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The study of behavioral response and control effectiveness of the *Sitophilus zeamais* L. (Coleoptera: Curculionidae) and different concentrations of essential oils

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Abstrac

The aim of this study was to evaluate the essential oil effects of different plants on their behavioral response (attractiveness/repellence) and the control effectiveness of the *S. zeamais*. Steam of eucalyptus plant (*Eucalyptus* sp) essential oils, sassafras (*Ocotea odorifera),* nim (*Azadirachta indica*) and citronela (*Cymbopogon nardus*) were used in order to test the control effectiveness in five concentrations (16,62; 33,25; 66,5 and 133 mL of oil/liter of hexane) and a control where it was used only solvent. For the Behavioral response test it was employed the concentration of 133 $mL.L^{-1.}$ The attractiveness or repellence in relation to the essential oils with bioassay in olfactometer in "Y", (that consists in a glass tube which works in a horizontal position, and the air flow is produced through sucking in the vacuum). Each lower arm for testing the olfactometer received a filter of paper full of volatile of a hybrid maize grain (Premium) previously tested and other volatile of a hybrid maize grain added to essential oil. It was observed important differences on the effectiveness between the two employed concentrations. The essential oils of sassafrás and citronela presented a better control effectiveness in the 133 $ml.L^{-1}$concentration (97,5 and 96,25 % of control) followed by Eucalyptus oil and nim which presented the lowest death rate. It was also observed an interaction between the concentration and exposure time on the observed death rate. In relation to the Behavioral response, it was observed that the sassafras and citronella essential oils were the ones that presented the lowest rate of insect attractiveness by the volatile of the hybrid (17,22 e 21,67 % respectively) in comparison to the control that was around 40,0 %. The maize extract treatments with Eucalyptus oil and nim did not influence significantly in terms of attractiveness.

Key words: attractiveness, repellence, insect, maize grain, *Zea mays*.

Introduction

The primary pests in the storage of grains are greatly important when all the damages caused by them are considered. The alternative methods for the control of pests are becoming more relevant when compared to the conventional ones, they now receive more attention from researchers, industries and consumers.

The problems resulted from the current control practices in the storage units have contributed to the reduction in the use of chemical insecticides. The main objective of the storage

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ecosystem is to preserve the grains with the minimum loss in quantity and quality.

In the storage, the destructive potential of the pest insects and other organisms (fungus and acarus) is narrowly related to the interactions between factors: a) abiotic as: collection and storage structures; intragranular environment; impurities and foreign matters. B) Biotic as: kind of grain, seed, product and by-product; insects, fungi and acarus; and the ecological relations. The damage to the grain or the loss of quality can be avoided if there is understanding of these factors, knowledge on how they interact and elaboration of a correct handling program for each condition (Jayas et al., 1995).

The process of loss of quality that the grains are subject to and its magnitude is the result of the interaction between insects, fungi, acarus, the physical state of the stored product, and where and when occurs the intervention from the man in this process (Pinto Junior, 1994; 1999).

The infestation of the mass of grains can propitiate the fungal development and change the physical ambient (temperature and humidity level) of the grains, promoting their deterioration, as well as an alteration on the nutritional balance (protein, carbohydrate and lipid levels). The successive generation of insects shall enlarge these damages, resulting in economical losses or even the impossibility of commercialization of the product (Robert et al., 1991).

Plant products have had a fundamental role in the traditional methods for the control of pests, just like the pyrethrum. However, the synthetic insecticides are frequently used for this activity.

Since the 60s, insecticides have been used as a tool in the control of insects of stored grains, aiming to keep the quality of the seeds, grains and its by-products. The challenges the researchers have faced in regarding the handling and control of pests of stored products have greatly increased in the last years, especially in Brazil, which is a country of many contrasts. There are two opposing sides: in one of them we find the top technology identifying and resolving part of the problems, in the other we can see the lack of information, which results in the use of inadequate practices of handling and control, as an example, the use of insecticides without any criteria or necessary care to avoid the contamination and residues of active ingredients in the products and their derivatives (Pinto Junior, 1999).

The aim of this study was to evaluate the effects of the essential oils of nim, eucalyptus, sassafras and citronella in the attractiveness and control of adults of *Sitophilus zeamais* L.

Materials and methods

Insect breeding

The insects were collected in different regions of the State of Paraná and were kept in mass creation, in separate recipients for the preservation of their origin. They were kept in temperature and moisture conditions (27 ± 2 ºC and 65 to 80 %, respectively) without any distinction of neither sex nor age. One hundred adult individuals of each origin were kept in 500 g of maize in a glass jar. After three weeks of oviposition, the insects were removed from the grains by sieving with a 2,0 mm mesh sieve. The subsequent generation emerged was used in the different biotests.

Essential oils extraction

For the experiment, the essential oils from eucalyptus (*Eucalyptus* sp), sassafras (*Ocotea odorifera*), nim (*Azadirachta indica*) and citronella (*Cymbopogon nardus*) were used, all of them obtained from around 500 g of fresh leaves of each vegetal species to be tested, through the Clevenger Set, at the Biochemistry Laboratory in the Environmental and Agrarian Sciences Department from the São José dos Pinhais Campus of the Pontifícia Universidade Católica do Paraná.

The oils extracted were used right after being extracted and after a storage period in a freezer at a -4 ºC temperature. Different doses of the essential oils (16.62, 33.25, 66.5 and 133 mL of oil/liter of hexane) were used in order to test its

effectiveness in the control of pests in laboratory conditions.

Biotests of insecticide activity

Tests were performed to check the toxicity of the vapors from the essential oils to different groups of pests that attack grain storage environments, structures and affected areas. Petri plates were used as the exposure chamber, with paper filters impregnated with the different doses of essential oils extracted of different plants, applied with graded pipettes.

Attractiveness or repellence biotests

To check the attractiveness of the storage pests before the volatile released by the maize hybrid Premium, biotests were performed in an olfactometer in "Y". It consists of a glass tube which works horizontally, and the air flow is produced by vacuum suction. Each of the olfactometer´s smaller test arms received a paper filter of 5cm of diameter folded and impregnated with the volatile from the corn hybrid, and the other only with the control (hexane solvent). Twenty insects were liberated in the bigger arm of the olfactometer, and their response to the olfactory stimulus was verified after 3 repetitions in 5, 10 and 15 minutes.

Data analysis

The differences between the insecticide activities and repellence or attractiveness of the oils to the tested pests were determined according to the variance and regression analysis test, making use of software and comparison tests between means when appropriate.

Results and discussion

Insecticide activity biotest

The insecticide activity was variable according to the kind and concentration of the tested oils. The natural death rate of the insects in the marker parcels only with the hexane solvent varied from 5 to 12.5 %. The essential oil from nim did not interfere in this death rate number even in the higher concentrations. A death rate superior to 80 % could only be found with the application of the eucalyptus oil (133 $mL.L^{-1}$), citronella oil (66.5 and 133.0 $mL.L^{-1}$). The sassafras oil resulted in a death rate of 62.5 % even in the lowest concentration (16.62 $mL.L^{-1}$) (Table 1). In the general mean, the sassafras and citronella oils have the biggest insecticide potential of the pest resulting in a higher number of killed individuals (Table 2). These two species have already had their oils studied as a potential for the use in agriculture or industry (Barreiro and Fraga, 1999; Malerbo-Souza and Nogueira-Couto, 1998; Malerbo-Souza et al., 2003). For the eucalyptus oil it is proposed that new studies be performed with higher concentrations in order to try to reach the same death rate gotten with the sassafras and citronella oils.

The number of insects killed varied significantly

Table 1. Effectiveness of different essential oils of nim, eucalyptus, sassafras and citronella in the control of *Sitophilus zeamais* after 72 hours of exposure.

Concentration	Death rate (%)			
$mL.L^{-1}$	Nim	Eucalyptus	Sassafrás	Citronella
0	5.0	8.7	7.5	12.5
16,62	5.0	10.0	62.5	33.7
33,25	2.5	25.0	82.5	40.0
66,50	6.2	36.2	100.0	80.0
133,00	5.0	87.5	97.5	96.2

according to the exposure period, concentration and kinds of oils studied. The exposure period of 72 hours to the oils resulted in a higher death rate than the period of 24 hours (Table 3) and the higher concentrations resulted in a more intense insecticide effect too, nevertheless, the effect was not linear, which suggests that the biological response of the insects to the exposure to oils is more complex and can be affected by other factors as well (Table 4).

Attractiveness and repellence biotests

It could be verified in the variance analysis that the factors essential oil and exposure period show highly significant variations (F=6,0552 and F=7,0722 respectively) when compared to each other by the F test. The ambient factor (hexane + maize or hexane + maize + essential oil) does not show any significant difference.

The essential oils tested show significant differences, and it can checked that in the hexane+maize+eucalyptus treatment, the highest number of insects attracted can be found (2,59), similar to the marker. The best results regarding repellence were found in the sassafras and citronella oils use (1,44 and 1,53 insects, respectively), which did not differ statistically between each other, but were different from the eucalyptus oil (Table 5 and 6). Statistical differences could be observed in the exposure period too, once the highest average number of insects attracted was found between 10 and 15 minutes (Table 7).

Table 2. Average number of *Sitophilus zeamais* killed after the exposure to essential oils.

Essencial oil	Death rate*
Nim	0.55 c
Eucalyptus	3.77 b
Citronella	7.80 a
Sassafrás	9.65 a

Means followed by different letters differ statistically by the Tukey Test at 5 %.

Table 3. Effect of exposure period to the essential oils in the death rate of *Sitophilus zeamais*.

Exposure period (horas)	Death rate
24	2.95 b
72	7.94 a

Means followed by different letters differ statistically by the Tukey Test at 5 %.

Table 4. Effect of the concentration of the essential oils in the average death rate of *Sitophilus zeamais* during the exposure to the essential oils.

Concentration ($mL.L^{-1}$)	Death rate
0	9.88 a
16,62	7.53 b
33,25	4.94 c
66,6	3.84 c
133,0	1.03 d

* Means followed by different letters differ statistically by the Tukey Test at 5 %.

Table 5. Percentage of *Sitophilus zeamais* in the olfactometer after exposure to essential oils.

	Position in the olfactometer after 15 minutes		
Treatment	Central arm[2]	Left arm[3]	Right arm[4]
Extract[1]	40.0 a	18.3 a	41.6 a
Extract + Nim	45.0 a	28.9 a	26.1 ab
Extract + Eucalypto	33.8 a	25,6 a	40.6 a
Extract + Sassafrás	56.7 a	26,1 a	17.22 b
Extract + Citronella	53.8 a	24.4 a	21,7 b

* Means followed by different letters differ statistically by the Tukey Test at 5%.

[1] Extract = extract of Premium corn hybrid

[2] Central Arm = no product

[3] Left Arm = only extract with solvent esquerdo

[4] Right Arm = maize extract with solvent added of essential oil (133,0 $ml.L^{-1}$ of solvent)

Table 6. Effect of the kind of oil in the attractiveness (%) of *Sitophilus zeamais*.

Essencial oill	Attractiveness (%)
Eucalyptus	12.9 a
Sassafrás	7.2 b
Citronella	7.6 b
Nim	9.1 ab

* Means followed by different letters differ statistically by the Tukey Test at 5 %.

Table 7. Effect of the treatment period in the olfactometer in the attractiveness (%) of *Sitophilus zeamais*.

Time (min)	Attractiveness (%)
5	6.5 b
10	10.4 a
15	10.9 a

* Means followed by different letters differ statistically by the Tukey Test at 5 %.

Conclusion

The sassafras and citronella oils showed the best repellence potential and caused the highest death rate of *Sitophilus zeamais*. The nim oil showed repellence potential but did not cause the death of the insects and the eucalyptus oil showed low repellence and only in the highest concentrations caused the death of the insects.

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