PS7-31 – 6291

Toxic effect of vegetable extracts on adults of *Sitophilus zeamais* Mots. 1855 (Col., Curculionidae)

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Abstract

This study sought to evaluate the potential use of vegetable extracts and essential oils in the stored corn grain protection against *S. zeamais*, through the determination of mortality effect on exposed individuals to treated grains.The biossay was done at the Laboratory of Entomology, DAG/ UEM. The extracts of *Piper nigrum* were obtained through the maceration of the grains with acetone and methanol and the essential oils of *Ocimum basilicum* and *Eucalyptus globulus* were acquired. To study the poisonous effect of the extracts on adults of *S. zeamais*, 6 concentrations they were tested (50; 25; 12.5; 6.25; 3.125 and 1.5625 %) of each extract, in 40 g of corn previously disinfectant, containing 20 adults of *S. zeamais* 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 0.1 mL in 40 g of corn. The evaluations were made to the 1, 3, 5, 7, 10, 15, 20 and 30 days after the treatments of the grains. The insects died were counted in each experiment, being discarded after each evaluation. Significant differences were not observed in the mortality of *S. zeamais* in the different concentrations of *P. nigrum* in relation to the control, indicating the use of insufficient dosages to promote the wanted poisonous effect. As for the essential oils, *E. globulus* stood out promoting adults' mortality, however at low levels of efficiency. It is indicated like this the need of new tests of the tested treatments seeking to obtain significant control of the populations of insects of products stored with the use of derived natural products of plants.

Key words: botanical insecticide, natural insecticide, essential oil, *Ocimum basilicum, Eucalyptus globulus.*

Introduction

Cereals constitute the largest source of food, for human beings, as well as for animals, thus about 90 % of the grains produced for their consumption come from cereals such as, wheat, maize and rice, that represent the basis of the feeding of almost all of the people living in the world (Hall, 1971; McFarlane, 1989; Garcia-Correia, 2002). Considering that nowadays the population growth is a reality, the world need for food is growing daily, consequently, there is a demand for the maintenance of quality and quantity or amount of the mentioned products, so that they can get to the final consumers regularly.

According to previous studies, from 80

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million tons of grains annually produced in Brazil, 20,0 % is wasted during the crop process or in the transport and in the storage system. The losses due to attack of plagues or curses during the storage get to 10,0 %, thus being considered one of the main problems faced by the farmer in the period after-crop. The curses or plagues have been causing physical losses and are responsible for the loss in the quality of both, grains and by-products, when they are sent to commercialization and consumption. Another factor that contributes to make the problem more serious is the reduced number of registered insecticides to be used in the control of weevils on stored grains pests, thus hindering the alternation of active ingredients (Conab, 2005).

Many species of curses can be found in stored products and their by-products. Among them, insects are seen as the agents highly responsible for the losses in the period after-crop.

Most of the species is cosmopolitan, although they have been disseminated all over the world, mainly due to the commercial exchanges. Synthetic insecticides that act in response to contact, ingestion or for fumigation have been used broadly in the control of insects, but on the other hand, the excessive application of products denominated insecticides, pesticides or agricultural defensive has been favoring the appearance of resistant insects, thus reducing the natural population of enemies. In addition to those facts other factors should be mentioned, such as, the contamination of food, pollution of rivers, soil erosion, desert growing process, intoxication and death of animals, among others (Medina et al., 2002; Garcia-Correia, 2002; Rodriguez et al., 2003; Vendramin & Rodriguez, 2003).

Usually, Coleoptera order contains the largest number of species and, among them, some of the most important infestation or curses of the grains and stored by-products. In the Curculionidae family, about 40.000 species are described and within the main primary curses listed are the weevils or carterpillars of stored grains. Although that family contains many destructive agricultural curses, just the *Sitophilus* species are regarded as significant storage curses. The three species, *S. zeamais* Motschulsky, *S. oryzae* (L.) and *S. granarius* (L.) are known as destructive of stored cereals. The cosmopolitan and abundant species are found in tropical areas, presenting high reproduction capacity, great number of individuals obtained in each progeny and high amount of generations, thus enabling a few insects to form a considerable population, in a short period of time (Harein & Davis, 1992; Pedersen, 1992; Haines, 1995; Gallo et al., 2002).

In several parts of the world, products, seen as ecologically more compatible, have been studied in order to be used for the control of stored grains curses or plagues. Considering that, chemical compounds found in plants, originating from the secondary metabolism of vegetables, have biological activities developed by them along their existence, having been useful to guarantee their survival, once after specific studies, they have been used as medicine, insecticide, repellent, larvae insecticide, anti-microbial and so on.

Essential oils (or natural essences), resins, alkaloids, flavonoids, bitter principles (bitters), among others, are seen as substances or secondary compounds. Such substances can be found in plants in the form of compounds whose components complete and reinforce their action on the organism. Even when the medicinal plant possesses only one active principle, it presents a superior beneficial effect to the one produced by the same substance obtained by chemical synthesis (Worwood, 1995; Silva et al, 1995).

The secondary compounds mentioned can represent several important functions for the plant, such as, interactions plant-plant (alelopathy), plant-animal (anti-herbivore, attraction of pollinating agents) and plant-insects (plagues or curses). Regarding the latter, such compositions could take part in the plant defense responses, in vegetable organs response, that is, in the plant that produces them or in any other systems, acting as insecticide substances, similarly to synthetic insecticides (Stangarlin et al., 1999).

Essential oils are mixtures of volatile organic substances, with consistence similar to the oil, being defined by a group of properties, among which - smell, flavor and high concentration. Although the chemical constitution of the essential oils is very differentiated, they are substances that possess physical properties in common, such as, characteristic smell, high refraction index, and most of them are optically active and, many times, their specific rotation is an useful property when regarding characterization (Robbers et al., 1997).

The potential use of medicinal plants for the control of stored grain curses has been shown in different studies. Hill (1990) reports that 2,000 species of plants, which produce chemical substances able to act against insects are known. The substances can have different effects. They can be poisonous, repellent, phagos restrainer, ovocide and can affect the insects' hormonal system. Moreover, a great number of essential oils can reduce the reproduction of several insects found in stored products and they can also affect by hindering the growth, the development and the reproduction of some herbivore insects.

Su (1977) in studies regarding the use of *Piper nigrum* as a 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 the attack of *S. oryzae*. It was verified that the persistence of the extract of *P. nigrum* was superior to 60 days.

The fumigation with essential oil of *Mentha piperita*, in the concentration of 15 μl/liter, for a 3-hour-period, has caused mortality superior to 75 % in adults of *Tribolium castaneum*, *Rhyzoperha dominica*, *Oryzaephilus surinamensis* and *S. oryzae* (Shaaya et al., 1991). When evaluating the efficiency of *M. piperita* essential oil in the control of *Tribolium castaneum*, Mishra and Kumar (1983) verified that 4 ml/liter applied in fumigation, for 48 hours, has caused 91 % of mortality in the 1st larvae instar, 71% in the 2nd instar and 60 % of mortality in adults. Klingauf et al. (1983) concluded that the exposition to 6 μl/liter, through fumigation with essential oil of *M. piperita*, for 3 hours, has lead to mortality of 100 % in *Sitotroga cerealella* and 50 % in adults of *Acanthoscelides obtectus*.

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. 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 *Limoneno* (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).

Thus, this work was developed aiming at evaluating the potential of the essential oil of *Eucalyptus globulus Labill.*, *Mentha piperita L.* and *Ocimum basilicum L.*, in the control of *Sitophilus zeamais* found in stored maize.

Materials and methods

The pre-test was carried out at the Laboratory of Entomology, Department of Agronomy, of a State University – Universidade Estadual de of Maringá, PR.

The reproduction stocks of *Sitophilus zeamais* was obtained from adults found in infested maize samples, that were previously put in glass flasks containing non-infested maize at low temperature (-10 ºC) for 96 h. The removal of the insects was made 10 days after infestation. The adults emerging in the flasks were used when carrying out the test and in the maintenance of the weevil reproduction stock.

The essential oils of eucalyptus (*Eucalyptus globulus*), mint pepper (*Mentha piperita L.*) and basil (*Ocimum basilicum L.*), were obtained by steam dragging by using 1000 grams of pricked leaves - fresh weight - collected at 'Horto of medicinal plants' and in the area of experimental cultivation of the university – Universidade Estadual de Maringá.

After disinfestations, the maize (40 g) was submitted to treatments with 1 ml of essential oil of each different plant species, in amounts considered enough to cover the surface of the grains. For each treatment, the grains were placed into the glass flasks and the oil flowed by the walls of the flask, with aid of a graduate pipette, without any accumulation in the bottom of the flask. Afterwards, the flask was closed and agitated manually for 4 minutes, and the treated grains were placed on a surface covered with plastic and then exposed to air for a period of 3 hours. The control treatment was constituted by identical amounts of maize grains submitted to treatment with 1 ml of water.

The infestation of the treated grains was accomplished soon after the air exposure and the flasks containing the different treatments were maintained at acclimatized room during the whole testing or rehearsal period. The counting of alive insects was accomplished daily, for 60 days. The insects that have not presented any reaction when touched with a brush, were considered as dead or exterminated.

The experimental outlining was entirely casualized (OEC), with four treatments and four replications, being each sample constituted of 20 insects (0 - 72 hours/age). After the variance analysis, the averages were compared among themselves by Tukey Test, in a level of 5 % of probability, and then compared to the control treatment by Dunnett Test ($P \leq 0.05$).

Results and discussion

The *O. basilicum* essential oil, after 60 days of the infestation of maize grains, presented the largest number of alive insects, contrary to the effect caused by *Eucalyptus globulus* oil; 60 days after the treatment, a reduced number of insects (10.25) remained alive, thus constituting about 50 % of mortality (Figure 1).

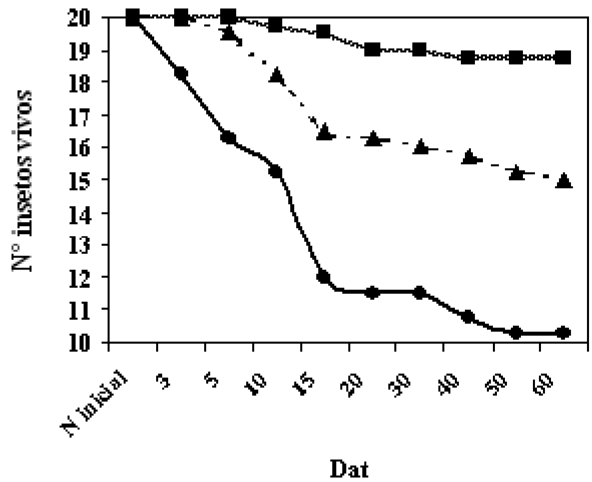

Figure 1. Adult mortality of *Sitophilus zeamais* when treated using essential oil of *Ocimum basilicum* ( ), *Menhta piperita* ( ) and *Eucalytpus globulus* ( ). n = 20 adults/replicate in 4 replicates) on 3, 5, 10, 15, 20, 30, 40, 50 and 60 days after treatments (dat).

Similar result was obtained by Nakano and Cortez (1967), as well as by Anjos and Santana (1994), who verified that the active ingredients contained in the leaves of *E. citriodora* and in other species of the same family have shown to be promising for the control of plagues or curses of stored grains and against ants (cutters) belonging to Atta family.

Procopio et al. (2003) evaluated, in laboratory conditions, the repellence level and *S. zeamais* adults' survival in corn grains treated with several vegetable species powders that possess essential oils in their compounds and verified that, besides the insecticide effect, *Eucalyptus citriodora*, as well as the other species of the same family, have shown strong repellence activity, being this biological activity quite intense.

In Table 1 contains the results obtained with the different treatments made with the essential oils - about *S. zeamais* survive number- where the effectiveness of the oils can be observed. The largest activity effect was provided by *E. globulus* essential oil, being followed by *M. piperita* oil. The essential oil of *O. basilicum* provided similar values to the control treatment, thus, the result was different from the result obtained by Obeng-Ofori et al. (2000) when testing *Ocimum* species in the control of *S. zeamais*, once each of the substance topically applied or impregnated in the maize grains were considered highly poisonous in the use against the weevil.

Duke (2005) reports that it was proved that, *O. basilicum* plant has in its elements, the following chemical compounds, with insecticide activity: α-pinen, α-terpinen, α-terpineol, α-tujon, anethol, benzeldeid, carvon, estragol, eugenol, furfural, limonen, linalool, menthol, methilchavicol, methilcinamol, ocimen, rutin and timol. Considering the scientific proof of the insecticide effect of such species, the result obtained was contrary to the ones in other studies, due to reasons registered and reported by Cruz, Nozaki and Batista (2001), who state that the place, the cultivation time, the collection time, the preparation form, the application method and concentration of the products originating from medicinal plants are decisive factors to obtain good results, because vegetable and plant active principles are unstable and are not distributed in a homogeneous way in the plant. It is common to observe the existence of more than two hundred components in essential oils, and usually the components are essential for their biological activity, can be altered by the absence of just one of the components. It is emphasized that, not always, high concentrations provide the best results.

When evaluating Table and Figure 1, it can be observed in comparison with the control treatment (18.50), that *E. globulus* essential oil (10.25) is suitable for the technique and dosage used in the present study. Thus, new studies and researches regarding this research line are necessary, because due to the population boom, a great amount of food is necessary and it is important to avoid the excessive use of synthetic pesticides to have food with good quality and without resistant noxious elements. When stimulating the use of natural products, extracted from vegetables, this experiment will be made positive in accordance to the results obtained. In conclusion, the essential oil of *E. globulus* has provided larger insecticide effect, whereas, the essential oil of *M. piperita* provided insecticide activity, however, inferior to the one found in *E. globulus*, regarding *S. zeamais*, the essential oil of *O. basilicum* has not presented insecticide biological activity. Concerning future researches, seeking for the improvement of techniques on phytochemicals use is an urgent need.

Table 1. Effect with essential oils of *Eucalyptus globulus*., *Menta piperita* e *Ocimum basilicum* of survive of adult (nº) of *Sitophilus zeamais*.

Essential oils (1ml)	*Eucalyptus globulus*	*Menta piperita*	*Ocimum basilicum*
	10.25 a $^{(-)}$	15.0 b ^{ns}	18.75 c ^{ns}
Control	18.50		
C.V.	12.3 %		

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.

References

Anjos, N., Santana, D.L.Q., 1994. Alterações deletérias no comportamento de *Atta laevigata* (F. Smith) e *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae), causadas por folhas de *Eucaliptus* spp. Anais da Sociedade Entomológica do Brasil 23, 25-30.

Conab. Companhia Nacional de Abastecimento. Available in <http:// www.conab.gov.br/>.

Cruz, M.E.S.; Nozaki, M.H.; Batista, M. A., 2000. Plantas Medicinais: Plantas Medicinais e Alelopatia. Biotecnologia Ciência e Desenvolvimento, Brasília/BR, 15, 28-34.

Duke, J., 2005. USDA, ARS, National Genetic Resources Program. 2005. *Phytochemical and Ethnobotanical Databases*. Online. Nat. Germplasm Resources Lab., Beltsville, Maryland.

Gallo, D., Nakano, O., Silveira Neto, S., Carvalho, R.P.L., Baptista, G.C., Berti Filho, E., Parra, J.R.P., Zucchi, R.A., Alves, S.B., Vendramin, J.D., Marchini, L.C., Lopes, J.R.S., Omoto, C., 2002. Entomologia Agrícola. Piracicaba: FEALQ, 920 p.

García-Correia, M.A., 2002. Bioatividade da Erva-de-Santa-María, *Chenopodium ambrosioides* L. (Chenopodiaceae), en relaçao a *Sitophilus zeamais* Mots., 1855 (Col.: Curculionidae). Dissertação (Mestrado) . Área de Concentração: Entomologia, Universidade de São Paulo, São Paulo, Brasil.

Haines, C.P., 1995. Grain storage in the tropics. In: Jayas, D., White, N.D.G., Muir, W.E. (eds.). Stored-grain ecosystems. New York: M. Dekker, pp.169-197.

Hall, D. W., 1971. Manipulación y almacenamiento de granos alimenticios en las zonas tropicales y subtropicales. Cuadernos de fomento agropecuario Nº 90, FAO. Roma, Italia.

Harein, P.K., Davis, R., 1992. Control of stored-grain insects. In: Sauer, D.B. (ed.). Storage of Cereal Grains and their Products. St. Paul: American Assoc. of Cereal Chemists, pp.491-534.

Hill, D.S., 1990. Pest of stored products and their control. London, Brit. Library, 274p.

Klingauf, F., Bestman, H.J., Vostrowsky, O., Michaelis, K., 1983. The effect of essential oils on insect pests. Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie 4, 123-126.

McFarlane, J.A., 1989. Guidelines for pest management research to reduce stored food losses caused by insect and mites. Bulletin N° 22. Overseas Development Natural Resources Institute. London, England.

Medina, S., Ortega, L.A., González, H.H. Y Villanueva, J.J., 2002. Influencia de arvenses sobre el complejo mosca blanca-virosis-parasitoides en Veracruz, México. Manejo Integrado de Plagas y Agroecología 65, 75-81.

Mishra, R.C., Kumar, J., 1983. Evaluation of *Mentha piperita* L. oil as a fumigant against red flour beetle, *Tribolium castaneum* (Herbst). Indian Perfumery 27, 73-76.

Nakano, O., Cortez, J., 1967. Ensaio de controle às pragas do milho armazenado, com óleo de eucalipto (*Eucaliptus citriodora* Hooker) e sua eficiência comparada ao malathion. Revista da Agricultura, 42, 95-98.

Obeng-Ofori, D., Jembere, B., Hassanali, A., Reichmuth, Ch., 2000. Effectieveness of plant oils and essential oil of *Ocimum* plant species for protection of stored grains against damage by stored product beetles. Proceedings of the 7th International Working Conference on Stored-product Protection. Beijing – China, pp. 799-808.

Pedersen, J.R., 1992. Insects: identification, damage and detection. In: Sauer, D.B. (ed.). Storage of Cereal Grains and their Products. St. Paul: American Association of Cereal Chemists, pp. 435-491.

Prates, H.T., Santos, J.P., Waquil, J.M., Oliveira, A.B., 2000. The potencial use of substances extracted from Brazilian flora to control stored grain pest. Proceedings of the 7th International Working Conference on Stored-product Protection. Beijing – China. p. 820-825.

Procópio, S.O., Vendramim, J.D., Ribeiro Júnior, J.I., Santos, J.B., 2003. Bioatividade de diversos pós de origem vegetal em relação a *Sitophilus zeamais* MOTS. (Coleoptera: Curculionidae). Ciênc. Agrotec. 27, 1231-1236.

Robbers, J.E., Speedie, M.K., Tyler, V.E., 1997. Pharmacognosy and Pharmacobiotechnology. Baltimore, USA: Williams & Wilkins, 372p.

Rodríguez, C., Silva, G., Vendramim, Y.J., 2003. Insecticidas de origen vegetal. En: Silva, G., Hepp, R. (Eds.) Bases para el manejo racional de insecticidas. Universidad de Concepción, Fac. Agron. Chillán, Chile. pp. 89-111.

Shaaya, E., Ravid, U., Paster, N., Juven, B., Zisman, U., Pissarev, V., 1991. Fumigant toxicity of essential oils against four major stored-product insects. Journal of Chemical Ecology 17, 99-504.

Sighamony, S., Annes, I., Chandrakala, T., Osmani, Z., 1986. Efficacy of certain plant products as grain protectants against *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.). Journal of Stored Product Research 22, 21-23.

Silva, I., Miranda Neto, M.H., Franco, S.L., Cardoso, M.L.C., Molinari, S.L., Sant'ana, D.M.G., Conegero, C.I., Iwanko, N.S., 1995. Noções sobre o organismo humano e utilização de plantas medicinais. Cascavel, Assoeste, 203 p.

Stangarlin, J.R., Schwan-Estrada, K.R.F., Cruz, M.E.S., Nozaki, M.H., 1999. Plantas medicinais e controle alternativo de fitopatógenos. Biotecnologia Ciência & Desenvolvimento 11, 16-21.

Su, H.C.F., 1977. Insecticidal properties of black pepper to rice weevils and cowpea weevils. Journal of Economic Entomology 70, 18-21.

Vendramim, J., Rodríguez, C., 2003. Insecticidas y resistencia vegetal.. In: Silva, G., Hepp, R. (Eds.) Bases para el manejo racional de insecticidas. Universidad de Concepción, Fac. Agron. Chillán, Chile. pp. 53-67.

Worwood, S., 1995. Aromaterapia. Um Guia de A a Z para o uso terapêutico dos óleos essenciais. São Paulo: Ed.Best Seller, 251 p.