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RELATIVE TOXICITY OF MUSTARD ESSENTIAL OIL TO INSECT-PESTS OF STORED PRODUCTS[1]

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ABSTRACT - The relative toxicity of the mustard (*Brassica rapa* L.) essential oil (MEO) on young and old larvae, pupae, and adults of *Sitophilus zeamais* and *Callosobruchus maculatus* was determined using concentration-response bioassays. The respiration rate of adults was measured to determine its influence on its toxicity. Different developmental stages of both species differed significantly in their response to MEO, with the adults being much more susceptible than the immature stages. Although adult mortality did not differ between species, the adult respiration rate of *C. maculatus* was significantly higher than that of *S. zeamais*. Thus, the toxicity oil for adults was not influenced by natural respiration rate of species investigated.

Keywords: Biofumigation. *Callosobruchus maculatus.* Grains. *Sitophilus zeamais*.

TOXICIDADE RELATIVA DO ÓLEO ESSENCIAL DE MOSTARDA PARA INSETOS-PRAGA DE PRODUTOS ARMAZENADOS

RESUMO - A toxicidade relativa do óleo essencial de mostarda (*Brassica rapa* L.) (OEM) para as fases de larvas jovens e velhas, pupas e adultos de *Sitophilus zeamais* e *Callosobruchus maculatus* foi determinada usando bioensaios de concentração-resposta. A taxa respiratória dos adultos foi mensurada para determinar se há influencia desta característica sobre a toxicidade do óleo. Observou-se variação de resposta para o OEM entre os estágios de ambas as espécies, onde os adultos foram mais susceptíveis que os estágios imaturos. Embora a mortalidade dos adultos não tenha diferido entre as species, a taxa respiratória dos adultos de *C. maculatus* foi significativamente maior que a de *S. zeamais*. Desta forma, a toxicidade do óleo para os adultos não foi influenciada pela taxa respiratória natural das espécies investigadas.

Palavras-chave: Biofumigação. *Callosobruchus maculatus.* Grãos. *Sitophilus zeamais*.

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INTRODUÇÃO

The search for botanical insecticides to control stored-product insect intensified recently (ISIKBER et al., 2006; COITINHO et al., 2010; SILVA et al., 2012) due to the phasing out of methyl bromide, for its negative environmental impact including its role in the depletion of ozone layer, and also due to the appearance of phosphine resistant populations (PIMENTEL et al., 2009).

Plants produce secondary metabolites which frequently act as natural defense against phytophagous insects and plant pathogens (POTENZA et al., 2004; ISMAN, 2008; GUERRA et al., 2009). Some plants of the family Brassicaceae (Cruciferae) produce glucosinolates whose hydrolytic products are toxic to insects, nematodes, bacteria and fungi (DHINGRA et al., 2004; MANSOUR et al., 2012). The glucosinolates are enzymatically hydrolyzed at the sugar moiety producing isothiocyanates. Allyl isothiocyanate (AITC) is the most toxic compound formed from allyl glucosinolate hydrolysis, and appears to be the most promising product for biofumigation (SANTOS et al., 2011). 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 (DHINGRA et al., 2004).

Most of the researches with new biofumigants emphasize the lethal effect of the compounds on the adult phase of insects, ignoring the importance of their toxicity to immature stages and the factors associated with the toxicity (SOUSA et al., 2009; COSTA et al., 2014; FOUAD et al., 2014). This may be a mistake for management of stored grain insects, due to at least two reasons: (1) the grains may act as a physical barrier against the fumigants for the species that present their young development phases within the grains; (2) the efficacy of the fumigants may be lower in differents species, developmental stages and population of insects with lower respiration rate (PIMENTEL et al., 2007). The respiratory system is the main entrance route of fumigants into the insect body. Therefore, the capture, absorption and consequently the toxicity of the compounds may increase with the respiration rate of the insects. However, this relationship is not always observed (SOUSA et al., 2008; SOUSA et al., 2012).

So this study aimed to evaluate the relative toxicity of the mustard essential oil (MEO) vapors of wild mustard (*Brassica rapa* L.) to young and old larvae, pupae and adults of *Sitophilus zeamais* Motschulsky and *Callosobruchus maculatus* (F.). The respiratory rate of the adults was also evaluated.

MATERIAL AND METHODS

The experiments were conducted at the Sector of Pre-processing and Storage of Agricultural Products, Department of Agricultural Engineering, Federal University of Viçosa (UFV), Viçosa, MG, Brazil. The MEO was extracted from mustard seeds. The botanical samples were collected at agricultural crops of Viçosa, subsequently dried at 40 °C and ground in a wiley mill to obtain a fine powder.

For extraction 5 kg seeds were ground to pass through 0.5 mm sieve, and the powder was mixed with in 10 L of water. The mixture was left to stand for 4 hours at room temperature to allow for enzymatic hydrolysis glucosinolates and then hydrodistilled using a stationary distillator at 100 ºC. The distillate containing water and the MEO was collected into several fractionation funnels, and then allowed to stand for 2 hours to separate the oil from the water phase. The water phase was discarded and the collected MEO was dehydrated by passing through a 5-cm column of anhydrous sodium sulfate. It was then emulsified with 10% Tween® 20 and refrigerated until use. The MEO was diluted with soybean oil (1:9 v/v) before use, for easy handling and measurements.

Maize and bean grains infested with young and old larva e, pupae and adults of *S. zeamais* and *C. maculatus* were obtained by the modified technique of Martinazzo et al. (2000). *Fifty* unsexed *adults S. zeamais* of 5-9-day-old were put in 200 g of corn grain. After 5 days, the adults were removed. The grains were stored to obtain the young and old larvae and pupae (11, 22 and 33 days after infestation, respectively). For *C. maculatus*, f*ifty* unsexed *adults* of 5-9-day-old were put in 200 g of beans. After 4 days, the adults were removed. The beans were stored to obtain the young and old larvae and pupae (8, 16 and 24 days after infestation, respectively).

The toxicity of MEO was determined using concentration-response bioassays. The concentration -response curves were established by increasing concentrations of MEO with an exposure period of 24 hours. Preliminary tests were carried out to estimate the maximum and minimum concentrations to be used for the concentration-response bioassays, and five to six exposure intervals were established within the range of concentrations recognized for each stage.

The bioassays were carried out in sealed glass jars of 800 mL capacity. The required amount of MEO was applied onto a 1.5 cm^2 filter paper disc in a held in a Petri dish at bottom of the jar. To avoid the direct contact between the MEO and the infested grains or insects, the dishes were covered with a muslin cloth. Each jar received 10 g of maize or bean grains containing larvae, pupae, or 50 adults of either *S. zeamais* or *C. maculatus*. The control jars were treated in a similar way but without the use of the MEO. Adult mortality of *S. zeamais* and *C. maculatus* was evaluated after 24 hours exposure

fects possibly not observed at the first assessment mortality. For the immature stages, the toxicity was evaluated 35 and 45 days after the oviposition for *C. maculatus* and *S. zeamais*, respectively, by counting the number of adults emerged from the grains. The period of exposure of grains infested with early life stages of the two species was also 24 hours. All bioassays were carried out in four replicates.

The respiration rate of *S. zeamais* and *C. maculatus* adults was determined by analyzing CO_2 production with the use of a TR2 carbon dioxide analyzer (Sable System International, Las Vegas, USA). This analysis could not be carried out for immature phases since they develop inside the grains, thus confounding CO_2 accumulation for these phases. A series of 25 mL flasks, containing 20 adults was placed in a completely hermetic system and CO_2 production in each flask was measured at 25 ºC, after an acclimatizing period of 10 hours. Three replicates were used for each population. CO_2-free air was fluxed for 2 min through the flasks, at 600 mL min^{-1} flow rate.

The concentration-response bioassays data were subjected to probit analysis to generate concentration-mortality curves (PROC PROBIT; SAS INSTITUTE, 2002). The confidence intervals for the toxicity ratios (TRs) were calculated following Robertson e Preisler (1992), and the lethal concentration (LC) values were considered significantly different (P<0.05) if the confidence limits on the TR did not include the value 1. Carbon dioxide production was subjected to analysis of variance (P<0.05) (PROC GLM; SAS INSTITUTE, 2002), and subsequent Tukey's HSD test, if there was significant variation among species.

RESULTS AND DISCUSSION

Response variation to the essential oil was observed among the development stages of *S. zeamais* and *C. maculatus* (Table 1), where the TR (at LC_{50}) ranged from 1.0- to 5.09-fold and 1.0- to 3.23-fold, respectively. The adults of both species was more susceptible than the immature stages, and there was not significant difference between the immature stages (*S. zeamais*: 3.82-5.08-fold, *C. maculatus*: 3.23-3.28-fold). The majority of the slopes of the concentration–mortality curves were similar between the developmental stages. These results indicate uniform susceptibility of immature stages of both species. The respiration rate of the *S. zeamais* was significantly lowest than that of *C. maculatus* ($F_{4,5}$ = 97.36; P>0.0006), with the mean CO_2 production of 0.92 ± 0.052 and 0.37 ± 0.018 µmol $insect^{-1}$ $hour^{-1}$, respectively (Figure 1).

Table 1. Relative toxicity of the mustard essential oil for development stages of *Sitophilus zeamais* and *Callosobruchus maculates*, with exposure period of 24 hours, under constant conditions of temperature (27±2 °C) and relative humidity (70±5%).

Species	**Stage**	**Slope (± S.E.M.)**	**LC_{50} (95% FI) (ppm)**	TR_{50} (95% LC)	χ^2	***P***
Species	Stage	Slope (± S.E.M.)	LC_{50} (95% FI) (ppm)	TR_{50} (95% LC)	χ^2	*P*
S. zeamais	Adult	7.16 ± 0.69	1.21 (1.16–1.28)	–	3.90	0.14
	Young larvae	4.73 ± 0.45	4.64 (4.30–5.00)	3.83 (3.49–4.18)	5.83	0.12
	Old larvae	3.59 ± 0.42	5.83 (5.26–6.46)	4.81 (4.23–5.45)	4.28	0.23
	Pupae	4.04 ± 0.58	6.17 (5.52–6.86)	5.09 (4.39–5.89)	4.08	0.25
C. maculatus	Adult	6.90 ± 0.68	1.20 (1.15–1.26)	–	4.19	0.12
	Young larvae	8.12 ± 0.45	3.94 (3.83–4.05)	3.28 (3.11–3.47)	5.65	0.13
	Old larvae	6.00 ± 0.44	3.89 (3.73–4.04)	3.24 (3.09–3.41)	3.04	0.39
	Pupae	8.76 ± 0.53	3.88 (3.74–4.02)	3.23 (3.06–3.42)	4.38	0.22

S.E.M. = Standard error of mean; LC = Lethal concentration; FI 95 % = Fiducial Interval at 95 % probability; TR = Toxicity Ratio; χ^2 = Qui-square; *P* = Probability.

The toxic effect observed for different development stages indicates that MEO vapors can penetrate the grains and kill the internal larvae and pupae within 24 hours. The low dose required for mortality of adults of *S. zeamais* and *C. maculatus* may be due the adults are located unprotected outside the grains. Since the larva and pupae of *S. zeamais* and *C. maculatus* are located within the grains, it appears that grain tissues act as a physical barrier against the MEO penetration. Although not examined, it is possible that if the fumigation had been carried out for a longer period, the lethal concentration of MEO vapors could be lower.

The toxicity of MEO was similar for the

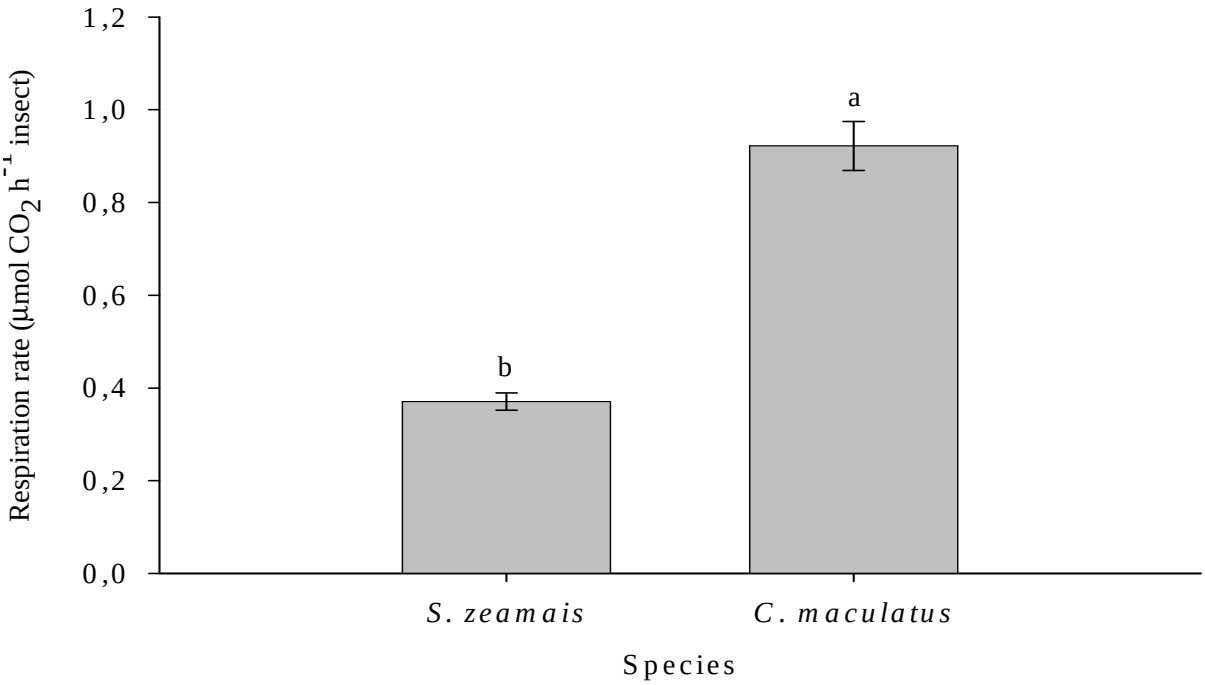

Figure 1. Natural respiratory rate of adults of *S. zeamais* and *C. maculates*.

adults of *S. zeamais* and *C. maculatus*, despite variation in the respiration rates. Similar results were observed in another study (SOUSA et al., 2008), since there was no correlation between respiratory rate of insects (sixteen populations of *T. castaneum*, 11 populations of *R. dominica* and nine populations of *O. surinamensis*) and toxicity to ozone gas. However, the gas exchange system of insects is the major entry route for the toxic gases into the insect body, which means that the amount of gas uptake in an insect is determined by its respiration rate (COTTON, 1932; PIMENTEL et al., 2007).

The relationship between the toxicity of MEO and respiration rate should not be ignored, as the earlier study showed a significant increase of CO_2 production by the adults of *Blattella germanica* (L.) and *Periplaneta americana* L. exposed to sub-lethal concentrations of AITC (TSAO et al., 2002). These authors suggested that like the insecticide dinitrophenol, the AITC might also act on the energy metabolism, where AITC may be acting as an inhibitor of oxidative phosphorylation, and thus interrupting ATP formation.

The results of this study suggest that the MEO holds promise as an alternative to methyl bromide and phosphine. The use of insecticides which effectively control insect developmental stages within the grains is one of the prerequisites for a good fumigant, because it avoids fumigant reapplication, thus reduce the quantity applied to the grain mass and also avoids selection of resistant individuals (MCKENZIE, 1996). It must be pointed out that the use of MEO as much safer fumigants in pest control

CONCLUSIONS

The mustard essential oil presented toxicity for the different developmental stages (larvae, pupae and adults) of *S. zeamais* and *C. maculatus*. The adult stage of both species was investigated more susceptible that the immature stages. The toxicity oil for adult phase is not influenced by natural respiratory rate.

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