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MONOTERPENOIDS AS FUMIGANTS IN THE MANAGEMENT OF *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE): OVIPOSITION DETERRENCE AND MORTALITY OF DEVELOPMENTAL STAGES

George N. Mbata[1*], Dana Smith[1], and Kendra Mcneal[1]

[1] Department of Biology, Fort Valley State University, Fort Valley, Georgia 31030, USA.
*Corresponding author's e-mail: *gn1185mbata@aol.com* or *mbatag@fvsu.edu*

ABSTRACT

Monoterpeniods have been demonstrated to cause mortality in certain stored-product insect pests. The current report investigated the prospects of using monoterpenoids in the management of all stages of the cowpea beetle, *Callosobruchus maculatus* (Fabricius). Newly emerged males and females (36 – 48 h), that were observed mating were exposed to cowpea seeds treated with the following monoterpenoids; *E*-anethole, estragole, *S*-carvone, linalool, L-fenchone, geraniol, γ-terpinene and *DL*-camphor at the following concentrations 66.7, 33.3, 16.7, 8.33 and 0 µL/L to determine the effect of the fumigants on egg laying. Treated *C. maculatus* females did not lay eggs even when exposed to sublethal doses of the monoterpenoids, while control adult beetles exposed to 0 µL/L laid several eggs. However, mated *C. maculatus* females laid eggs on cowpea seeds treated with monoterpenoids if the treated seeds were aerated for one week. The monoterpenoids did not exhibit residual toxicity to the cowpea beetles. Exposure of the developmental stages of the beetle, which include eggs, young larvae (first instar), 4th instar, pupae and adults to different concentrations of the monoterpenoids over 24 h period generated varying levels of mortality. The developmental stages of the beetle that were most tolerant to the monoterpenoids were the 4th instar, and the pupae. All the monoterpenoid evaluated were effective in causing mortality to the stages of *C. maculatus*. These monoterpenoids could be further investigated for the postharvest management of seed beetles of grain legumes.

Key words: pest management, dried beans, monoterpenoids, methyl bromide alternative, *Callosobruchus maculatus*

INTRODUCTION

The cowpea weevil, Callosobruchus maculatus (F.) (Coleoptera: Bruchidae), is a worldwide pest of cowpea, *Vigna unguiculata* (L.) Walpers (Fabales: Fabaceae). Infestation of cowpea by this bruchid commences in the field before mature seeds are harvested (Huignard et al., 1985). Infestation level of cowpea is very low at harvest and may sometimes be undetectable (Huignard et al. 1985). The cowpea weevil multiplies very fast in storage, giving rise to a new generation every month, and losses up to 30 percent in three months (Ouedraogo et al., 1996). Barring containment of the pest, complete loss of cowpea could occur within six months of storage (Caswell 1961). The most effective pest management tool used in the

disinfestation of commercial quantities of cowpea is fumigation with synthetic insecticides such as methyl bromide or phosphine gas (Mbata 2004). Use and production of the fumigant methyl bromide was scheduled to end in developed countries by January 2005 and worldwide by 2015 under the terms of the Montreal Protocol (United Nations Environment Programme 1998). Uses of other insecticides in stored products are facing restriction, and pest populations are evolving resistance to chemical insecticides (Phillips et al., 2000). Several traditional measures for protecting harvested cowpea are in use in subsistence agriculture, but their efficacy is often unverified (Alabeek 1996). Monoterpenoids, which are volatiles from plants, are being proposed here for the management of *C. maculatus* populations in post harvest storage of cowpeas.

Monoterpenoids are 10-carbon, secondary plant chemicals that are major components of essential oils extracted from leaves or fruits of herbs such as *Eucalyptus*, *Ocimum* spp., *Carum carvi* L. (caraway), *Coriandrum sativum* L., and many others (Rice and Coats 1994; López et al., 2008). Most monoterpenoids are volatile and have distinct aromas or flavors which may be pleasant to humans. The monoterpenoids are believed to aid plants in chemical defense against phytophagous insects and are now being exploited as insecticides. Monoterpenoids that have been investigated for insecticidal actions include *E*-anethole, estragole, *S*-carvone, linalool, *L*-fenchone, geraniol, γ-terpinene and *DL*-camphor (Lopez et al., 2008; Pascual-Villalobos et al., 2004; Pascual-Villalobos and Ballesta-Acosta 2003). Many monoterpenoids have been found to be effective against several postharvest insects (López et al., 2008). The authors hypothesize that some or most of these monoterpenoids will deter oviposition in exposed mated females of *C. maculatus*, cause mortality of exposed developmental stages and adults of *C. maculatus*.

MATERIALS AND METHODS

Insects

The cowpea weevil colony used in this study was obtained from laboratory rearing facility of Grain Marketing and Production Research Center, USDA-ARS, Manhattan, Kansas, and has been maintained for in the rearing facility for ten years at the Department of Biology, Fort Valley State University, Georgia. The beetles were reared on cowpea seeds in 1-liter wide-mouth glass jars at 30 ± 0.5°C, 70 ± 5% r.h., and a photoperiod of 12:12h (L:D) as described by Shu et al., (1996).

Eggs, 6 –24 h old (except where otherwise specified), first and last (fourth) instars, 24 h old pupae and adults were used in these experiments. Females of *C. maculatus* glue their eggs on cowpea seeds and the eggs are easily discernable. Seeds bearing 1 to 3 eggs were sorted to obtain a total of thirty seeds with a collective number of sixty eggs per jar and were transferred to 1000 ml rearing jars. The larvae of *C. maculatus* feed and develop internally, and could not be discerned externally by observing the surface of seeds. Radiography was used previously to follow larval and pupal development in this beetle at 30 ± 0.5°C, 70 ± 5% r.h. (Mbata and Reichmuth 1996, Mbata et al., 2000), and these developmental schedules were used to estimate the life stages present in infested seeds tested in this study. Eggs hatch into first instar after 2 d. Following hatching, the color of egg changes from clear to cream-white because of frass deposition in the eggshell. The last instar (4^{th}) is attained between 14 – 17 d while the pupal stage is attained between 18 - 21 d from the day eggs were laid. Thus, in these experiments, 3 d old and 16 d old developing individuals were assumed to be first and last instars, respectively. The number of eggshells with the appearance of successful hatching was used to estimate the number of larvae in seeds used in experiments requiring larvae.

Seeds from cultures of desired ages bearing first and last instars were sorted as described for the eggs and placed in ventilated glass vials with each vial containing 50 larvae. The pupae used in the experiments were from infested seeds that were 19 -20 d old, and could be seen through an opaque pre-emergence "window" in the cotyledons of the seed. Seeds bearing 30 pupae were placed in ventilated vials as described above for eggs. Mated adults used were 36 h old and 6 adults comprising 3 males and 3 females were placed in 1000 ml along with either treated or control pristine uninfested seeds in rearing jars as described for the eggs. Filter papers held in place with a metal lid having hollow-core were used as covers for jars and treatments with monoterpenoids were conducted by injecting the monoterpenoids with 100 µL syringes through the filter papers onto test seeds, which were either uninfested or bore developmental stages. The monoterpenoids investigated are *E*-anethole, estragole, *S*-carvone, Linalool, *L*-fenchone, geraniol, γ-terpinene and DL-camphor. The monoterpenoids were obtained from Sigma-Aldrich Co. LLC, St. Louis, MO 63178, USA. The concentration of monoterpenoid used was 66.7 µL L^{-1}, except when investigating mortality of adults, and oviposition by treated adults that 66.7, 33.3, 16.6 and 8.3 µL L^{-1} concentrations of monoterpenoids were used. As soon as the monoterpenoid was introduced into the system, filter papers used in covering the jars were replaced with metal plates. The jars used were air-tight canning jars. Eighteen jars were set up for each of seven trials and each trial consisted of 2 jars for each monoterpenoid and 2 for the control which had untreated infested seeds. The treatment jars were placed in a chamber maintained at 30.0 ± 0.5°C and 70 ± 5% r.h. The metal lids of the jars were replaced with filter papers after 24h and the jars replaced in the cooled chamber for up to 4 weeks or until the complete emergence of all surviving beetles. Seeds bearing eggs, larvae, and pupae were observed daily for adult emergence. The individuals that failed to emerge 2 wk following emergence of the last adult from control are deemed to have died. Adults treated in these studies were considered dead if they were immobile 24 h after treatment. Mortality values were recorded and analyzed.

Data analysis

Treatment effects were determined by using analysis of variance (ANOVA; Proc GLM), and differences in treatments were elucidated through Turkey's test and LSD ($\propto$ = 0.05) (SAS, 2001). Percentage data were arcsine of square root transformed.

RESULTS AND DISCUSSION

Mortality data resulting from treatments of cowpea seeds infested by life stages of *C maculatus* with monoterpenoids are shown in Tables 1 -3.

Exposure of adults beetles to cowpea seeds treated with concentrations (8.3 – 66.7 µL L^{-1}) of monoterpenoids showed that minute quantities of the different monoterpenoids had effect on the mortality of the beetles. The critical concentration of the monoterpenoids investigated that generated 100% mortality in adult beetles was 16.7 µL L^{-1}. Exposure of seeds infested with life stages, eggs, 1st instar, 4th instar, pupae and adults of *C. maculatus* with 66.7 µL L^{-1} of monoterpenoids generated mortality in the life stages that ranged between 85.3 and 100%. These mortality values were significantly higher than those of the control (Table 2).

The eggs and adults of the beetle were more susceptible to the monoterpenoids than the 4th instar and pupae since individuals from these late developmental stages survived to adulthood. All the eight monoterpenoids investigated in this study were effective against *C. maculatus*. In an earlier observation, DL-camphor and estragole were the only

monoterpenoids effective against diapausing larvae of *Plodia interpunctella* (Mbata et al., 2012 in press).

Table 1. Mortality (% ± SE) of adult *C. maculatus* exposed to different concentrations of monoterpenoids

Monoterpenoids	Concentration(μg L^{-1})			
	8.3	16.7	33.3	66.7
E-anethole	78 ± 14.5B	100 ± 0A	100 ± 0A	100 ± 0A
Estragole	62 ± 18.5BC	100 ± 0A	100 ± 0A	100 ± 0A
S-carvone	74 ± 21.3B	100 ± 0A	100 ± 0A	100 ± 0A
Linalool	72 ± 16.7B	100 ± 0A	100 ± 0A	100 ± 0A
L-fenchone	85 ± 18.5AB	100 ± 0A	100 ± 0A	100 ± 0A
Geraniol	77 ± 18.5B	100 ± 0A	100 ± 0A	100 ± 0A
γ-terpinene	67 ± 13.7BC	94 ± 18.9A	100 ± 0A	100 ± 0A
DL-camphor	50 ± 16.7C	91 ± 13.5A	91 ± 14.8A	100 ± 0A
Control	7 ± 1.8D	7 ± 1.8D	7 ± 1.8D	7 ± 1.8D

Values in rows or columns having different uppercase letters are significantly different ($P < 0.05$)

Table 2. Mortality (% ± SE) of life stages of *C. maculatus* exposed to monoterpenoids (66.7 μL)

	Eggs	1st instar	Fourth instar	Pupae	Adults
E-anethole	100 ± 0A	100 ± 0A	100 ± 0A	85.6 ±17.5A	100 ± 0A
Estragole	100 ± 0A	100 ± 0A	100 ± 0A	91.3 ± 9.2A	100 ± 0A
S-carvone	100 ± 0A	100 ± 0A	99.2 ± 2.7A	99.3 ± 2.2A	100 ± 0A
Linalool	100 ± 0A	100 ± 0A	98.3± 5.6A	85.3± 16.9A	100 ± 0A
L-fenchone	100 ± 0A	100 ± 0A	98.4 ± 5.4A	98.7 ± 3.3A	100 ± 0A
Geraniol	100 ± 0A	100 ± 0A	95.1 ± 9.2A	86.7 ± 13.6A	100 ± 0A
γ-terpinene	100 ± 0A	100 ± 0A	96.7 ± 10.5A	92.0 ± 15.4A	100 ± 0A
DL-camphor	100 ± 0A	100 ± 0A	97.5 ± 4.9A	88.7 ± 8.9A	100 ± 0A
Control	9.0 ± 3.7C	17.3 ± 5.2C	7.4 ± 1.8C	2.0 ± 0.6D	7.0 ± 1.8C

Values in rows or columns having different uppercase letters are significantly different ($P < 0.05$)

Mated adult females provided with seeds exposed to 8.3 μg L^{-1} of the monoterpenoids did not deposit eggs on the seeds. However, when seeds treated with monoterpenoids were aerated for 21 d following treatment mated females laid eggs on them (Table 3). It is probable that the monoterpenoids inhibited oviposition by the female beetles. In addition, it appears that the monoterpenoids did not exhibit residual toxicity to the beetles.

Table 3. Eggs (No. ± SE) laid by *C. maculatus* females on seeds treated with monoterpenoids (8.3 µL)

	1 hr Post treatment	21 d Post treatment
E-anethole	0C	27.5 ± 4.3A
Estragole	0.4 ± 0.2C	29.3 ± 6.9A
S-carvone	0C	25.3 ± 3.5A
Linalool	0.6 ± 0.2C	24.3 ± 7.4A
L-fenchone	0C	31.8 ± 4.7A
Geraniol	0C	24.5 ± 3.4A
γ-terpinene	0C	25.8 ± 5.1A
DL-camphor	3 ± 1.8B	27.0 ± 3.3A
Control	28.7 ± 2.7A	27.0 ± 3.1A

Values in rows or columns having different uppercase letters are significantly different ($P < 0.05$)

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