Donahaye, E.J., Navarro, S. and Leesch J.G. [Eds.] (2001) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, Fresno, CA. 29 Oct. - 3 Nov. 2000, Executive Printing Services, Clovis, CA, U.S.A. pp. 119-124

RESPONSE OF *TRIBOLIUM CASTANEUM* AND *SITOPHILUS ZEAMAIS* TO POTENTIAL FUMIGANTS DERIVED FROM ESSENTIAL OILS OF SPICES

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ABSTRACT

Essential oils derived from four spices - star anise, nutmeg, cinnamon and garlic - were found to have fumigant activity to *Tribolium castaneum* and *Sitophilus zeamais*. The fumigant activity of anethole, derived from star anise, was similar for both adults of *T. castaneum* and *S. zeamais,* when compared at the LC_{50} level. However, at the LC_{95} level, *S. zeamais* was ~200x more tolerant than *T. castaneum*. *S. zeamais* adults were 1.7x more susceptible than T *castaneum* adults to the fumigant action of nutmeg oil, (LC_{50} of 4.5 and 7.7 mg/cm^2 respectively). *T. castaneum* adults were twice as susceptible as *S. zeamais* adults to the fumigant activity of cinnamaldehyde, from cinnamon oil (LC_{50} of 0.28 and 0.54 mg/cm^2 respectively). *T. castaneum* larvae became progressively more tolerant with age. Three constituents of garlic oil were tested for fumigant activity to these two species of insects. Allyl disulfide, methyl allyl disulfide and diallyl trisulfide all had more potent fumigant action against *T. castaneum* adults than against their larvae or against *S. zeamais* adults. The larvae of *T. castaneum* became also more tolerant with age to the three compounds from garlic oil. Among these compounds, diallyl trisulfide was the most potent for both species of beetles (LC_{50} for *T. castaneum adults* = 0.83 mg/L air; LC_{50} for *S. zeamais* adults = 6.32 mg/L air). It was also the most potent of all the essential oils studied. These essential oils and their constituents, particularly diallyl trisulfide, have the potential to be developed as fumigants for stored-product protection.

INTRODUCTION

Currently fumigation and application of chemical grain protectants are measures commonly used to control pest infestation in grain and other dried foodstuffs. Methyl bromide (MB) is being gradually phased out (Anon. 1997) due to its status as an ozone-depleting substance (Banks, 1994). Although there are a number of alternative fumigants to MB, many of them are either in restricted use (e.g. ethylene oxide, carbon disulfide) or under research (e.g. carbonyl sulfide, methyl iodide), and much work is still needed before registration for use comes into effect (Banks, 2000). The race is on for the search for alternative fumigants that are safer for the environment.

Plants produce essential oils that have been found to possess insecticidal activities to various species of insects. Essential oils from many herbs and spices have been reported to have fumigant and other activities (Rice and Coats, 1994; Regnault-Roger and Hamraoui, 1995). Essential oils from Labiatae, Umbelliferae and Lauraceae have a fumigant toxic effect on *Acanthoscelides obtectus* Say (Regnault-Roger and Hamraoui, 1994). Shaaya *et al.* (1991, 1997), found that a number of essential oils from spices showed fumigant toxicity to stored-product insects. More recently, the fumigant toxicity of the essential oils from the spices anise, *Pimpinella anisum*, and cumin, *Cuminum cyminum*, to *Tribolium confusum* (J. du Val.) and *Ephestia kuehniella* Zeller has been studied (Tunç *et al*., 2000).

In our laboratory, we have been screening a large number of plant species for bioactivities against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais Motsch.* We found that essential oils from spices are potential insecticides against these stored-product insects. Among these, essential oils from star anise (Ho *et al*., 1997), nutmeg (Huang *et al*., 1997), cinnamon (Huang and Ho, 1998), and garlic (Ho *et al*., 1996; Chiam *et al*., 1999; Huang *et al*., 2000) have contact and fumigant activities to these insects.

This paper compares the fumigant toxicity of *trans*-anethole (extracted from star anise oil), essential oil of nutmeg, cinnamaldehyde (a constituent of cinnamon oil), and three constituents of garlic oil to *T. castaneum* and *S. zeamais*.

MATERIALS AND METHODS

Insects

Adults (2-4 week old) of *S. zeamais* and *T. castaneum* were obtained from laboratory cultures maintained in the dark at 30±1°C and 70-80% r.h. *S. zeamais* was cultured on whole wheat grain (*Triticum aestivum* L.) of 13.5% m.c., and *T. castaneum* was cultured on wheat flour ground from the whole wheat grain mixed with yeast (10:1, w:w). *T. castaneum* larvae used in the experiments were 12, 14, and 16 d old. All experiments were carried out in the dark in incubators set at 30±1°C and 70-80% r.h.

Spices

The spices from which essential oils were extracted were star anise, *Illicium verum* Hook f. (Illiciaceae), nutmeg, *Myristica fragrans* Houtt (Myristicaceae), cinnamon, *Cinnamomum aromaticum* Nees (Lauraceae), and garlic, *Allium sativum* L. (Amaryllidaceae).

Extraction of essential oils

Dried star anise fruit was ground, steam-distilled and extracted with *n*-hexane to obtain the essential oil. The oil was purified by liquid chromatography and the major component of oil, *trans*-anethole, was identified using NMR and IR spectrometry. Nutmeg seeds were ground to a powder and steam-distilled. The essential oil was extracted with *n*-hexane. Dried bark of cinnamon was ground to a powder and steam distilled and extracted with methylene chloride. The major constituent was confirmed to be cinnamaldehyde. Subsequently, cinnamaldehyde (98% purity) was purchased from Sigma, U.S.A. Peeled fresh garlic cloves were minced in a blender and steam distilled, and the oil was extracted with *n*-hexane. Constituents of the garlic oil were purified by reversed-phase HPLC, and identified using NMR and mass spectroscopy. Two constituents, methyl allyl disulfide and allyl trisulfide, were isolated for the study. A third compound, allyl disulfide (80% purity) was purchased from Aldrich, U.K.

Fumigant toxicity bioassays

Fumigation vessels were set up according to the method of Huang *et al*. (1997). A filter paper (2 cm diam.) was placed on the underside of the screw cap of a glass vial (2.5 cm diam., 5.5 cm high), and impregnated with aliquots of 25 μL of an appropriate concentration of the chemical solution, or solvent alone (*n*-hexane or acetone, depending on the solvent used in making dilutions) in the controls. After evaporation of the solvent, the cap containing the impregnated filter paper was screwed tightly onto a glass vial containing 10 *T. castaneum* (adults or larvae) or *S. zeamais* adults. Ten replicates were set up for each treatment and control. The vials were incubated at 30±1°C and 70-80% r.h. After 24 h, the insects were transferred to clean vials with culture media and returned to the incubators. End-point mortality was observed and recorded after 7 days.

RESULTS AND DISCUSSION

The fumigant toxicity of the essential oils of the spices and some of the constituents of the spices are presented in Table 1. At lower dosages, both *T. castaneum* and *S. zeamais* adults were equally susceptible to the fumigant toxicity of anethole. However, at the LC_{95} level, *S. zeamais* was about 200× more tolerant than *T. castaneum*. *S. zeamais* adults were about 1.7× more susceptible than *T. castaneum* adults to nutmeg oil at both LC_{50} and LC_{95} levels. In contrast, adult *T. castaneum* were twice as susceptible as adult *S. zeamais* to cinnamaldehyde. *T. castaneum* adults were also more susceptible than the larvae to cinnamaldehyde, which became progressively more tolerant with age.

TABLE 1
Fumigant toxicity of *trans*-anethole, nutmeg oil and cinnamaldehyde to *T. castaneum* and *S. zeamais*

Essential oil/compound	LC_{50} (mg/ L air)		LC_{95} (mg/L air)	
	T. castaneum	*S. zeamais*	*T. castaneum*	*S. zeamais*
trans-Anethole	208	228	256	>50000
Nutmeg oil	971	562	2228	1314
Cinnamaldehyde	35.2	67.9	40.2	224

(Data from Ho and Huang, 1997; Huang *et al*., 1997; Huang and Ho, 1998. All values converted to mg/L air).

A comparison is made in Table 2 of the fumigant toxicity of the three constituents of garlic oil, namely allyl disulfide, methyl allyl disulfide and diallyl trisulfide. All three compounds were more toxic to adults than to larvae of *T. castaneum*, and the larvae became progressively more tolerant with age. In addition, *T. castaneum* adults were more susceptible than *S. zeamais* adults. This trend is similar to that of cinnamaldehyde. At the LC_{50} level, the toxicity ratio of *S. zeamais* to *T. castaneum* is as follows: allyl disulfide = 5.2, methyl allyl disulfide = 2.8, diallyl trisulfide = 7.6. Of the three compounds, diallyl trisulfide is the most potent fumigant to both species of insects. It is 5× and 2× more potent than methyl allyl disulfide to adults of *T. castaneum* and *S. zeamais*, respectively, and 4.6× and 3× more potent than allyl disulfide to *T. castaneum* and *S. zeamais*, respectively. When compared to cinnamaldehyde, diallyl trisulfide is 4× and 11× more potent to *T. castaneum* and *S. zeamais*, respectively. Cinnamaldehyde is 28× and 8× more effective than nutmeg oil against *T. castaneum* and *S. zeamais*, respectively. The low potency of nutmeg oil is probably due to the crude extract used in the study. Isolation and purification of the major compounds may show increased potency.

TABLE 2
Fumigant toxicity of allyl disulfide, methyl allyl disulfide and diallyl trisulfide to *Tribolium castaneum* and *Sitophilus zeamais*

Compound	Insect	Life stage	LC_{50} (mg/L air)	LC_{95} (mg/L air)
Allyl disulfide	*T. castaneum*	Adults	7.2	15.9
		12-d larvae	36.7	175
		14-d larvae	134	-*
		16-d larvae	104	-*
	S. zeamais	Adults	22.5	32.8
Methyl allyl disulfide	*T. castaneum*	Adults	4.3	6.6
		12-d larvae	6.6	16.4
		14-d larvae	6.1	18.0
		16-d larvae	22.2	57.3
	S. zeamais	Adults	12.1	17.2
Diallyl trisulfide	*T. castaneum*	Adults	0.83	1.2
		12-d larvae	3.7	10.6
		14-d larvae	5.2	14.5
		16-d larvae	5.2	15.2
	S. zeamais	Adults	6.3	12.2

* Values are too high to be determined.
(Data taken from Chiam *et al.*, 1999; Huang *et al.*, 2000).

The data for toxicity of commonly used fumigants to adults of *S. zeamais* and *T. castaneum* (Anon. 1975) were adjusted to 24 h exposure using the assumption of a constant concentration by time (Ct) product for ease of comparison with our data. For *T. castaneum,* the LC_{50}'s of allyl disulfide, methyl allyl disulfide and diallyl trisulfide are 3.80 mg/L, 4.32 mg/L and 0.83 mg/L, compared with 40.3 mg/L for MB and 0.01 mg/L for phosphine. For *S. zeamais* adults, the LC_{50}'s of allyl disulfide, methyl allyl disulfide and diallyl trisulfide are 19.8 mg/L, 12.1 mg/L and 6.32 mg/L respectively, compared with 15.4 mg/L for MB and 0.008 mg/L for phosphine. Thus, these three compounds from garlic oil are much more potent fumigants than MB. Even cinnamaldehyde, with an LC_{50} of 35.2 mg/L, is more toxic than MB to *T. castaneum.*

Although anethole, nutmeg oil and cinnamaldehyde are less potent than the three constituents of garlic oil, all of them possess contact toxicity and other anti-insect properties to both species of beetles (Ho *et al.*, 1997; Huang *et al.*, 1998; Huang and Ho, 1998; Chiam *et al.*, 1999; Huang *et al.*, 2000). These are potentially useful grain protectants that have contact and fumigant toxicity. The three compounds from garlic oil, particularly diallyl trisulfide, can be developed as potential fumigants for stored products protection. However, field trials with suitable formulations need to be carried out to further assess the efficacies of these essential oils as fumigants.

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