ECOTOX – Brazil

J. Braz. Soc. Ecotoxicol., v. 6, n. 1, 2011, 9-13
doi: 10.5132/jbse.2011.01.002

JBSE

Evaluation of the Acute Effects of Organic Solvents on Adults of *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera, Curculionidae)

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(Received April 14, 2010; Accepted November 16, 2010)

ABSTRACT

The continuous use of synthetic insecticides has created problems for the environment and human health. With the objective of reducing such problems, ecofriendly measures have been taken and, in this case, plants have representing an important resource for pest control. Due to the increase of studies using organic extracts in bioassays and the difficulty of finding solubilizant agents that do not interfere in the results, the aim of this work was to evaluated the acute effect of different solvents on *Sitophilus zeamais* by contact and ingestion assays. The results suggest that pure dimethyl sulphoxide (DMSO) and Tween-20 solvents have toxic acute effects on adults of *S. zeamais*, but they can be used diluted at 1 or 5% with no toxic acute effect. On contrary, pure dichloromethane (DCM) and methanol (MeOH) did not cause any acute effect on target organism. Thus, DCM and MeOH solvents even in the pure form and the Tween-20 and DMSO diluted at 1 or 5% can be used as solubilizant agents for apolar to medium polarity plant extracts, involving contact or ingestion assay on adults of *S. zeamais*.

Keywords: plant extracts, stored grain pest, solvents, toxicity.

RESUMO

Avaliação do Efeito Agudo de Solventes Orgânicos Sobre Adultos de *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae)

O uso contínuo de inseticidas sintéticos tem criado problemas para a saúde humana e para o meio ambiente. Com o objetivo de diminuir estes problemas, medidas sustentáveis têm sido adotadas, e neste caso, espécies vegetais têm representado um importante recurso para o controle de pragas. Devido ao aumento de estudos usando extratos orgânicos e a dificuldade de encontrar agentes solubilizantes que não interfiram nos resultados, este trabalho propôs avaliar o efeito agudo de diferentes solventes sobre *Sitophilus zeamais* pelas vias contato e ingestão. Os resultados sugerem que os solventes dimetil sulfóxido (DMSO) e Tween-20 (ambos puro) causam efeito tóxico agudo em adultos de *S. zeamais*, mas os mesmos podem ser usados diluídos a 1 ou 5% sem causar efeito tóxico agudo. No entanto, os outros solventes testados diclorometano (DCM) e metanol (MeOH), ambos puro, não causaram efeito agudo no organismo alvo, por contato e ingestão. Assim, os solventes DCM e MeOH ambos na forma pura e o Tween-20 e o DMSO, diluídos a 1 ou 5% podem ser usados como solubilizantes para extratos de baixa e média polaridade, em ensaios de contato e ingestão contra adultos de *S. zeamais*.

Palavras-chave: extratos vegetais, pragas de grãos armazenados, solventes, toxicidade.

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INTRODUCTION

The maize weevil *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae) has been found mainly in corn grains and their sub-products. It is an important stored maize pest; however, it can be hosted on a high number of grains. *S. zeamais* is also capable to attack grains in the field phase. In Brazil, it has been considered one of the major maize stored pest, specially for the capacity of easily penetrate in the grain mass, high biotic potential, crossed infestation and for the fact that all insect phases are potential damage cause (Gallo *et al.,* 2002; Lazzari & Lazzari, 2009).

The principal stored grain pest control method still used today is based on successive applications of synthetic insecticides (Coitinho *et al.,* 2006; Souza & Trovão, 2009). In this case, liquefied fumigants are used (Gallo *et al.,* 2002; Lee *et al.,* 2004; Coitinho *et al.,* 2006; Souza & Trovão, 2009). These products have been successful, but, the indiscriminate and continuous use has been creating serious problems for the environment and the human beings health (Jacobson, 1982; Tapondjou *et al.*, 2002; Poletti & Omoto, 2003; Viegas Júnior, 2003).

With the objective of decreasing the damages caused by pesticides it is more desirable the adoption of sustainable and effective measures in the pest's control. With this philosophy, the number of studies involving the search for molecules less poisonous capable to act on certain population, taking into account time of degradation, specific target, action over beneficial insects, ecosystems and the human being health is increasing (Saito & Lucchini, 1998; Fazolin *et al.*, 2002; Lee *et al.*, 2004; Estrela *et al.*, 2006).

For this, the use of plants as insecticide source is an important resource with the objective of reducing stored product pest populations (Liu & Ho, 1999; Amaral *et al.*, 2006), and so, several botanical families have been studied (Oliveira & Vendramim, 1999; Almeida *et al.*, 2004; Papachristos & Stamopoulos, 2004; Paul *et al.,* 2009). In Brazil, the control of adult's *S. zeamais* through plant extractive or other substances of vegetable origin has been verified in some works (Procópio *et al.*, 2003; Tavares & Vendramim, 2005; Estrela *et al.*, 2006; Fazolin *et al.*, 2007; Coitinho *et al.*, 2006; Souza & Trovão, 2009).

In bioassays that evaluate insecticide, fungicide and bactericide properties, solvents have been used in the solubilization of compounds with low polarity (Mahasneh & El-Oqlah, 1999; Gonçalves-Gervásio & Vendramim, 2004; Inoue *et al.*, 2005; Kukić *et al.*, 2008). These compounds are still used as herbicide adjuvants or in the preparation of products that present inhibitory effect on the plant species development (Stahlman *et al.*, 1997). However, when organic extracts are used in toxicological assays, it's important to take into account the solvent toxicities used as extract solubilizants on the organism-target (Hammer *et al.*, 1999; Nascimento *et al.*, 2008), because it is known that solvents can interact with the organisms target causing the death of the same ones, or interfering on the result. Chagas *et al.* (2002) observed that essential oils when transformed in emulsified oils had their activity potentiated when tested on larvae ticks. Nascimento *et al.* (2008) observed that the emulsifier Tween-80 when added to the long pepper essential oil showed fungitoxic influence *Alternaria alternate* mycelia grown of (Fr.) Keissl. (1912) (Fungi: Hyphomycetes).

This research evaluated the acute effect of dimethyl sulphoxide, Tween-20, dichloromethane and methanol solvents on adults of *S. zeamais* by contact or ingestion assay, with the intention to eliminate any effect of these solvents used as solubilizants in future assays.

MATERIALS AND METHODS

The tests were accomplished at the Laboratory of Agricultural Entomology of the Amazonia National Research Institute (INPA), in Manaus, Amazon - Brazil. All *S. zeamais* specimens used in the experiments were from stocks cultures creation. All bioassays were made at 25 ± 2 °C temperature, 60 ± 10% relative humidity (r.h.) and 12:12 light:dark photoperiod conditions.

Four solvents were selected for the assay: dimethyl sulphoxide (DMSO) (pure, 1 and 5%), polysorbate surfactant (Tween-20) (pure, 1 and 5%), dichloromethane (DCM) and methanol (MeOH), both pure, through two administration ways: contact and ingestion. For each solvent concentration were performed two treatments: control group (without any chemical substance) and treatment group (with the respective solvent). Both assays (contact and ingestion) were full randomized with five repetitions, each one with 20 non-sexed adults between 10 and 20 days of age, totalizing 100 individuals for treatment.

Contact bioassay

The contact mortality assay was performed by using filter paper as proposed by Tavares & Vendramim, (2005). Filter papers (9.0 cm diameter) were impregnated with 1 mL aliquots of each solvent (treatment group), and the control group didn't received anything. The filters were air dried for 1 hour. Then, each paper was put into a Petri dish, and adults of *S. zeamais* were confined on it, taking the care of sealing each with a plastic film, to prevent the insects escape. The number of death individuals was count after 5 days and the specimens were considered dead with a complete absence of movement.

Ingestion bioassay

The ingestion assay was installed based on the work accomplished by Llanos *et al.* (2008) with minor modifications, using 20 g of mixed corn, type 2. Before the tests, the grains were maintained for about 2 months in freezers for expunge. Each treatment was performed impregnating the corn grains with 2 mL of each solvent and the control group without; the corn mass was homogenized and dried for 1 hour. The number of dead individuals in control and treatment were counted 15 days after the adult's confinement. The individuals were considered dead with total absence of movement.

Statistic analyze

Student's t-Test method was applied to all solvents at first, and for each concentration (MeOH and DCM, both pure, and for DMSO and Tween-20 pure, 1 and 5%) and analyzed

separately. After, ANOVA one three-way variance analyses was performed for DMSO and Tween-20 solvents, concentrations and administration way. A critical value of ($p < 0.05$) was selected as evidence of a difference significant. In this case, Tukey test was used as a *post hoc* test to determine which variable different significantly and the statistical analysis was performed by Statistica for Windows (StatSoft, 1997).

RESULTS

The first analysis suggest that only the solvents DMSO and Tween-20, both pure, are capable to cause a toxic acute effect on the adults of *S. zeamais*, by ingestion assay and DMSO by contact assay (Table 1 and 2). Dichloromethane (DCM) and methanol (MeOH), both pure, and with DMSO and Tween-20 diluted both at 1 and 5% were no toxic, because there was no observed significantly difference between treated and control group, on the two administration ways.

Analyzing the dead individuals percentage it was possible to observe that DMSO pure killed 100% of them, as for contact or ingestion, while Tween-20 pure killed 38% by contact and the total mortality was observed on the ingestion assay (Table 3).

Table 1 – Results of pure dimethyl sulphoxide (DMSO) assayed on *Sitophilus zeamais* individuals by contact and ingestion.

Administration way	Treatment	Means	d.f.	t-*value*	*p*
Contact	Control	0.60	8	60.7157	0.000000*
	DMSO (pure)	19.80			
Ingestion	Control	2.20	4**	14.4671	0.000133*
	DMSO (pure)	19.80			

*Significant differences ($p < 0.05$) found applying the Student's t-Test method; **Degrees of freedom adjusted to perform the inferential method. Original significance level: *p*-value = 0.000001 (d.f = 8).

Table 2 – Results of pure polysorbate surfactant (Tween-20) assayed on *Sitophilus zeamais* individuals by contact and ingestion.

Administration way	Treatment	Means	d.f.	t-*value*	*p*
Contact	Control	0.20	4*	3.6108	0.022540**
	Tween-20	7.60			
Ingestion	Control	3.40	4*	23.1931	0.000020**
	Tween-20	19.80			

*Degrees of freedom adjusted to perform the inferential method. Original significance level: *p*-value = 0.000001 (d.f = 8); **Significative differences ($p < 0.05$) found applying the Student's t-Test method.

Table 3 – Percentage of *Sitophilus zeamais* individuals dead by contact and ingestion assay, considering each solvent and their respective control.

Solvent used	Contact assay		Ingestion assay	
	Control	Treatment	Control	Treatment
Methanol	0	5	33	25
Dichloromethane	0	2	33	24
Tween-20 pure	0	38	17	100
Tween-20 1%	14	23	20	24
Tween-20 5%	14	22	20	38
Dimethyl sulphoxide pure	3	100	11	100
Dimethyl sulphoxide 1%	2	2	1	1
Dimethyl sulphoxide 5%	1	1	1	1

Analysing the results using ANOVA, the first analysis was confirmed. Differences were found between concentrations due to the exposure way. Thus DMSO pure had a significant effect ($p = 0.000126$) in both way, however, Tween-20 showed differences only in the ingestion experiments ($p = 0.000126$), with no significant contact ($p = 0.772315$), in other words, the effect using Tween-20 is smaller by contact than ingestion (Table 4).

The results suggest that the solvents: DMSO (1 and 5%), Tween-20 (1 and 5%), DCM and MeOH (both pure) can be used as solubilizant agents in future organic plant extracts bioassays to evaluate their toxicity effect on adults of the *S. zeamais*. The results also suggest that they will not probably interfere on result, for not causing negatives effects on the target.

DISCUSSION

In assays that look for plant extracts insecticidal activity should not be observed deleterious effect of the solubilizant agent on the organism target. However, it is often impossible to know in advance if the solvent chosen as solubilizant has toxic effect on individuals or not. So, it is essential to evaluate the solvents before perform toxicological assays with extracts. In agreement with our results some researchers working with organic extracts didn't observe negative effects caused by the solvents on the organism target.

Souza *et al.* (2007) studying methanolic and ethanolic extracts of *Annona coriaceous* Mart. in nymphs of *Dichelops melacanthus* Dallas, 1851 (Heteroptera: Pentatomidae), dissolved in DMSO (40%) and hexanic extracts dissolved in Tween (1:1) did not observe any negative effect of the solvents on these individuals. Prophiro *et al.* (2008) studying *Melia azedarach* L. green and ripe fruits crude extracts, dissolved in Tween-20 against *Aedes aegypti* Linnaeus, 1762 (Diptera: Culicidae), observed the larvae mortality inexistence in all of the control groups, as well as observed by Montenegro *et al.* (2006) in contact assay when extracts were dissolved in DMSO 1% aqueous solution.

Fonseca *et al.* (2006) evaluating antimicrobial activity of the fruit essential oil from *Vitex cymosa* Bert. used 10 µL of each oil diluted in Tween-80 (0.5%) were not mention any negative solvent effects. Kannathasan *et al.* (2008) testing larvicidal activity of fatty acid methyl esters of *Vitex* species against *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae) did not found any toxicity using DMSO at 5%. Similar result was found by Roel *et al.* (2000) and Cunha *et al.* (2006) who used acetone as a solvent and they did not found deleterious

Table 4 – Summary of ANOVA three-way analyses considering the solvents dimethyl sulphoxide (DMSO) and Tween-20, concentrations and administration way on *Sitophilus zeamais* individuals.

Effect / factor	d.f.	MS	F	*p*
Solvent	1	14.016666	2.1731267	0.14697
Concentration	2	1261.6666	195.60724	< 0.00001*
Administration way (AW)	1	150.41667	23.320414	< 0.00001*
Solvent × Concentration × AW	2	70.199997	10.883721	0.00012*
Residual	48	6.4499998		

* Significance levels ($p < 0.05$).

effect on control group, as well as Trindade *et al.* (2000) who used methanol as solubilizant agent.

In view of the importance of knowing how toxic the solvent used as solubilizant agent can be Sarti *et al.* (1996) when seeking trypanosome activity in plant extracts, determined in advance how much ethanol could be used to dilute the extracts to achieve a concentration that does not kill the parasites and they found that 10-30 $\mu L.mL^{-1}$ of ethanol in blood were no toxic. Hoold *et al.* (2003) studying Tween-80 realized that this surfactant provoked an increase in the membrane cellular permeability of the individuals studied, increasing their susceptibility. In addition, Santos *et al.* (2003) studying the effect of the Tween-80 (1%) on lettuce plantules, demonstrated that this surfactant affect the growth of plantules radicle and hypocotyl.

Prophiro *et al.* (2008) did not found negative effect when using Tween-20 pure over *A. aegypti*. As our results show toxic effect of Tween-20 pure over *S. zeamais*, these corroborates the importance of knowing how much toxic one solvent can be for each group studied. It is important to note that the susceptibility of individuals can be very variable among different species, or between individuals of the same species and also the variability of answers can be dependent on the solvent composition.

In summary, the solvents DMSO and Tween-20 can be used diluted at 1 or 5%, while DCM and MeOH can be used pure to perform the assays of plant extracts on adults of *S. zeamais*.

Acknowledgements – We thank the Programa de Pesquisa em Biodiversidade (PPBio/INPA/MCT) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CT-Agro/CNPq and PPBio/CNPq) for the financial support.

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