil. More abundant compounds are in **bold** font.

Compounds	Percentage (%)
p–cymene	0.02
Limonene	**23.05**
Limonene oxide I	0.80
Limonene oxide II	1.25
p-α dimethyl styrene	0.10
***p*-mentha-2,8-dien-1-ol cis**	**14.26**
***p*-mentha-2,8-dien-1-ol trans**	**5.63**
4′-methyl acetophenone	0.03
***p*-mentha-1(7),8-dien-2-ol**	**12.63**
***p*-mentha-1(7),8-dien-2-ol isomer**	**14.06**
cis carveol	0.75
Tr. Carveol	1.40
Carvone	**3.32**
Isoamyl hexanoate	0.13
Isoamyl octanoate	0.09
Perilla aldehyde	0.31
Phenethyl hexanoate	0.03
Phenethyl octanoate	0.01
Total	**77.87**

tolerance of stages developing inside the seeds which are protected from exposure to essential oil by the seed coat (Ketoh et al., 2002), and 2) variation in the susceptibilities of congeneric species. Therefore, emphasis needs to be put on strategies for application of essential oil to control critical stages like eggs and adults of all species with a view to protect grain from larval penetration and oviposition.

In this paper, we report the variation in fumigation activities of essential oils extracted from two *Cymbopogon* species against the egg and adult stages of two congeneric species, *C. maculatus* and *C. subinnotatus*.

2. Materials and methods

2.1. Plant materials

Plant materials of two *Cymbopogon* species were used in the experiments. *Cymbopogon nardus* L. Rendle material was collected in the southern region of Togo (Locality of Zeve in Zio) while *Cymbopogon giganteus* Chiov. material was harvested in the western region (Locality of Kpalime). Aerial parts of the two plant species collected were dried at 30 °C for 72 h before extraction.

Table 2
Main compounds in *Cymbopogon nardus* essential oil. More abundant compounds are in **bold** font.

Compounds	Percentage (%)
Limonene	1.39
Linalol	0.27
tr-verbenol	0.35
Citronellal	**30.58**
Citronellol	**7.65**
Neral	0.42
Geraniol	**23.93**
Geranial	0.74
Geranyl acetate	**8.68**
thymyl acetate	3.48
β-elemene	2.09
Germacrene D	1.28
Bicyclogermacrene	0.19
α-farnesene	0.17
tr., β-farnesene	0.33
Cadinene	1.18
Elemol	**12.04**
γ+β–eudesmol	0.26
β-eudesmol	0.15
Citryl tiglate	1.12
Total	**96.30**

2.2. Insects

Callosobruchus maculatus developing either on *Vigna unguiculata* or *V. subterranea* and *C. subinnotatus* developing on *V. subterranea* were collected from stores and mass-reared under laboratory conditions (28 ± 2 °C, 75 ± 10% relative humidity (r.h.) and 12 h: 12 h L:D) for six months using the method of Dick and Credland (1984). *Callosobruchus maculatus* and *C. subinnotatus* were reared separately on *V. unguiculata* and *V. subterranea* seeds bought from a market and frozen for 15 days to avoid any previous contamination. The moisture content of the seeds was then equilibrated at 13% before their use in tests. Fresh eggs (<1-day old) and newly-emerged adults (<1-day old) were used in the bioassays.

2.3. Chemical composition analysis

The essential oils were extracted from aerial parts of the plants by steam distillation. Crude extracts were washed with a NaCl solution (5%), and dried over sodium sulphate. Gas chromatography coupled with mass spectrometry (Perkin Elmer) was used to identify the main volatiles contained in each essential oil. GC–MS analysis was performed as described by Ketoh et al. (2002).

2.4. Toxicity tests

The tests were carried out on eggs and adults according to Ketoh et al. (2005). Forty eggs or 1-day old adults were exposed to five concentrations (5, 10, 20, 30 and 40 and 100 μL/L) of essential oil for 24 h in sealed l-L bottles containing 100 g of seeds. Fresh eggs removed from treated bottles were examined after hatching and penetration of the first instar larvae into the seeds. Treated adults were removed after 24 h from the treatment glassware and placed in non-treated Petri dishes for 24 h before mortality rates were recorded.

2.5. Statistical analysis

All experiments were repeated six times. The mortality rate at each developmental stage was subjected to analysis of variance (ANOVA) followed by Newman–Keuls multirange test using the software package STAT–ITCF Microstat, Version 5.0 (ECOSFT). The level of significance was set at $P < 0.05$. Abbott's formula was used to correct for control mortality rates when necessary.

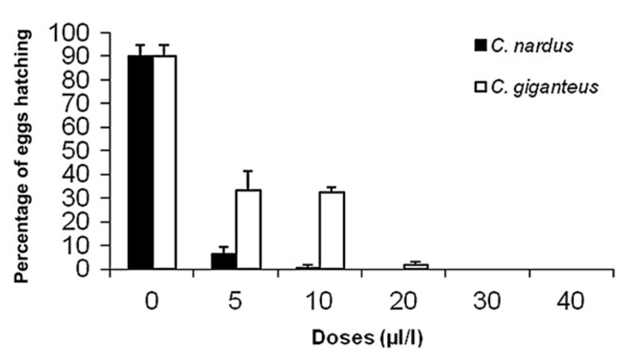

Fig. 1. Comparative effect of essential oils on *C. maculatus* egg hatching. *C nardus*: $F = 899.04$, df = 5,30, $P < 0.0001$. *Cymbopogon giganteus*: $F = 272.25$, df = 5,30, $P < 0.0001$.