SCAP
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The use of *Laurus nobilis* and *Mentha pulegium* essential oils against *Sitophilus zeamais* (Coleoptera: Curculionidae) on stored maize

Utilização de óleos essenciais na proteção de milho armazenado contra *Sitophilus zeamais* (Coleoptera: Curculionidae)

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ABSTRACT

Laboratory studies were carried out to evaluate the effects of some essential oils from *Laurus nobilis* and *Mentha pulegium* against *Sitophilus zeamais* on stored maize. The concentrated essential oils at different volumes of 0.5 µL, 1.5 µL, 2.5 µL, 5.0 µL and 10 µL, were poured on filter papers with 2 cm Ø each. For diluted oils, the fixed volume of 15 µL of different concentrations of 1:150v/v, 1:100 v/v, 1:75 v/v, 1:50 v/v and 1:10 v/v either in methanol or n-hexane were used to impregnate the filter papers. Treatments with the concentrated oils were more effective. All the concentrations used from *M. pulegium* provided 100% adult mortality and no progeny production were achieved. *L. nobilis* has revealed 100% adult mortality at 3.185 $\mu L/cm^2$. Regarding the treatments with diluted oils once again the oil from *M. pulegium* provided 100% adult mortality at concentrations of 1:50 v/v and 1:10 v/v. *L. nobilis* was not effective at any of the concentrations used. There were no significant differences between the solvents used.

Keywords: botanical insecticides, essential oils, *Laurus nobilis*, *Mentha pulegium Sitophilus zeamais*

RESUMO

Para avaliar o efeito dos óleos essenciais de *Laurus nobilis* e *Mentha pulegium*, no controlo de insetos adultos de *Sitophilus zeamais*, em milho armazenado, foram efetuados vários ensaios laboratoriais, utilizando-se óleos essenciais concentrados e diluídos, em metanol e n-hexano. Os tratamentos com os óleos essenciais concentrados apresentaram melhores resultados do que com os diluídos. Verificou-se 100% de mortalidade e ausência de descendência (F_1) em todas as concentrações testadas de óleo essencial de *M. pulegium*. No caso de *L. nobilis* só se obtiveram aqueles mesmos resultados com a concentração mais elevada. Em relação aos óleos essenciais diluídos, os valores de 100% de mortalidade e ausência de descendência (F_1) só foram atingidos para a concentração de 1:10 v/v, com *M.* pulegium e em ambos os solventes. O óleo de *L. nobilis* não foi eficaz em nenhuma das concentrações ensaiadas, não apresentando diferenças entre os solventes utilizados.

Palavras-chave: inseticidas naturais, *Laurus nobilis*, *Mentha pulegium*, óleos essenciais, *Sitophilus zeamais*

Introduction

Maize is one of the most important cereal in the world because is a staple food for millions of people especially in Africa and Central America. According to the World Bank (2011) in Kenya and Tanzania maize contributes over one third of people's daily caloric intake.

Traditional storage practices in developing countries cannot guarantee protection against major storage pests of food crops like maize, leading to 20-30% grain losses, particularly due to post-harvest insect pests and grain pathogens (Tefera *et al*., 2011).

Insect damage to stored grains has been of great concern to man throughout the ages. Storage pests, such as the maize weevil (*Sitophilus zeamais*), the rice weevil (*S. oryzae*), the red flour beetle (*Tribolium castaneum*), the bruchid beetle (*Callosobruchus maculatus*) and others cause quantitative and qualitative damage to grains (Padin *et al*., 2002). One of the most economically destructive maize storage insect in Sub-Saharan Africa is *S. zeamais*.

The control strategies against *S. zeamais* consist in the use of the traditional chemical control but the development of insect resistance to insecticides and consumer concern over the use of these products in food has resulted in the search for alternative methods of insect control. Consumers today expect a food product that is pesticide free or with low residue levels. The management of stored product pests, using substances of natural origin, is nowadays the subject of much research (e.g. Papachristos and Stamopoulos, 2002).

In this study two essential oils, of *Laurus nobilis* and *Mentha pulegium*, were tested to answer the questions about the fumigant toxicity of these essential oils, adult mortality, life cycle and the effects on the emergence of the F1 of *S. zeamais*, in order to establish new control practices with lower mammalian toxicity and low persistence in the environment.

Material and Methods

Maize and insects

The yellow maize grain was acquired from local commerce with an average moisture content of 14 ± 0.5%. Unsexed adult insects aged from 1 to 7 days were used. The stock cultures of insects and the biological tests were carried out in a single incubator at 27˚C and 75± 5% r.h.

Plant material and essential oils

In this study the essential oil of *L. nobilis* used was obtained by hydro-distillation of air-dried leaves in a modified Clevenger-type apparatus for 3-h. The leaves were collected in the Agronomy College Campus. The extracted oil was dried over anhydrous sodium sulphate and stored in a sterilized amber bottle at 4 ^{0}C until used. The essential oil of *M. pulegium* was supplied by the company "Segredo da Planta".

Laboratory procedures

For the bioassays, plastic jars of 25 mL capacity containing 10 g of maize and 10 adult insects aged from 1 to 7 days old were used. The concentrated essential oils at different volumes of 0.5 μL, 1.5 μL, 2.5 μL, 5.0 μL and 10 μL, were applied on filter papers with 2 cm Ø each corresponding respectively to 0.159 μL/cm^2, 0.478 μL/cm^2, 0.796 μL/cm^2, 1.592 μL/cm^2 and 3.185 μL/cm^2. The treated filter papers were attached to the under surface of the plastic jars screw caps. The caps were screwed tightly and hermetically sealed with parafilm. Five replicates per treatment and control were set up. All the replicates were kept in an incubator at 27 ^{0}C and 75% ± 5% r.h..

After 24-h of exposure the adult insects were examined for mortality and thereafter were re-examined after 48-h and 168-h, on maize grain. After the last observation at 168-h the adult insects were removed and presumably only the young eggs or the early stage of larvae remained in the maize kernels which were kept at 27 ºC and 75% ± 5% r.h. during three weeks for the F_1 progeny evaluation. The development index and the life cycle were also evaluated.

Similar procedures were also adopted to evaluate the effects of the diluted essential oils. The fixed volume of 15 μL of different concentrations of 1 :150 v/v, 1 :100 v/v, 1 :75 v/v, 1 :50 v/v and 1 :10 v/v of diluted oils either in methanol or n-hexane were used to impregnate the filter papers of 2 cm Ø each. After the evaporation of the solvents during 2 – 3 minutes the filter papers were transferred into the plastic jars which were hermetically sealed. Five replicates per treatment and each control (methanol, n-hexane and non-treated ambient) were set up.

Data processing and analysis

Haryadi and Rahayu (2002) criteria was adopted to calculate the developmental index, using the following formula: DI = (ln F_1/ADD) x100, where DI = developmental index of *S. zeamais*; F_1 = number of adult insects emerged from F_1 and ADD = average duration of development.

For the analysis of the results it was used ANOVA statistical analysis and Tukey test for the means comparison.

Results and Discussion

The *M. pulegium* concentrated oil caused 100% mortality of *S. zeamais* after 24-h of exposure and complete inhibition of F_1 progeny at the lowest dosage of 0.159 μL/cm^2. The *L. nobilis* concentrated oil revealed 100% mortality and complete inhibition of F_1 progeny at the highest dosage of 3.185 μL/cm^2 after 168 h (Table 1).

Table 1. - Parent adult mortality and F1 progeny of Sitophilus zeamais treated with concentrated oils of *Laurus nobilis* and *Mentha pulegium*.

Plant species	volume (μL)	dose (μL/cm^2)	mortality (%)			F_1 progeny
			24-h	48-h	168-h	
L. nobilis	control	0	0	0	0	151
	0.5	0.159	0	0	0	88
	1.5	0.478	0	0	0	82
	2.5	0.796	6	24	40	80
	5.0	1.592	16	26	96	0
	10,0	3.185	96	96	100	0
M. pulegium	control	0	0	0	0	151
	0.5	0.159	100	100	100	0
	1.5	0.478	100	100	100	0
	2.5	0.796	100	100	100	0
	5.0	1.592	100	100	100	0
	10.0	3.185	100	100	100	0

The effects of *L. nobilis* in the duration of the development differed statistically between the dosage of 0.159 μL/cm^2 and the dosages of 0.478 μL/cm^2 and 0.796 μL/cm^2. This difference may be caused by suppression of feeding (Table 2). The results obtained in this study are according to Haryadi and Rahayu (2002). For the 1.592 μL/cm^2 and 3.185 μL/cm^2 dosages there was no progeny.

Table 2 - Average duration of development (ADD), life cycle, mean of F1 progeny and developmental index (DI) of *Sitophilus zeamais* from maize grain treated with concentrated oil of *Laurus nobilis*

Parameter	dosage (µL/cm^2)			
	0	0.159	0.478	0.796
ADD	36^a	37^a	39^b	39^b
Life cycle	33	34	32	36
Mean of F_1	30.2	17.6	16.4	16
DI	9.4^a	7.8^a	7.1^a	7.1^a

Means within the same line followed by the same letter are not significantly different (Tukey – P≤0.005)

The *M. pulegium* diluted essential oil at 1:10 v/v and 1:50 v/v with *n*-hexane caused 100% mortality after 168-h and a complete inhibition F_1 progeny. For the dilution with methanol 100% of mortality after 168-h and a complete inhibition F_1 progeny was obtained with a dosage 1:10 v/v. The methanol and *n*-hexane diluted oil of *L. nobilis* presented a weak effect in the mortality and in the F_1 progeny (Table 3).

Table 3 - Adults mortality and F1 progeny of *Sitophilus zeamais* treated with Laurus nobilis and *Mentha pulegium* diluted oils on n-hexane and methanol after 168h.

	n-hexane			methanol		
Plant species	dilution (µL)	mortality (%)	F_1 progeny	dilution (µL)	mortality (%)	F_1 progeny
L. nobilis	control	0	151	control	0	139
	n-hex	0	155	MeOH	0	109
	1:150	0	89	1:150	0	113
	1:100	0	114	1:100	2	70
	1:75	0	143	1:75	4	105
	1:50	0	120	1:50	12	64
	1:10	10	114	1:10	18	39
M. pulegium	control	0	107	control	0	130
	n-hex	0	128	MeOH	10	104
	1:150	4	148	1:150	6	122
	1:100	32	47	1:100	38	50
	1:75	90	42	1:75	72	20
	1:50	100	0	1:50	88	13
	1:10	100	0	1:10	100	0

The reduction and the absence of F_1 progeny may be attributed to the repellent effects of the substances in the essential oils and extracts, which disturb the oviposition behaviour of the parent insects. Another possibility is that there are substances in the essential oils and extracts that are toxic to *S. zeamais* larvae during their early development.

The results obtained are according to similar experiments performed by other authors. The essential oil of *L. nobilis* at the concentration of 0.1-0.74 mg/kg caused decrease on the adult longevity and fecundity of *S. granarius* (Boeke *et al*., 2001). Rozman *et al*. (2007) have demonstrated the fumigant activity of some major constituents present in the essential oils of *Lavandula* *angustifolia*, *Rosmarinus officinalis*, *Thymus vulgaris* and *L. nobilis* against adult insects of *S. oryzae*, *Rhyzoperta dominica* and *T. castaneum*.

Lee *et al*. (2001) reported substantial efficacy of *M. arvensis* oil as well as its constituents, menthone, linalool and α-pinene against *S. oryzae*. Similarly, Varma and Dubey (2001) reported complete inhibition of *S. oryzae* and *T. castaneum*, through the treatment of wheat samples with *M. arvensis* essential oil.

Lee *et al*. (2003) proved that plant monoterpene pulegone in preliminary fumigant analyses successfully controlled insect pests, and recommended this substance as suitable fumigant because of their high volatility, fumigant efficacy and safety.

In conclusion, the effects of the essential oils from *M. pulegium* have revealed differences in the development and in the F1 progeny of *S. zeamais,* in comparison with *L. nobilis* oils. *Mentha* species has all the potential to restrict synthetic insecticides as well as to be used in IPM (Integrated Pest Management) for control of various insects pests.

More studies are needed to further define the repellent effect, antifeedant effect and toxicity to storage insect pests of the active principles present in those essential oils.

These compounds may be suitable as fumigants because of their high volatility, effectiveness, and their safety

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