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Toxicity of mustard essential oil to larvae and pupas of *Sitophilus zeamais* (Coleoptera: Curculionidae)

R.R. da Costa², A.H. de Sousa^{1,*}, L.R.D'A. Faroni², O.D. Dhingra³, M.A.G. Pimentel¹

Abstract

The toxicity of vapors of the essential oil of mustard (*Brassica rapa* L.) to first and third-instar larvae and pupa of *Sitophilus zeamais* (Coleoptera: Curculionidae) and lethal concentration for 50 and 95 % mortality (LC₅₀ and LC₉₅) for each larval instar and pupa was estimated. The bioassays were carried out in sealed 800-ml glass jars containing 10 g of maize grains infested with *S. zeamais* larvae and pupas. The required amount of the oil was imbibed on to 1.5 cm² filter paper disc in a Petri dish placed on jar's bottom. The dish was covered with a muslin cloth to avoid direct contact of grains carrying larvae and pupas with the oil. The infested grains were exposed to the oil vapors for 24 h. After fumigation adult insects emerging from the grains were counted 45 days after the beginning of oviposition, which was 72 hours. Each experiment was repeated three times. LC₅₀ and LC₉₅ for first and third-instar larvae and pupa were respectively 4.63 and 10.32; 5.17 and 13.29; and 6.17 and 15.78 ml/l jar volume. It was concluded that first-instar larvae were more susceptible than third-instar larvae, and that the pupa stage was most resistant to the vapors of mustard essential oil.

Key words: Allyl isothiocyanate, insecta,

Brassica rapa, storage, grains.

Introduction

Severe attack by insects is one the major factors that affects grain quality and their commercial value during storage. *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae) is one the most destructive pest of stored grains in Brazil. This pest has a wide host range, high biotic potential, high capacity to penetrate the grain mass and can infest grains in the field as well in the storage (Loeck, 2002). The control of this pests is difficult because it completes young developmental stages inside the grain. Female individually inserts the eggs into small cavities made by chewing activity. The cavity is then covered with a waxy secretion, sealing the egg into the grain, the eggs hatch in about six days and the larvae feed on grains internally. Pupation takes place within the grain after fourth instars and the cycle is completed in about 35 days at about 27 °C and 70 % relative humidity (Rees, 1996; Loeck, 2002; Lorini, 2002).

In Brazil, *S. zeamais* is controlled by the use of grain treatment with phosphorated and pyrethroids insecticides, although fumigation with phosphine is most common. The extensive and indiscriminated use of phosphine is resulting is

¹Department of Animal Biology, Federal University of Viçosa (UFV), Viçosa, MG, 36570-000, Brazil.

²Department of Agricultural Engineering, Federal University of Viçosa (UFV), Viçosa, MG, 36570-000, Brazil.

³Department of Phytopathology, Federal University of Viçosa (UFV), Viçosa, MG, 36570-000, Brazil.

* Corresponding author. Department of Animal Biology, Universidade Federal de Viçosa, Viçosa, MG, 36570-000, Brazil. Phone: +55-31-3899-1919; Fax: +55-31-3899-2537. E-mail address: adalberto@insecta.ufv.br (AH Sousa).

the appearance of resistant populations (Bell et al., 1977; Bengstone et al., 1999). Therefore, it is imperative to find alternative controls, preferably eco-friendly plant derived bioactive compounds. Most commonly used botanical extracts with insecticidal activity are essential oils and organic solvent extracts.

Plants produce secondary metabolites many of which act as natural defense against insects and disease causing microorganisms. (Potenza et al., 2004). More than 100,000 secondary metabolites from about 200,000 plant species have been identified, which include alkaloids, terpenoides, flavonoides and others which can have insecticidal properties (Vendramim & Castiglioni, 2000; Potenza et al., 2004). Some plants in the Brassicaceae (Cruciferae) family, such as mustard (*Brassica rapa*, *B. juncea*), cabbage (*B. oleracea*) and crambe (*Crambe abyssinica*) produce glucosinolates as secondary metabolites that help plants defend themselves against phytophagous insects, fungi and other pests (Tsao et al., 2002; Noble et al., 2002; Dhingra et al., 2004). The glucosinolates are enzymatically hydrolyzed to the sugar moiety and isothiocyanates (Borek et al., 1997; Noble et al., 2002). Allyl isothiocyanate (AITC) is the most toxic compound formed from allyl glucosinolate hydrolysis (Mayton et al., 1996), and possibly the most important for biofumigation (Noble et al., 2002). Among the *Brassica* species, the highest concentrations of AITC are found in some mustard, horseradish and wasabi species; and there is considerable variation among the cultivars of the same species (Olivier et al., 1999; Yu et al., 2003). The *in planta* AITC content can be affected by the region of production and cultural practices (Sultana et al., 2002; Yu et al., 2003). AITC is widely used as a flavoring agent by the food industry and is classified as generally regarded as safe (GRAS) by the Food and Drug Administration of the United States (Furia, 1972). As flavoring agent AITC can be hydro-distilled from macerated mustard leaves or wet-ground seeds, or can be synthesized chemically.

In search for alternative methods to control insect pests of stored grains, this initial

exploratory study was done to evaluate the toxicity of the vapors of the essential oil of wild mustard (*Brassica rapa* L.) to the larvae of the first and third-instar and pupa of *S. zeamais*.

Materials and methods

Extraction of the essential oil

The essential oil was extracted from mustard seeds using the following procedure: Five kilogram of mustard seeds were ground to pass through a 0.5 mm screen and soaked in 10 l of water. The mixture was allowed to stand for 4 h at room temperature for hydrolyzing glucosinolates and then hydrodistilled using a stationary distillator at 100 °C and air flow of 5 l/min. The distillate containing water and the essential oil was collected into several separatory funnels, and allowed to stand for 2 h to separate the oil from the water phase. The water phase was discarded and the collected essential oil was dehydrated by passing through a 5-cm column of anhydride sodium sulfate. It was then emulsified with 10 % Tween® 20 and refrigerated until use. The essential oil was diluted with soybean oil (1:9, v/v) before use for easy handling and measurements.

Obtaining different developmental phases of *S. zeamais*

The following procedure was used to obtain larvae of the first and the third instars and pupa of *S. zeamais*. Lots of clean corn (300 g) were exposed for 72 hours to 500 adult insects of *S. zeamais*, in a 800-ml glass jar, to obtain the ovipositioned grains. Knowing the number days required to obtain each phase of the insect after the oviposition until emergence of the adults and the optimum conditions for their development (Golebiowska, 1969; Rees, 1996), the ovipositioned grains were incubated at 27 ± 2 °C and relative humidity of 65 ± 5 % until the period required for completion of the each developmental stage (7 to 12 days for larvae of the first instar, 19 to

24 days for the larvae of the third instar and 31 to 36 days for pupae).

Concentration-response bioassay

The bioassays were done in sealed glass jars of 800 ml capacity. The concentrations of the essential oil used for bioassay on larvae of the first instar varied between 2.25 and 8.1 ml/l jar volume, with interval of 1.46 ml, while for assays on larvae of the third instar and the pupa, the concentrations varied between 2.25 to 11.25 ml/l jar volume with the interval of 2.25 ml. The required amount of essential oil was applied onto a 1.5 cm² disc of filter paper held in a Petri dish in the bottom of the jar. To avoid the direct contact between the oil and the infested grains or insects, the plates were covered with a muslin cloth. Each jar received 10g of corn grains carrying larvae or pupae of *S. zeamais*. The jars treated in similar manner but without the use of the essential oil served as control. The jars were sealed for 24 h and then opened to remove the Petri plate containing the oil carrying paper disc, and to allow for dissipation of vapors of the essential oil. The efficacy of the fumigation with the essential oil on each developmental stage was determined, 45 days after the oviposition, by

counting the number of adults emerging from the grains. All bioassays were done in three replications and repeated.

Data analysis

The data were analyzed by the Probit analysis according to Finney (1971), using the SAS's PROC PROBIT software (SAS Institute, 1989), which generated the concentration-response curve.

Results

The three developmental stages of *S. zeamais* showed different sensitivity to the toxic effects of the mustered essential oil (Figure 1). The larvae of the first instar were most sensitive followed by the larvae of the third instar. The pupas were most resistant of the three stages of the insect assayed. The mean LC₅₀ for larvae of first and third instar, and pupa was calculated to be 4.63, 5.17 and 6.17 ml/l jar volume, respectively, while the LC₉₅ for the respective developmental stage was estimated to be 10.32, 13.29 and 15.78 ml/l jar volume (Table 1).

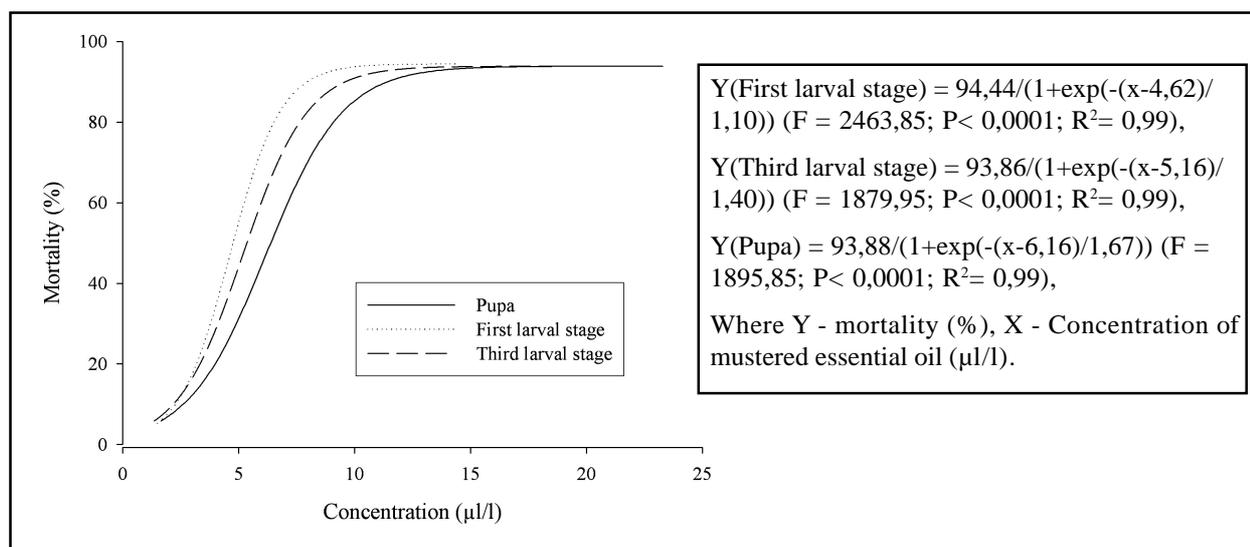


Figure 1. Probit concentration-response curve of the toxicity of mustered essential oil towards larvae of the first and third instars, and pupas of *Sitophilus zeamais*.

Table 1. Lethal concentration of mustered essential oil for 50 % (LC₅₀) and 95 % (LC₉₅) mortality of the larvae of the first and the third instar, and pupa of *Sitophilus zeamais*.

Developmental stage	Inclination ± SEM ¹	LC ₅₀ (FI 95 %) µl/l	LC ₉₅ (FI 95 %) µl/l	χ ²	p
First instar	4.73 ± 0.45	4.63 (4.29 - 4.99)	10.32 (9.00 - 12.50)	5.83	0.12
Third instar	4.01 ± 0.39 (4.65 - 5.69)	5.17 (11.36 - 16.57)	13.29	2.83	0.41
Pupa	4.03 ± 0.58	6.17 (5.51 - 6.85)	15.78 (12.68 - 22.96)	4.08	0.25

¹ S.E.M. = Standard error of mean; LC = Lethal concentration; FI 95 % = Fiducial Interval at 95 % probability; χ^2 = Qui-square; p = Probability.

Discussion

The results of this initial exploratory study show that the vapors of essential oil of mustered can penetrate the grain and kill, within 24 h, the internal larvae and the pupa of *S. zeamais* in the infested grains, at relatively low concentrations, like other grain fumigants such as phosphine and methyl bromide. The lethal concentration for 50 or 95 % mortality, however differ significantly among the developmental stages of the insect. Pupae proved to be most resistant to the essential oil.

Among the many factor that can affect toxicity of fumigant insecticides on the insect pests of the stores grains, the developmental stage of the insect most important because of the different respiratory intensity, and the pupa along with the eggs have the least respiratory rate (Bijok, 1996; Emekci et al., 2002; Mahroof, 2003). Consequently the developmental stage of low respiration rate absorbs less quantity of the fumigant in a given time period (Gonçalves et al., 2000). In this study the essential oil, did have the direct contact with the infested grains, thus the bioactive compound penetrated the larvae or pupa through respiratory system, which may explain the requirement for higher lethal concentration for pupa. Although not examined, it is possible that if the fumigation can be carried out for longer period, the lethal concentration of the

vapors of the essential oil in the atmospheres could have been lower.

According to Chapman (1998) during the pupal period oxygen consumption initially declines and then rises again, due to changes in the enzymes systems regulating energy release. The higher lethal concentration required the mortality of larvae of the third instar, compared to the larvae of the first instar, also might be related to its lower metabolic rate (Gonçalves et al., 2000). It is well accepted that the developmental phases of insects with higher metabolic rate are more susceptible to gas exchange disequilibria incited by an insecticide. (Emekci et al., 2001), resulting in the higher energy expense for homeostase maintenance (Hostetler et al., 1994; Harak et al., 1999)

Tsao et al. (2002) reported that in cockroaches (*Blattella germanica* (L.) (Dictyoptera: Blattellidae) *Periplaneta americana* L. (Blattodea: Blattidae) CO₂ production rate increased when exposed to sub lethal concentrations of AITC (the major component of the mustered essential oil), which led to the belief that like the insecticide dinitrophenol, the AITC might also act on the energy metabolism, where AITC may be acting as inhibitor of oxidative phosphorylation, and thus interrupting ATP formation.

The data from this initial study strongly suggest that fumigation with mustered essential

oil has the potential to control insect pests in stored products independent of development phase, thus warranting detailed and extensive research to develop it into an effective grain fumigant to substitute methyl bromide or phosphine.

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