Available Online at ESci Journals

International Journal of Entomological Research

ISSN: 2310-3906 (Online), 2310-5119 (Print)

http://www.escijournals.net/IJER

EVALUATION OF EXTRACTS AND ESSENTIAL OILS FROM “POEJO”, *CUNILA ANGUSTIFOLIA* (LAMIALES: LAMIACEAE) LEAVES TO CONTROL ADULTS OF MAIZE WEEVIL, *SITOPHILUS ZEAMAIS* (COLEOPTERA: CURCULIONIDAE)

[a]Marcoandre Savaris, [b]Sandra M. Sabedot-Bordin, [c]Caroline E. Mendes, [d]Neusa F. Moura, [e]Cristiano M. Teixeira, [e]Flavio R.M. Garcia*

[a] Postgraduate in Entomology, Federal University of Paraná, Curitiba, Paraná, Brazil.

[b]Community University Region Chapecó, Avenida Senador Atílio Fontana, Brazil.

[c] Federal University of São Carlos, São Paulo, Brazil.

[d] Federal University of Rio Grande, Avenida Paulo Gama, Porto Alegre, Brazil.

[e] Department of Ecology, Zoology and Genetics, Biology Institute, Federal University of Pelotas, Pelotas, Brazil.

A B S T R A C T

The maize weevil, *Sitophilus zeamais* is a major pest of stored grain whose control has been performed with the use of synthetic insecticides, which in many cases are being banned due to issues involving risks to human and animal health and to the environment. In order to avoid the use of these insecticides, alternative control has been sought, including the use of plant extracts. In this study, “poejo”, *Cunila angustifolia* leaves extracts and essential oils have been used against S. zeamais. The results of the bioassay for the control of S. zeamais showed statistical significance (P <0.05) for all sources of variation. The essential oil reached 100% efficiency at all different concentrations, the hexane extract at 0.32% showed 38.70% efficiency, while the aqueous and crude extracts showed no insecticidal effect on adults of S. zeamais. The highest mortality rate was found after 96 h of application of the extracts.

Keywords: Weevil, insecticide, pest, stored, grains.

INTRODUCTION

Maize weevil, *Sitophilus zeamais* (Motschulsky, 1855) is a major pest of stored grain whose control is performed with the use of insecticides. These substances cause serious damage to the environment and human health, making it necessary to use other control methods (Lovatto *et al*., 2012). The presence of this insect causes severe losses by reducing the weight of agricultural products and consequently its commercial and nutritional value (Potenza *et al*., 2004). The current paradigm of relying almost exclusively on synthetic chemicals for pest control may need to be rethought. New pesticides, including those based on natural products, are being discovered and developed to replace those compounds no longer accepted due to new requirements for registration (Dayan *et al*., 2009). Plants with insecticidal purpose have been currently one of the most studied alternative methods to control stored product pests. This method is widely used throughout the world, due to ease of application and the nature of the substrate to be protected; it is preferred to use powders in relation to other plant derivatives (Procópio *et al*., 2003). Bioactive organic compounds produced by plants include repellents, growth inhibitors, sterilizing and toxins that form an extensive chemical defense against invading pathogens and insects (Cavalcante *et al*., 2006). These common compounds in vegetables such as essential oils have insecticidal activity by the presence of terpenoids (Viegas, 2003; Smaniotto *et al*., 2010; Marangoni *et al*., 2012). Among the species indicated for use as an insecticide is “poejo”, *Cunila angustifolia* Bentham, 1834 (Lamiales: Lamiaceae). The plant C. angustifolia is a species native to tropical America, widely distributed throughout the world (Xifreda and Mallo, 2001). This species has in parts of its structures,

* Corresponding Author:

Email: flavio.garcia@pq.cnpq.br

such as leaves and flowers, oils and volatile chemical constituents like linalool, cineole, menthofuran, limonene, isomenthone, menthone, pulegone, sabinene, terpinene and trans-piperitone oxide (Manns, 1995; Bordignon *et al*., 1997; Bordignon *et al*., 1999; Echeverrigaray *et al*., 2003; Savaris *et al*., 2012). In nature, these compounds have an important role in restricting the palatability of plants to herbivores or leading them to completely avoid such plants (Raven *et al*., 2001). In this context, the study aimed to evaluate the efficiency of extracts and essential oils of *C. angustifolia* in the control of *S. zeamais* under laboratory conditions.

MATERIALS AND METHODS

Obtaining oil and extracts and their composition: The volatile oil of the leaves was obtained by steam distillation (yield 0.9%) and then subjected to analysis in GC/MS: SE-54 fused silica column (25m x 0.25 mm), temperature (50-250 °C at 4 °C/min); E.I 70 eV. The identification of compounds was performed in comparison with mass spectrum and retention index of the literature, calculated by enrichment with a hydrocarbon mixture. Leaves were dried in the shade, and then macerated and extracted by percolation with ethanol/water exhaustively. The solvent was evaporated under reduced pressure on a Randolph rotary evaporator, resulting in a viscous dark residue named crude extract (CE). The same procedure was carried out, but the extraction was done with water only, obtaining the aqueous extract (AE). The crude extract was dissolved in a hydroalcoholic solution (3:7), underwent partition with hexane, and exhaustively extracted to obtain the hexane fraction (EH). Dilutions were made of the oil, crude, aqueous and hexane extracts at a ratio of 1 gm solution to 4 ml solvent. For the oil, it was used ethyl ether [$(C2H5)2O$] as solvent, for the crude and hexane extracts, alcohol ($CH3OH$), and for the aqueous extract, water ($H2O$).

Application of the oil and extracts: With the aid of pipettes, solutions were separated at concentrations of 0.04 ml, 0.08 ml, 0.16 ml and 0.32 ml, which were impregnated on 9 cm diameter filter paper discs. After evaporated the solvent of each concentration, filter papers were placed on petri dishes, then received 10 adults of *S. zeamais*. The exposure time of insects on the plate was determined by survival of ten individuals, being adopted exposure times of 24 h, 48 h, 72 h, 96 h, and 120 h.

Bioassay: Bioassays were conducted in a completely randomized design with 17 treatments and four replications. The treatments used were aqueous, crude and hexane extracts and essential oil at 0.04 ml, 0.08 ml, 0.16 ml, 0.32 ml and the control with distilled water. The experiment was conducted at 25±3 °C, relative humidity 70±10% and photoperiod of 14 h. *C. angustifolia* plants and insects were collected on farms of Chapecó (27° 5′ 45″ S, 52° 37′ 4″ W), State of Santa Catarina.

Data analysis: It was run a bifactorial analysis of variance, being the factor A the treatments and factor B the hours after application, on the number of live insects with values transformed into square root of X + 0.5, and the mean values grouped by Duncan test at 5% probability. Moreover, it was calculated the percentage efficiency by the Abbott equation (1925).

RESULTS AND DISCUSSION

The analysis of the volatile constituents of C. angustifolia identified 93.4% of the compounds present in the species, and the major substance was pulegone with 56.1% share in the composition, followed by isomenthone with 11.2% and b-caryophyllene with 6.8%. The metabolism of pulegone can lead to the formation of menthofuran, a compound with high toxicity that can produce a lethal effect against mite Tyrophagus putrescentiae Schrank, 1781 (Gordon *et al*. 1982, 1987; Sánches-Ramos and Castañera, 2000). The results of the bioassay for the control of S. zeamais evidenced statistical significance ($P < 0.05$) for all sources of variation (Table 1).

Table 1. Summary of analysis of variance of live individuals of S. zeamais in laboratory (T=25±3°C, RH=70±10% and photoperiod=14h).

Sources of variation	Live insects	
	DF	MS
Treatments	16	17.13*
Hours	4	0.08*
Interaction	64	0.05*
Residual	255	0.01

Coefficient of variation = 4.025%; DF = degrees of freedom; MS = mean square; *Significant at 5% probability.

Treatments at different concentrations of the essential oil of C. angustifolia were the most different from control with percentage efficiency of 100%, followed by hexane extract at 0.32% with 38.70% efficiency (Table 2). The other extracts used showed no insecticidal effect on adults of S. zeamais, since the mortality values were statistically similar to the control. The results of low efficiency of aqueous, crude and hexane extracts in controlling adults of S. zeamais must be better understood, as it may mean that substances of these extracts do not exhibit insecticidal activity specific to the target Coleoptera of this work. The very high efficiency of C. angustifolia oil in controlling S. zeamais suggests the need to conduct further experiments using lower concentrations, seeking greater savings and less environmental impact. In relation to time after application, it was observed that the highest mortality of insects occurred 96 h after application of the leaves extracts and essential oils (Figure 1). Besides insects, oils from Cunila and other representatives of the family Lamiaceae has demonstrated efficacy in controlling other arthropods such as ticks Rhipicephalus (Boophilus) microplus Canestrini, 1887 (Acari; Ixodidae). Using a treatment based on essential oil from leaves of Hesperozygis ringens (Benth.) Epling, 1936 (Lamiaceae), Ribeiro *et al*. (2010) achieved inhibition of oviposition in 95% hatching rate of larvae at the highest concentration, and all concentrations were lethal to 100% of the larvae. According to Gazima *et al*. (2011), the plant Tetradenia riparia (Hochst) Codd, 1983 (Lamiaceae), shows high activity against ticks on R. (B.) microplus. These authors observed high mortality of engorged females at low concentrations of the essential oil of this plant, reduced number and weight of eggs, reduced egg hatch and larval mortality. Another representative of the same family is Lavandula angustifolia Mill., 1768 (lavender). The essential oil of this plant when tested on engorged females of Rhipicephalus (Boophilus) annulatus (Say, 1821) Neumann, 1897 (Acari; Ixodidae), allowed to obtain considerable results of mortality, reaching 100% of deaths, and reduced oviposition and egg weight according to the concentration (Pirali-Kheirabadia and Silva, 2010; Alves *et al*., 2012).

Table 2. Mean number of live individuals of *S. zeamais* per treatment and percentage efficiency in assessment, after application of extracts and essential oil of *C. angustifolia*.

Treatment	Number of live insects	% efficiency
Control	10.00± 0.1 a	
CE 0.08	10.00± 0.1 a	0
AE 0.32	9.80± 0.1 ab	2.04
CE 0.16	9.79± 0.1 ab	2.07
HE 0.08	9.79± 0.1 ab	2.07
HE 0.04	9.69± 0.1 abc	3.1
AE 0.16	9.58± 0.1 abc	4.2
CE 0.32	9.54± 0.1 abc	4.6
AE 0.08	9.48± 0.1 bc	5.3
CE 0.04	9.23± 0.1 c	7.7
HE 0.16	8.65± 0.1 d	13.5
AE 0.04	7.93± 0.1 e	20.8
HE 0.32	6.14± 0.1 f	38.7
O 0.32	1.14± 0.1 g	88.6
O 0.16	0.00± 0.1 h	100
O 0.04	0.00± 0.1 h	100
O 0.08	0.00± 0.1 h	100

CE = crude extract; AE = aqueous extract; HE = hexane extract; O = essential oil. Mean values followed by different letters in the same column are significantly different at 5% (Duncan).

Results of the treatment with five species of Cunila are reported by Apel *et al*. (2009) for this family of ticks, where experiments using the essential oil from the leaves indicated that C. angustifolia and Cunila incana Bentham, 1834 caused 100% mortality of larvae at the lowest concentration. Moreover, Mentha rotundifolia

(L.) Hudson, 1762 another plant of the family Lamiaceae, when used as extract and applied on egg masses of the nematode Meloidogyne incognita (Kofoid & White) Chitwood 1949, reduces the hatch rate for up to 2 2% (Echeverrigaray *et al*. 2010). Maedeh *et al*. (2011) studied the fumigant, contact and repellent activity of the essential oil of Satureja hortensis L., 1753 (Lamiaceae), and reported a mortality rate of up to 98.33% of the moth Plodia interpunctella Hübner, 1813 (Lepidoptera, Pyralidae) by fumigation, and by contact, the highest mortality observed was 80%. Also, the adults of P. interpunctella were repelled satisfactorily, reaching 80% repellency. Methanol extracts of Ajuga iva (L.) Schreb., 1774 have toxic substances with significant insecticidal activity, acting to prevent the emergence of the F1 generation, which could be used as a potential shield against Tribolium castaneum Herbst., 1797 (Coleoptera, Tenebrionidae) (Jbilou *et al*., 2006).

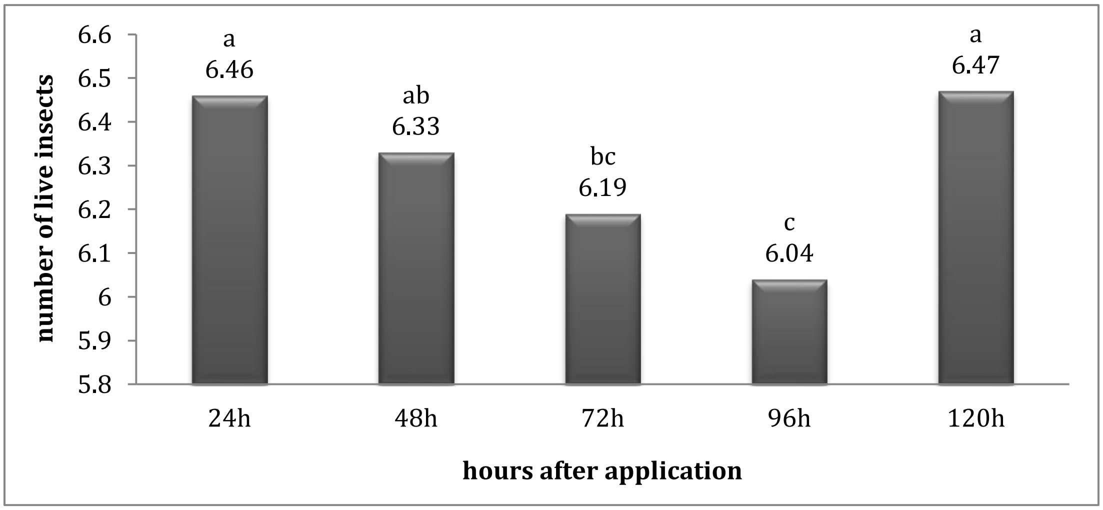

Figure 1. Mean number of live individuals of S. zeamais subjected to application of extracts and essential oil of C. angustifolia at different hours after the application in laboratory.

Species belonging to the family Lamiaceae also demonstrated significant activity against the bean weevil Acanthoscelides obtectus Say, 1859. This was observed for the essential oils of Mentha microphyla, Rosmarinus officinalis and Oreganum vulgaris in the control of egg, larva, pupa and adult (Papachristos and Stamopoulos, 2002a; 2002b; 2004). Another Coleoptera, the lesser mealworm Alphitobius diaperinus Panzer, 1797 was subjected to treatment with C. angustifolia in larval and adult stages in a study by Prado *et al*. (2013). In the experiments, the authors observed 100% efficiency for both larvae and adults when tested at concentrations of 5 and 10%. They observed thus that the oil of C. angustifolia has larvicidal and insecticidal effect against larvae and adults of A. diaperinus, in vitro and in vivo. Savaris *et al*. (2012) registered insecticidal effect of C. angustifolia against adults of A. obtectus, and considered promising the use of the plant in controlling this pest. However, as well as the effects obtained in S. zeamais, the extracts and the hexane fraction of C. angustifolia showed a low efficiency of the control in all of the tested doses, compared to the effect of essential oil that was 100% after 24 hours of exposure. The efficiency obtained by the oil of C. angustifolia meets the indices recommended by Garcia (2014), which are 80 to 90% efficiency to prevent the development of pest resistance.

CONCLUSIONS

Under the conditions of this assay, it is concluded that:

1°- The essential oil of C. angustifolia is effective against S. zeamais under laboratory conditions, at all concentrations tested;

2°- Aqueous, crude and hexane extracts are ineffective against S. zeamais;

3°- At 96h after application, it was reached the highest mortality of S. zeamais.

The use of extracts of Lamiaceae plants offers a promising field to be exploited in pest control given the characteristics of their constituents.

REFERENCES

Abbot, W.S. 1925. A method of computing the effectiveness of insecticide. Journal of Economic Entomology, 18(2): 265-267.

Alves, W.V., E.R. Lorenzetti and F.C. Gonçalves. 2012. Utilização de acaricidas a base de plantas no controle de *Rhipicephalus (Boophilus) microplus*: Uma contribuição para a produção e desenvolvimento sustentável. RevistaBrasileira de Agropecuária Sustentável, 2(2): 14-25.

Apel, M.A., V.L.S. Ribeiro, S.A.L. Bordignon, A.T Henriques and G.L. von Poser. 2009. Chemical composition and toxicity of the essential oils from *Cunila* species (Lamiaceae) on the cattle tick *Rhipicephalus (Boophilus) microplus*. Parasitology Research, 105(3): 863–868.

Bordignon, S.A.L., E.P. Schenkd and V. Spitzer. 1997. The essential oil composition of *Cunila microcephala* and *Cunila fasciculata*. Phytochemistry, 44(7): 1283-1285.

Bordignon, S.A.L., E.P. Schenkd and V. Spitzer. 1999 . The Essential Oil Composition of *Cunila angustifolia* (Lamiaceae). Journal of Essential Oil Research, 11(2): 145-148.

Cavalcante, G.M., A.F.C. Moreira and S.D. Vasconcelos. 2006. Potencialidade inseticida de extratos aquosos de essências florestais sobre mosca-branca. Pesquisa agropecuária brasileira, 41(1): 9-14.

Dayan, F.E., C.L. Cantrell and S.O. Duke. 2009. Natural products in crop protection. Bioorganic & Medicinal Chemistry, 17(12): 4022–4034.

Echeverrigaray, S., F. Fracaro, A.C.A. Santos, N. Paroul, R. Wasum and L.A. Serafini. 2003. Essential oil composition of south Brazilian populations of *Cunila galioides* and its relation with the geographic distribution. Biochemical systematics and ecology, 31(5): 467-475.

Echeverrigaray, S., J. Zacaria and R. Beltrão. 2010. Nematicidal activity of monoterpenoids against the root knot nematode *Meloidogyne incognita*. Phytopathology, 100(2): 199-203.

Garcia, F.R.M. 2014. Zoologia Agrícola. 4ª ed. Ed. Rígel, Porto Alegre, Brasil.

Gazima, Z.C., I.G. Demarchi, M.V.C. Lonardoni, A.C.L. Amorim, A.M.C. Hovell, C.M. Rezende, G.A. Ferreira, E.L. Lima, F.A. Cosmo and D.A.G. Cortez. 2011. Acaricidal activity of the essential oil from *Tetradenia riparia* (Lamiaceae) on the cattle tick *Rhipicephalus (Boophilus) microplus* (Acari; Ixodidae). Experimental Parasitology, 129(2): 175-180.

Gordon, W.P., A.C. Huitric, C.L. Seth, R.H. Mcclanahan and S.D. Nelson. 1987. The metabolism of the abortifacient terpene, (R)-(+)-pulegone, to a proximate toxin, (R)-(+)-menthofuran. Drug Metabolism and Disposition, 15(5): 589-594.

Gordon, W.P., A.J. Forte, R.J. Mcmurty, H. Gal and S.D. Nelson. 1982. Hepatotoxicity and pulmonary toxicity of pennyroyal oil and its constituent terpenes in the mouse. Toxicology and Applied Pharmacology, 65(3): 413-424.

Jbilou, R., A. Ennabili and F. Sayah. 2006. Insecticidal activity of four medicinal plant extracts against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). African Journal of Biotechnology, 5(10): 936-940.

Lovatto, P.B., G. Schiedeck and F.R. M. Garcia. 2012. A interação co-evolutiva entre insetos e plantas como estratégia ao manejo agroecológico em agroecossistemas sustentáveis. Interciencia, 37(9): 657-663.

Maedeh, M., I. Hamzeh, D. Hossein, A. Majid and R.K. Reza. 2011. Bioactivity of essential oil from *Satureja hortensis* (Lamiaceae) against three stored-product insect species. African Journal of Biotechnology, 10(34): 6620-6627.

Manns, D. 1995. Linalool and cineole type glucosides from *Cunila spicata*. Phytochemistry, 39(5): 1115-1118.

Marangoni, C., N.F. de Moura and F.R. M. Garcia. 2012. Utilização de óleos essenciais e extratos de plantas no controle de insetos. Revista de Ciências Ambientais, 6(2): 95-112.

Papachristos, D.P. and D.C. Stamoupolous. 2002a. Repellent, toxic and reproduction inhibitory effects of essential oil vapours on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). Journal of Stored Product Research, 38(2):117 128.

Papachristos, D.P. and D.C. Stamoupolous. 2002b. Toxicity of vapours of three essential oils to the immature stages of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). Journal of Stored Product Research, 38(4): 365-373.

Papachristos, D.P. and D.C. Stamoupolous. 2004. Fumigation toxicity of three essential oil on the eggs of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). Journal of Stored Product Research, 40(5): 517-525.

Pirali-Kheirabadia, K. and J.A.T. Silva. 2010. *Lavandula angustifolia* essential oil as a novel and promising natural candidate for tick *Rhipicephalus*

(Boophilus) annulatus control. Experimental Parasitology, 126(2)184-186.

Potenza, M.R., J.D. Felicio, V. Arthur, M.H. Rossi, M. Nakaoka Sakita, D. de F. Silvestre and D.H.P. Gomes. 2004. Efeito de produtos naturais irradiados sobre *Sitophilus zeamais* Mots. (Coleoptera; Curculionidae). Arquivos do Instituto Biológico, 71(4): 477-484.

Prado, G.P. do, L.M. Stefani, A.S. da Silva, L.F. Smaniotto, F.R.M. Garcia and N.F. de. Moura. 2013. *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) Susceptibility to *Cunila angustifolia* Essential Oil. Journal of Medical Entomology, 50(5): 1040-1045.

Procópio, S. de O., J.D. Vendramim, J.I. Ribeiro Júnior, J.B. dos Santos. 2003. Bioatividade de diversos pós de origem vegetal em relação à *Sitophilus zeamais* Mots. (Coleoptera: Curculionidae). Revista Ciência Agrotécnica, 27(6): 1231 1236.

Raven P.H., R.F. Evert and S.E. Eichhorn. 2001. Biologia Vegetal. 6ªed. Koogan. Rio de Janeiro, Brasil. Ribeiro, V.L.S., J.C. Santos, S.A.L. Bordignon, M.A. Apel, A.T Henriques and G.L. von Poser. 2010. Acaricidal properties of the essential oil from *Hesperozygis ringens* (Lamiaceae) on the cattle tick *Riphicephalus (Boophilus) microplus*. Bioresource Technology, 101(7): 2506-2509.

Sánches-Ramos, I. and P. Castañera. 2000. Acaricidal activity of natural monoterpenes on *Tyrophagus putrescentiae* (Schrank), a mite of stored food. Journal of Stored Product Research, 37(1): 93-101.

Savaris, M., S. Lampert, F.R.M. García, S.M. Sabedot-Bordin and N.F. de Moura. 2012. Atividade Inseticida de *Cunila angustifolia* sobre adultos de *Acanthoscelides obtectus* em Laboratório. Ciencia y Tecnología, 5(1): 1-5.

Smaniotto, L.F., N.F. de Moura, R.B. Denardin and F.R.M. Garcia. 2010. Bioatividade da *Cabralea canjerana* (Meliaceae) no controle de adultos de *Acanthoscelides obtectus* (Coleoptera, Bruchidae) em laboratório. Biotemas, 23(2): 31-35.

Viegas, C.J. 2003. Terpenos com atividade inseticida: Uma alternativa para o controle químico de insetos. Química Nova, 23(3): 390-400.

Xifreda, C.C. and A.C. Mallo. 2001. Citas nuevas o críticas para La flora argentina III: *Cunila angustifolia* (Lamiaceae: Mentheae). Darwiniana, 39(1-2): 175-178.