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Evaluation of toxic effect of plant extracts on adults of *Sitophilus oryzae* L., 1763 (Col., Curculionidae)

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Abstracts

The natural products of plants come as an alternative ecologically more compatible in substitution to the synthetic insecticides. This study had the objective to evaluate the action of vegetable extracts and essential oils on *S. oryzae* in the adult phase in stored wheat, through the assessment of toxic effect on exposed individuals to wheat grains treated. The biossay was done at the Laboratory of Entomology, DAG/UEM. The extracts of *P. nigrum* were obtained through the maceration of the grains with acetone and methanol and the essential oils of *Ocimum basilicum* and *Eucalyptus globulus* acquired of specialized companies.To study the toxic effects of the extracts on adults of *S. oryzae*, six concentrations were tested (50; 25; 12.5; 6.25; 3.125 and 1.5625 %) of each extract, in 40 g of wheat previously disinfectant, containing 20 adults of *S. oryzae* of 0-72 h of age. The experiments consisted of 4 repetitions for treatment. The same conditions were repeated for the experiments with essential oils of *O. basilicum* and *E.globulus*, where each portion received oil 0.1mL in 40 g of wheat. The evaluations were made to the 1, 3, 7, 10, 20 and 30 days after the treatments on grains. The insects dead were counted in each experiment, being discarded after each evaluation. The extracts of *P. nigrum* didn't control in an effective way the *S. oryzae* in the different concentrations. The other essential oils provided quite promising results, and the insecticide activity was reached by the three essential oils, being emphasized the oil of *E. globulus* with 100 % of mortality, *O. basilicum* and *M. piperita*, species that it has been proven as botanical insecticide potentials being proceeded, because the secondary metabolics of plants have been used as pesticides or models for synthetic pesticides, there is, however the need of complemental researches.

Key words: grain weevil, botanical insecticide, natural insecticide, essential oil, aromatic medicinal plants.

Introduction

When observing the history of the civilization along time, it can be seen that the human being has been using practices that, although empiric, constituted of techniques for the conservation of grains. With the coming of the agro-chemical industry, the natural practices gave place to the use of insecticides.

The use of chemical products in order to control agricultural curses or plagues became a constant in the last 50 years thus, such a method of control has been highly recommended. Chemical compounds artificially synthesized, on the other hand, have been used in an irrational way, besides not being incorporated to the

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integrated handling of pests, thus, increasing problems such as, the resistance of insects, the appearance of secondary pests, extinction of natural enemies, consumers´ intoxication, lefts of residue in food - above the limits allowed by law and environmental contamination.

The cereals globally considered as the most important vegetable species in men and animals´ feeding need to be stored for a long period of time, which is fundamental to supply the constant world demand for food.

The pests are seen as the cause of physical losses, besides being responsible for the loss in the quality of the grains and by-products at the moment they are being sent to the market and consumption. In Coleoptera (Curculionidae family) there are significant species, due to the damage and losses by weevils or woodworms (*Sitophilus oryzae* and *S. zeamais)* caused in maize, wheat and rice.

Considering the circumstances, the need to make a more rational control of pests is evident. Such a control can be done through an integrated handling, where the insecticide vegetables would be included in a process known as botanical insecticides or phito-insecticides. The rediscovering of plants that possess in their elements, metabolic or secondary compounds with insecticide property has been disseminating in the last 35 years; and in some countries of Latin America, researches seeking for plants with substances biologically active against pests, which cause smaller environmental impact, is growing with promising results (Garcia-Belt, 2002).

Mechanisms of natural defense frequently involve secondary metabolic then, the phito-chemical study of cultivated plants, associated to the natural resistance characteristics, can be used as a new alternative against pests. Extracts coming from plants, or their active components, have been used in the control of noxious insects, as a form of practicing a sustainable agriculture. Such metabolics are also known as natural products, and the most common examples in that research line, are found in the successful attempts to control some insects. There are substances with insecticide effect, as the ones obtained from eucalyptus (*Eucalyptus* spp.*)*, from chrysanthemum (*Chrysanthemum cinerariaefolium*) and from nim (*Azadirachta indica*), that have originated many similar substances, which are more efficient as insecticides, and that were synthesized and commercialized more than a decade ago (Voss and Roman, 2005).

Saito (2004) argues that a class of substances that has deserved a lot of attention are the substances that make part of the essential oil of some plants. The essential oils, or volatile oils, can be found in aromatic plants and can present attractive, repellent, and even poisonous activity to insects and microorganisms. Substances that have been used in the control of insects, and are present in the essential oil compounds are, for instance, the ones found in oils extracted from pine tree resin (*Pinus* sp.), the *nerol* extracted from the essential oil of '*capim limão*' (*Cymbopogon citratus*), the *limone* extracted from the oil of fruit peel belonging to several species of Citrus (orange, lemon), and some substances obtained from plants and used as food seasoning, such as, *eugenol* extracted from Indian clove (*Eugenia caryophyllata*), menthol extracted from mint (*Mentha piperita*), *piperin extracted* from black pepper (*Piper nigrum*) and sulfurate substances obtained from garlic extract (*Allium sativum*).

Baptista et al., (2005) when evaluating the insecticide activity of medicinal plants essential oil against pests, such as, the fall armyworm (*Spodoptera frugiperda)*, verified that the essential oils of *Ocimum gratissimum* (basil), *Ocimum basilicum* (sweet basil) and *Ruta graveolens* (rue) were poisonous for caterpillar, and the calculated LD50 were: *O.gratissimum* (0,518 µL/insect), *O.basilicum* (0,33 µL/insect) and *R.graveolens* (0,220 µL/insect). Maschio (1998) describes that *citral*, the main component of lemon grass oil (*Cymbopogon citratus* (D.C.) *STAPF*, presents properties against larvae and works as insect repellent. According to researches, the action of species against larvae in the extermination of the yellow fever mosquito is larger than the action of usual chemical insecticides.

Hill (1990) reports that, 2000 species of plants, which produce chemical substances able to act against insects are known. The substances can have poisonous and repellent effect, and can work as phagos restrainer, ovicide and can affect the insects' hormonal system. Moreover, a great number of essential oils can reduce the reproduction system of several insects found in stored products and they can also hinder the growth, the development and the reproduction of some herbivore insects. The main groups of chemical substances are the terpins, which are abundant compounds of many superior plants essential oils, such as, tannins and alkaloids.

Su (1977) in studies regarding the use of *Piper nigrum* as protection to stored products, verified the high toxicity of the raw extract or essence of the purified and of the powder of black pepper dry fruits on adults of *Sitophilus oryzae* and *Callosobruchus maculatus*, which was attributed to the synergic action of the pepper chemical compounds. Sighamony et al. (1986) compared the efficiency of *Piper nigrum* acetone extract to the efficiency of several essential oils extracted from native plants of India in the protection of wheat grains stored against *S. oryzae*. It was verified that the persistence of the extract of *P. nigrum* was superior to 60 days.

Obeng-Ofori et al. (2000) when testing species of *Ocimum* in the control of *S. zeamais* found that each substance, either topically applied or impregnated in the maize grains, was highly poisonous for the weevil. Jembere et al. (1995) proved the bioactivity of both, the extract and the essential oil of *Ocimum kilimandscharicum* on *S. zeamais*, *S. cerealella* and *Rhyzopertha dominica*. Weaver et al. (1991) evaluated the effectiveness of linalool, a component of *Ocimum canum*, on stored products pests. Dube et al. (1989) researched the fungi and insecticide properties of *Ocimum basilicum*.

Prates et al. (2000) concluded that the ingestion test and the contact with the grain, impregnated with the substance, has proved to be more efficient than the contact test made with the paper filter. Cineol and Limonen (terpins) and essential oils of *Eucalyptus globulus* and *E. camaldulensis* have shown poisonous effect in insects through the cuticle and in ingestion and fumigation, causing 100 % of mortality of *S. zeamais* in a dilution 2:8 (essential oil: acetone).

Considering the facts above exposed, the main aim of the present study was to evaluate the potential use of organic extracts of *Piper nigrum* and essential oils of *Eucalyptus globulus*, *Ocimum basilicum* and *Mentha piperita* in order to protect stored wheat, so that they can be used in the integrated handling of pests, specifically *Sitophilus oryzae*.

Materials and methods

The *Sitophilus oryzae* stocks of reproduction and the tests were carried out at the Laboratory of Entomology, DAG/UEM. The stocks of *S. oryzae* reproduction has started from adults obtained from samples of infested wheat. The adults were previously put in glass flasks, containing wheat disinfested at low temperature (-10 ºC) for 96 h. The removal of the insects was made 10 days after the infestation. The adults emerging in the flasks were used in the tests and also in the maintenance of the reproduction stock. Thus, two tests were performed:

Regarding the First Test – that was made with black pepper extract (*P. nigrum*), obtained in the concentrations of 50 and 1.5625 %, by using two solvents, acetone and methanol. Pepper grains (500 g) were triturated in an electric mill, thus obtaining a fine powder that was conditioned in dark color flasks and then conserved at 0 ºC temperature up to the preparation of the extract. The solvents were added in the pepper powder, in 1:1 proportion (vegetable powder weight: solvent volume), remaining for 10 days. After that period, each mixture was filtered and the obtained solution was kept in flasks; the volume was adjusted to concentrations previously planned, placed in dark flasks separately and stored in refrigerator ± 5 °C.

After the disinfestations, the wheat was submitted to different treatments, with 1 mL each 40 g, an amount enough to cover the surface of the grains without any accumulation in the bottom of the flask. For each treatment, the grains were placed in glass flasks and the solution was flowed by the walls of the flask, with the aid of a graduate pipette. The flask was closed and manually agitated for 4 minutes, and the treated grains were dried in open air for 4 hours, on a surface covered with plastic.

The experimental outlining was entirely casualized or at random (OEC), in factorial scheme/outline 2 x 2 +3, with 4 treatments and 4 replications, being used 2 types of extracts (methanol and acetone) in 2 concentrations (50 and 1.5625 %), besides 3 additional control treatment: methanol, acetone and water).

The Second test - aimed at evaluating the effect of plants such as, eucalyptus (*Eucalyptus globulus*), basil (*Ocimum basilicum*) and mint pepper (*Mentha piperita*), on *S. oryzae* adults. The vegetable species were collected at ‘Horto of medicinal plants’ and in the ‘area of experimental cultivation’ of the university – Universidade Estadual de Maringá. The essential oils were obtained through distillation - ´steam dragging´ - by using 1,000 grams - fresh weight of pricked leaves of each plant, that is, each oil constituted a different treatment.

After the disinfestations, the wheat was submitted to the different treatments, which used 30 g of grains, conditioned in flasks of acrylic, then adding 1 mL of each essential oil with the aid of a graduate pipette. The control treatment was constituted by identical amounts of wheat grains submitted to the treatment with 1 ml of water. After submitting the wheat to the treatments and after manual to agitation for 4 minutes, the grains were exposed to the air, on a surface covered with plastic.

The infestation of the treated grains and exposure to air, was made with 20 insects (0-72 hours/age). The flasks were maintained at acclimatized room during the testing period. The counting of exterminated insects was accomplished daily for 30 days, insects that have not presented any reaction when touched with a brush were considered killed.

The experimental outlining was entirely casualized or at random, (OEC), with 4 treatments and 4 replications. The averages were compared among themselves by Tukey Test , in level of 5 % of probability and, compared to the control treatment by Dunnett Test ($P \leq 0.05$).

Results and discussion

1st Test - Concentrations of Raw Extract of *Piper nigrum*:

The variance analysis revealed that there was only significant effect, in level of 5 % for test F, regarding the factor - Concentrations of Raw Extract of *Piper nigrum*. There was factorial difference from the control treatment, but, there was no difference among each other, that is, the methanol control treatment has not presented any difference, neither from acetone nor from the control treatment with water. The type of extract used has not influenced in the mortality of insects. But, the concentration presented differences, that is, when using a concentration of 50 %, a larger mortality occurred, whereas, the opposite happened when a concentration of 1.5625 % was used (Table 1). Regarding statistics, such difference in the insects’ mortality was significant in level of 5 % for test F.

The result obtained in the present test, proving the insecticide activity of *P. nigrum* plant, was inferior to the results obtained in the control of *Sitophilus zeamais* by Nagamo et al. (2001), who evaluated the essential oil of this species in natural concentrated form and not diluted, as it is the case of the extract obtained from grains. The authors verified that, *P. nigrum* oil caused larger mortality than 96 %, after 48 hours of the application, that is, after the contact of insects with the product, a result considered highly promising.

Besides the results obtained by the authors (Nagamo et al., 2001), it is worthwhile mention that, a black pepper synthesizes a wide variety of secondary compounds that present insecticide activity and are found in the species vegetable organs, a fact already proved by science, regarding: - pinen, - terpinen, - terpineol, carvon, cariophilen ocide, d-limonen, eugenol, limonen, linalool, methilcinamal, miristicin, ocimol, piperin and rutin, and besides those compounds, also borneol and camphor, that possess repellent activity among others (Duke, 2005). Piperin is the most abundant compound/element of black pepper fruit-extract, but despite that, piperin does not seem to be the main responsible for the activity presented by this extract. However, that cannot be left apart, once it is significant to mention that when acting synergistically with other substances, piperin can make the toxicity of the extract strong (Nagamo et al., 2001).

Such information, is similar to the results obtained by Cruz et al. (2000) who emphasize that factors such as, place, cultivation time, collection time, preparation form, application method and concentration of products originating from medicinal plants are decisive factors in the obtaining of successful or promising results, because vegetable active principles are unstable and are not distributed in a homogeneous way in the plant. A single species can contain more than two hundred compounds, among them, the essential oil (composed by a mixture of volatile substances), and usually the vestigial components are essential for their biological activity, that can be changed just in the absence of one of the compounds.

2nd test – Evaluation of plant effects / where essential oils were used:

In this test (Figure 1) it can be observed aspects such as, where some essential oils of three species were used; and that the percentage of efficiency Abbott - reached by *Eucalyptus globulus* oil - was of 100 %, followed by *Ocimum basilicum* and *Mentha piperita* oils, with 79.2 % and 43.1 %, respectively.

The result obtained with the application of *E. globulus* oil, corroborates several previous studies accomplished with this species used in the control of curses in the fields, as well as, in stored grains. Duke (2005) states that it was proved that *E. globulus* species has in its composition, the following chemical compositions:

- Insecticide activity: 1,8 - cineol, - pinen, - terpinen, anethol, benzaldehyde, estragol, eugenol, limonen, linalool, menthol, methilchavicol, methilcinamat, ocimen, rutin and timol.
- Repellent activity: 1,8 - cineol, - pinen, - terpinen, benzaldehyde, cafeic acid, camphor, citronelal, ferulic or antioxidant acid, - terpinen, geraniol, limonen, linalool, linalool acid, mircen, p-cimol, piperiton, timol, verbenon.

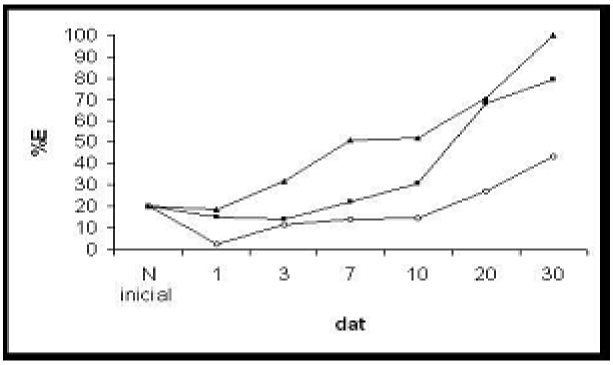

Figure 1. Adult mortality (Percentage efficiency Abbott - %E) of *Sitophilus oryzae* when treated using essential oil of *Ocimum basilicum* ( ), *Mentha piperita* ( ) and *Eucalytpus globulus* ( ) (n = 20 adults/replicate in 4 replicates) on 1,3,7,10,20 and 30 days after treatments (dat).

Table 1. Effect of concentrations of acetonic and methanolic extracts of de black piper (*Piper nigrum*) of adult survive of *Sitophilus oryzae*.

Concentration (%)	Methanolic extract (*P. nigrum*)	Acetonic extract (*P. nigrum*)	Means
50	14.75	13.75	14.25 A
1.5625	18	18.5	18.25 B

Means followed by the different letters are significantly different at the 5 % level by the F test (4 replicates).

- Feeding inhibiting activity: pinen, terpinen, isoquercitin, limonen, luteolin, rutin, terpinolen.

The insecticide effect of 1,8 - cineol was already confirmed for the *Rhyzopertha dominica* (F.) and for the beetle *Tribolium castaneum* (Herbst), which cause great economical losses in the cereal storage. According to the authors above mentioned, 1,8-*cineol* or *eucaliptol*, a natural product produced in the plants secondary metabolism is an element/compound also found in the essential oil extracted from leaves of several species of *Eucalyptus* spp. (Myrtaceae). The concentration of that substance in the eucalyptus leaves can vary according to the species, the variance is: *Eucalyptus citriodora* (55 %), *Eucalyptus globulus* (71 %), *Eucalyptus punctata* (66 %), *Eucalyptus maculata* (51 %), *Eucalyptus maidesii* (70 %), *Eucalyptus smithii* (84 %) and so on (Chagas et al., 2002).

Other essential oils also provided quite promising results (Table 2). Regarding the evaluation previously proposed in the present study, the insecticide activity was reached by three essential oils, that is, *E. globulus*, *O. basilicum* and *M. piperita* oil, extracted from species seen as natural or botanical potential insecticide, once the secondary plant metabolic have been used as pesticides or as models for synthetic pesticides, thus, they can cause poisonous interference in the biochemical and physiologic functions of herbivore insects. However, most of these compounds are not poisonous (or possess a low level of toxicity) for mammals, since they are usually recognized as safe by '*United States Food and Drug Administration*'. Considering that, such compounds are used in many products for human use, such as, seasonings, perfumes and countless medicinal formulas of: expectorants, decongestants, topical/external painkillers and antiseptics. Nevertheless, it is necessary to go on making researches and seeking for the maximum potential that these species can offer.

In conclusions, extracts in general, independently on the form (methanol or acetone), in the concentration of 50 %, can kill larger number of insects; a contrary result when compared to the one obtained when small concentration (1.5625 %) were used. *Eucalyptus globulus* essential oil provided 100 % of insect (*Sitophilus oryzae*) mortality, being followed, in terms of effectiveness, by *Ocimum basilicum* oil and then by *Mentha piperita* oil, which has shown a smaller efficiency or activity against insects.

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Table 2. Effect with essential oils concentrations of *Eucalyptus globulus*, *Menta piperita* e *Ocimum basilicum* of adult (nº) of *Sitophilus oryzae*.

Essential Oils	*Eucalyptus globulus*	*Ocimum basilicum*	*Mentha piperita*
	0 a (-)	3.75 b (-)	10.25 c (-)
Control	18.50		
C.V.	29.2 %		

Means followed by the same letters are not significantly different at the 5 % level by the Tukey test (4 replicates).

(-) Means were low level to control by Dunnett test ($P \leq 0.05$).

ns No significant.

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