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Scientific paper

Biofumigation of plant species on *Sitophilus zeamais* in stored maize

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Abstract

The objective of this study was to evaluate the repellent effect and toxicity of volatile substances from plant species in the form of ethereal, ethyl, aqueous extracts and powders on adults of *Sitophilus zeamais*. The experiments were conducted at the Laboratory of Plant Science of the Camilo Castelo Branco University, Campus Descalvado/São Paulo State. It were used the *Chenopodium ambrosioides*, *Corymbia citriodora* and *Cucurbita pepo* plant species. The experimental design was completely randomized with six replications. In repellency tests were used doses of 5µl, 10µl, 15µl, 20µl, and 25µl (for the ethereal, ethyl and aqueous extracts) and 0.3 g (for powder) with assessments after 24 hours. In the toxicity tests it was used the dose of 25µl (for the ethereal, ethyl and aqueous extracts) and 0.3 g (for powder) with assessments after 48 hours. Based on the obtained data it can be concluded that the ethereal (excepting *C. pepo*), ethyl, aqueous extracts and powders of all the tested plants showed repellent effect to adults of *S. zeamais*. All extracts and powders from the tested plants showed toxic effects to adults of weevil.

Keywords: Plant insecticides; maize weevil; plant extracts; alternative control.

Efeito da biofumigação com substâncias voláteis de espécies vegetais sobre *Sitophilus zeamais* em milho armazenado

Resumo

O objetivo desse trabalho foi avaliar o efeito repelente e a toxicidade de substâncias voláteis provenientes de espécies vegetais na forma de extratos etéreos, etílicos, aquosos e pós sobre adultos de *Sitophilus zeamais*. Os experimentos foram conduzidos no Laboratório de Fitotecnia da Universidade Camilo Castelo Branco, Campus de Descalvado/SP. Utilizou-se as espécies vegetais *Chenopodium ambrosioides* (parte aérea de Erva de Santa Maria), *Corymbia citriodora* (folhas de Eucalipto cheiroso) e *Cucurbita pepo* (sementes de Abóbora). O delineamento experimental foi inteiramente casualizado, com 6 repetições. Nos testes de repelência utilizaram-se doses de 5µl, 10µl, 15µl, 20µl e 25µl (para os extratos etéreos, etílicos e aquosos) e 0,3g (para pó) com avaliações após 24 horas. Nos testes de toxicidade utilizou-se a dose de 25µl (para os extratos etéreos, etílicos e aquosos) e 0,3g (para pó) com avaliações após 48 horas. Pelos dados obtidos pode-se concluir que os extratos etéreos (excetuando-se de Abóbora), etílicos, aquosos e pós de todas as plantas testadas mostraram efeito repelente aos adultos de *S. zeamais*. Todos os extratos e pós provenientes das plantas testadas mostraram efeitos tóxicos aos adultos do gorgulho.

Palavras-chave: Plantas inseticidas; gorgulho do milho; extratos vegetais; controle alternativo.

Efecto de la biofumigación con sustancias volátiles de especies vegetales sobre *Sitophilus zeamais* en maíz almacenado

Resumen

El objetivo de este estudio fue evaluar el efecto repelente y la toxicidad de las sustancias volátiles de las especies de plantas en forma de etéreo, etilo, acuosa y después de la de los adultos de *Sitophilus zeamais*. Los experimentos se llevaron a cabo en el Laboratorio de Botánica de la Universidad Camilo Castelo Branco, Campus Descalvado / SP. Utilizamos *Chenopodium ambrosioides* especies de plantas (disparar Yerba Santa Maria), *Corymbia citriodora* (Eucalyptus deja olor) y *Cucurbita pepo* (semillas de calabaza). El diseño experimental fue completamente al azar con seis repeticiones. En las pruebas de repelencia

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se utilizaron dosis de 5µl, 10µl, 15µl, 20µl, y 25 l (para el etéreo, etilo y acuosa) y 0,3 g (polvo) con evaluaciones después de 24 horas. En los ensayos de toxicidad se utiliza la dosis de 25 l (para el etéreo, etilo y acuosa) y 0,3 g (polvo) con evaluaciones después de 48 horas. A partir de los datos obtenidos, se puede concluir que el etéreo (exceptuando la calabaza), etilo, acuosa y polvo de todas las plantas examinadas mostraron efecto repelente a los adultos de *S. zeamais*. Todos los extractos y polvos de plantas examinadas mostraron efectos tóxicos en adultos del gorgojo.

Palabras clave: insecticidas vegetales, extractos de plantas gorgojo del maíz, control alternativo.

Introduction

Maize grains and other stored cereals are infested by many insect species. The chemical control of this plague is generally done with fumigants insecticides and protectors that, despite being effective, can cause intoxications to the applicators, presence of toxic residues in the grains and selection of resistant populations (LORINI, 2003; RIBEIRO et al., 2003; BENHALIMA et al., 2004).

In the corn ears storing, using rustic structures, as wooden storehouses, the weight losses caused by insects and rodents have affected around 7% of the total maize production in Brazil (SANTOS and MANTOVANI, 1997). These losses can occur before, during and after the harvest, in the transportation, industrialization and storing of the grains, being influenced by many factors, such as the poor structure of the unities of storing, the high moisture content, impurity of the grains and the presence of plagues (TAVARES, 2002). Among these plagues which attack the maize grains during the storing, stand out the *Sitophilus zeamays* (Mots.), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae), being considered primary plagues for having the capacity of piercing the seed coat of seeds and grains.

The use of plants insecticides is actually one of the most studied alternative methods in the whole world for the control of stored-products plagues, as the beetles of the genus *Sitophilus*. Despite of the importance that this genus presents in Brazil, according GALLO et al. (2002), the number of studies in this research line is quite reduced, especially when is considered the enormous diversity of the national flora.

In some countries, producers have already used plants for protection of stored grains against the plagues infestation. For the ease of application and for the nature of the substrate to be protected, it has been preferred the use of powders in relation to other plant derivatives. OLOB et al. (1981) mention that, in the African southeast, producers use tobacco powder to control insects infestations during the storing of maize. For LAGUNES and RODRÍGUEZ (1989) states that some Mexican farmers use the plants *Sambucus mexicana* Presl. ex DC. and *Piper auritum* Sieber ex Kunth., together in stored maize, in the proportion of 10g of mixture per kilogram of maize, obtaining protection against plagues for four months. In Brazil, the use of Eucalyptus Citriodora leaves (*Corymbia citriodora*) between layers of ear corns is a relatively common practice between the small producers (SANTOS et al., 1984).

The control of plagues of stored-products with the use of plant powders can be result of the repellency or toxicity of the same, which reflects in the lower growth of the insects population. With regard to the *S. zeamais*, promising results have been obtained with the use of *Azadirachta indica*, *Chenopodium ambrosioides*, *Dennettia tripetala* E.G. Baker, *Hippocratea* sp., *Hyptis suaveolens* Poit., *Mentha spicata* L., *Ocimum gratissimum* L., *Ocimum kenyense* Ayob. ex A.J. Patn, *Piper nigrum* L., *Piper guineense* C. DC., *Pneumus boldus* Lyons. and *Ricinus communis* powders.(CHAKRABORTY and GHOSE, 1988; LAGUNES and RODRÍGUEZ, 1989; KOSSOU, 1989; ARLEU et al., 1990; ARAYA-GONZALEZ et al., 1996; OKONKWO and OKOYE, 1996; BEKELE et al., 1997; BANJO et al., 2001; NTONIFOR and MONAH, 2001).

This study had as aim to assess the effect of repellency and toxicity using ethereal, ethyl, aqueous extracts and powders of aerial part of the "Erva de Santa Maria" *C. ambrosioides*, Eucalyptus Citriodora leaves (*Corymbia citriodora*) and pumpkin seeds (*Cucurbita pepo*) on adults of *S. zeamais* in grains of stored maize.

Materials and Methods

The experiments were conducted in the Plant Sciences Laboratory of the Camilo Castelo Branco University, at a temperature of 25±2°C, relative humidity of 60±10% and 12 hours of photophase (photoperiod or light phase), with the weevil *S. zeamais* in stored maize.

The insects used in the experiments were

kept in breeding stocks, confined in glass receptacles (with the mouth sealed with fabric of the type tulle attached by elastic) containing grains of wheat. This receptacles remained in B.O.D. (28 ± 2°C, photophase of 12 hours and relative humidity of 70± 5%), being that at every 30 days, this material was sieved and the adults used to initiate the infestation in new receptacles.

The plant species used were: aerial part of the "Erva de Santa Maria" (*C.* ambrosioides), Eucalyptus Citriodora leaves (*C. citriodora*) and pumpkin seeds (C. *pepo*). This were collected in several places of the Experimental Center of the Unicastelo (CEU) and transferred to laboratory. From these were prepared the following types of formulations:

- Ethereal, ethyl, aqueous extracts from the distillation through dragging (process adapted and described by GUIMARÃES et al., 2000).

- Powders from the structures of the plants which remained drying for 48 hours in greenhouse with forced air circulation at 40ºC and grounded in a mill.

The extracts and powders were kept individually per species, in hermetically sealed glass receptacles, until use.

Repellency Tests

In these tests were used the doses of 5, 10, 15, 20 e 25µL of the extracts and 0.3g of the powders. The experimental design was completely randomized, with 6 repetitions (arenas) for each plant species and dose. Each unit was composed of 20 insects recently emerged and not sexed from the breeding stock. Observations were taken after 24 hours, taking note of the total of insects in the control and in the treatment. In the bioassays were used arenas formed by five circular plastic cases, with diagonal interrelation. Each sample contained 10g of maize and the treatments were disposed in a manner to insert in the receptacles.

For the tests with the extracts, it as fixated in the receptacles covers filter paper of 5.5 of diameter, soaked with the aid of a micropipette with the different doses. For the tests with powders, the used dose was added directly to the grains.

From the observed data, it was applied the Preference Index (PI), (equation 1), quoted by PROCÓPIO et al. (2003), where:

$$\text{I.P.} = \frac{(\%IPT - \%Ipt)}{(\%IPT + \%Ipt)} \qquad (1)$$

(**I.**P. = PI)

PI= Preference Index
%IPT= % of insects in the test-plant
%Ipt= % of insects in the control
Where:
- I.P.: -1.00 to – 0.10, repellent test plant;
- I.P.: -0.10 to + 0.10, neutral test plant
- I.P.: +0.10 a +1.00, attractive test plant.

Toxicity Test

For the tests seeking to verify the toxicity of the extracts and powders of the different plant species on the weevil, it was done in plastic receptacles, added 10g of maize seeds, carrying out 6 repetitions for each plant species, using 20 adult insects with 24 hours of age per recipient.

For the plant extracts, it was fixated in the receptacles covers, circles of filter paper of 5.5 of diameter, soaked with the highest dose tested in the anterior bioassay (25µL), thus starting the process of biofumigation. In the control treatment without the extract use, was added the same quantity of distilled water. Because there is no direct contact of the insects in the grains with the oil, the cases will have subdivisions with cloth of the type voil. For the plants powder, it was added in the receptacles the quantity stipulated for treatments (0.3g of powder). In the control treatment it was used only the grains without addition of powders.

After 48 hours of liberation of insects, all the receptacles were open and it was made the counting of the number of dead insects in each treatment. The obtained data were statistically analyzed by the F test, being the averages compared by the Tukey test, at the level of 5% of probability.

Results and Discussion

Repellency Tests

In the Table 1 are found the repellency data of adults of *S. zeamais* per extracts and powders of the "Erva de Santa Maria" aerial part. The ethereal extracts showed in all used concentrations repellents to weevil adults, being that the PI varied from -0.18 to -0.41. Now the ethyl extract also showed in all used concentrations repellents to weevil adults, being that the PI vaied from -0.18 to -0.50. In the same way the aqueous extracts presented repellency to the adults, with PI varying from -0.23 to -0.45. When using powders, the PI was of -0.73, having in this methodology the higher repellence when compared

to the ethereal, ethyl, aqueous extracts.

LAGUNES and RODRÍGUEZ (1989) observed that the plant "Erva de Santa Maria" presented repellent effect on the *S. zeamais*.

According to RODRÍGUEZ (2000) the insecticidal properties of the *C. ambrosioides* are proved in relation to plagues in stored grains which belong to the families Bruchidae, Anobiidae, Bostrichidae, Tenebrionidae and Curculionidae, as is the case of the tested species, i. e. *S. zeamais*.

As reported by TAVARES and VENDRAMIM (2005b) powders of *C. ambrosioides* present residual insecticidal effect against *S. zeamais* adults, for the maximum period of 5 days. Now SILVA et al. (2005) verified high mortality of adults from this plague through the mixture of maize grains with powders of *C. ambrosioides*.

In Table 2 are found the repellence data to *S. zeamais* adults by extracts and powders of Eucalyptus Citriodora.

Table 1. Repellence to adults of *Sitophilus zeamais* by ethereal, ethyl, aqueous extracts and powders of aerial part of the "Erva de Santa Maria" (*Chenopodium ambrosioides*).

Aerial part of the "Erva de Santa Maria" Extract/Concentrations	**Attracted adults (%)[1]**	**PI.[2]**
Ethereal Extract 5µl	29.17 a	-0.41 R
Control	70.83 b	
Ethereal Extract 10µl	30.00 a	-0.40 R
Control	70.00 b	
Ethereal Extract 15µl	40.83 a	-0.18 R
Control	59.17 b	
Ethereal Extract20µl	34.17 a	-0.38 R
Control	65.83 b	
Ethereal Extract 25µl	35.00 a	-0.30 R
Control	65.00 b	
Ethyl Extract 5µl	36.67 a	-0.27 R
Control	63.33 b	
Ethyl Extract 10µl	30.00 a	-0.40 R
Control	70.00 b	
Ethyl Extract 15µl	40.83 a	-0.18 R
Control	59.17 b	
Ethyl Extract 20µl	28.33 a	-0.43 R
Control	71.67 b	
Ethyl Extract 25µl	31.67 a	-0.37 R
Control	68.33 b	
Aqueous Extract 5µl	38.33 a	-0.23 R
Control	61.67 a	
Aqueous Extract 10µl	34.17 a	-0.32 R
Control	65.83 b	
Aqueous Extract 15µl	27.50 a	-0.45 R
Control	72.50 b	
Aqueous Extract 20µl	31.67 a	-0.37 R
Control	68.33 b	
Aqueous Extract 25µl	35.00 a	-0.30 R
Control	65.00 b	
Powder 0.3g	13.33 a	-0.73 R
Control	86.67 b	

[1] *Means followed by the same letter, within each treatment, do not significantly differ, by the T Test at the level of 5% of probability.*

[2] *Classification: A = attractive; R = repellent; N = neutral.*

The ethereal extracts showed in all used concentrations repellents to weevil adults, being that the PI varied from -0.22 to -0.62. The same happened using the aqueous extracts, with PI between -0.28 and -0.53. Now the ethyl extracts also showed in all used concentrations repellents to weevil adults, with the PI varying between 0.27 to -0.42, except in the concentration of 15µl where the plant behaved as neutral. When using powders, the PI was of -0.45. Noting that the best PI was obtained in the ethereal and aqueous extracts.

Results presented by ALMEIDA et al. (1999) confirmed the influences of the Eucalyptus Citriodora and Pepper extracts with control around 85.75% to 100%, respectively, of the weevil *Sitophilus* spp. when compared to the mortality in the control, whose efficiency on adults of this insect was none (0.0%).

PROCÓPIO et al. (2003) verified that between the several assessed plant species, the only one that had repellency on *S. zeamais* adults was the powder of Eucalyptus Citriodora (*C. citriodora*) leaves, differing significantly from all other plants.

Table 2. Repellence on adults of *Sitophilus zeamais* by ethereal, ethyl and aqueous extracts and powders of leaves of Eucalyptus Citriodora (*Corymbia citriodora*).

Eucalyptus Citriodora leaves Extract/Concentrations	Attracted adults (%)[1]	PI[2]
Ethereal Extract 5µl	32.50 a	-0.35 R
Control	67.50 b	
Ethereal Extract 10µl	27.50 a	-0.45 R
Control	72.50 b	
Ethereal Extract 15µl	31.67 a	-0.37 R
Control	68.33 b	
Ethereal Extract 20µl	19.17 a	-0.62 R
Control	80.83 b	
Ethereal Extract 25µl	39.17 a	-0.22 R
Control	60.83 a	
Ethyl Extract 5µl	36.67 a	-0.27 R
Control	63.33 b	
Ethyl Extract 10µl	36.67 a	-0.27 R
Control	63.33 b	
Ethyl Extract 15µl	47.50 a	-0.05 N
Control	52.50 a	
Ethyl Extract 20µl	33.33 a	-0.33 R
Control	66.67 b	
Ethyl Extract 25µl	29.17 a	-0.42 R
Control	70.83 b	
Aqueous Extract 5µl	35.83 a	-0.28 R
Control	64.17 b	
Aqueous Extract 10µl	25.00 a	-0.48 R
Control	75.00 b	
Aqueous Extract 15µl	28.33 a	-0.51 R
Control	71.67 b	
Aqueous Extract 20µl	21.67 a	-0.53 R
Control	78.33 b	
Aqueous Extract 25µl	28.33 a	-0.51 R
Control	71.67 b	
Powder 0.3 g	27.50 a	-0.45 R
Control	72.50 b	

[1] *Means followed by the same letter, within each treatment, do not significantly differ, by the T Test at the level of 5% of probability.*

[2] *Classification: A = attractive; R = repellent; N = neutral.*

In the Table 3 are found the data of the repellency to adults of *S. zeamais* per extracts and powders of Pumpkin seeds.

The ethereal extracts showed in all used concentrations, neutrals or attractive to weevil adults, being that the PI varied from -0.03 to +0.33. In the opposite way, the ethyl extracts had in all used concentrations repellents to weevil adults, and the PI varied from -0.23 to -0.68. In the same way the aqueous extracts were repellents to the adults, with a PI varying from -0.23 to -0.53. When using powders, they also showed repellents with a PI of -0.30.

Toxicity Tests

In the Table 4 are found the results obtained in the bioassays of mortality of weevil adults in the different treatments.

Table 3. Repellency to *Sitophilus zeamais* adults by ethereal, ethyl and aqueous extracts and powders of Pumpkin seeds (*Cucurbita pepo*).

Pumpkin Seeds Extract/Concentrations	**Attracted adults (%)[1]**	**PI[2]**
Ethereal Extract 5µl	48.33 a	-0.03 N
Control	51.67 a	
Ethereal Extract 10µl	45.83 a	-0.08 N
Control	54.17 a	
Ethereal Extract 15µl	59.17 a	+0.18 A
Control	40.83 b	
Ethereal Extract 20µl	60.00 a	+0.21 A
Control	40.00 a	
Ethereal Extract 25µl	66.67 a	+0.33 A
Control	33.33 b	
Ethyl Extract 5µl	38.33 a	-0.23 R
Control	61.67 a	
Ethyl Extract 10µl	34.17 a	-0.32 R
Control	65.83 b	
Ethyl Extract 15µl	27.50 a	-0.45 R
Control	72.50 b	
Ethyl Extract 20µl	15.83 a	-0.68 R
Control	84.17 b	
Ethyl Extract 25µl	31.67 a	-0.37 R
Control	68.33 b	
Aqueous Extract 5µl	38.33 a	-0.23 R
Control	61.67 a	
Aqueous Extract 10µl	34.17 a	-0.32 R
Control	65.83 b	
Aqueous Extract 15µl	31.67 a	-0.37 R
Control	68.33 b	
Aqueous Extract 20µl	21.67 a	-0.53 R
Control	78.33 b	
Aqueous Extract 25µl	35.67 a	-0.28 R
Control	64.17 b	
Powder 0.3 g	35.00 a	-0.30 R
Control	65.00 b	

[1] *Means followed by the same letter, within each treatment, do not significantly differ, by the T Test at the level of 5% of probability.*

[2] *Classification: A = attractive; R = repellent; N = neutral.*

It can be observed that all treatments, i. e. when was used the different extracts and powders of the different plant species, they significantly differed from the control.

The ethyl extracts of the "Erva de Santa Maria", Pumpkin and Eucalyptus Citriodora, the aqueous extracts and powders of the same plants, beyond the ethereal extracts of "Erva de Santa Maria" and Pumpkin, did not statistically differed between themselves, presenting percentages of mortality between 23.00 and 45.00%.

The ethereal extract of Eucalyptus Citriodora, besides the statistical difference presented with the control, also differed statistically from the aqueous extract of the same plant and from the ethyl extract of the "Erva de Santa Maria". This ethereal extract i. e. from the Eucalyptus Citriodora presented the best mortality result, reaching 52.00% of mortality in the tested insects.

In agreement with TAVARES and VENDRAMIM (2005a) the mortality of *S. zeamais* adults occasioned by fruits and whole plant (with fruits) powders of *C. ambrosioides* significantly differed of the ones found in the control.

Table 4. Mortality (48 hours) of *Sitophilus zeamais* adults in maize grains treated with ethereal, ethyl and aqueous extracts and powders of plant origin.

Treatments[1]	Mortality (%)[2]
Control	0 a
"Erva de Santa Maria" (AP) – ethyl extract	23.00 b
Eucalyptus Citriodora (LE) – aqueous extract	24.00 b
Pumpkin (S) – ethereal extract	29.00 bc
Eucalyptus Citriodora (LE) – powder	30.00 bc
"Erva de Santa Maria" (AP) – ethereal extract	33.00 bc
Pumpkin (S) – ethyl extract	35.00 bc
Eucalyptus Citriodora (LE) – ethyl extract	35.00 bc
Pumpkin (S) – aqueous extract	36.00 bc
"Erva de Santa Maria" (AP) – aqueous extract	40.00 bc
"Erva de Santa Maria" (AP) – powder	40.00 bc
Pumpkin (S) – powder	45.00 bc
Eucalyptus Citriodora (LE) – ethereal extract	52.00 c
F	2.54*
CV	33.34

[1] *LE = leaves; S = seeds; AP = aerial part.*

[2] *Means followed by the same letter, within each treatment, do not significantly differ, by the T Test at the level of 5% of probability.*

Conclusions

Through the obtained data it can be concluded that the ethereal (except the Pumpkin), ethyl, aqueous extracts and powders of all tested plants had a repellent effect of *S. zeamais* adults. All the extracts and powders from the tested plants showed toxic effects to weevil adults. The plants "Erva de Santa Maria", Eucalyptus Citriodora and Pumpkin are control alternatives of this important plague of stored maize.

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