Toxic effects of essential plant oils in adult *Sitophilus oryzae* (Linnaeus) (Coleoptera, Curculionidae)

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ABSTRACT. Toxic effects of essential plant oils in adult *Sitophilus oryzae* (Linnaeus) (Coleoptera, Curculionidae). Stored grains are subject to losses in quality nutritional value and in sanitation from the time they are stored to the time they are consumed. Botanical insecticides may offer an alternative solution for pest control. The objective was to test the insecticidal properties of the essential oils of *Cymbopogon citratus* (leaf), *Zingiber officinale* (root) and *Mentha* sp. (leaf). The efficacy of these oils was tested to control the rice weevil, *S. oryzae*, using hydrodistillation. Chemical analysis of the essential oils was carried out by gas chromatography. Major components of *C. citratus* were geranial (48%) and neral (31%), of *Z. officinale* were α-zingibereno (13%), geranial (16%), neral (10%) and α-farneseno (5%) and of *Mentha* sp. was menthol (92%). Bioassays were carried out by fumigation and topical application. In topical application assays, the essential oil of *C. citratus* had greater toxicity (LC_{50} 0.027 μL mL^{-1}) and shorter exposure time than the oils of the other two plants. After 24 h and 48 h, 70% and 100% mortality of *S. oryzae* occurred, respectively. In fumigation assays, essential oil of *Z. officinale* had a lower LC_{50} (1.18 μL cm^{-2}) and 70% mortality after 24 h exposure. Therefore, we recommend the use of essential oils of *C. citratus* and *Z. officinale* to control the rice weevil *S. oryzae*.

KEYWORDS. Medicinal plants; stored product pests; botanical insecticides.

RESUMO. Efeito tóxico de óleos essenciais de plantas medicinais em adultos de *Sitophilus oryzae* (Linnaeus) (Coleoptera, Curculionidae). Grãos armazenados estão sujeitos a perdas na qualidade física, sanitária e nutricional, desde sua maturação fisiológica até o momento do consumo. Uma alternativa para o manejo de pragas são os inseticidas botânicos. O presente trabalho teve como objetivo avaliar o efeito inseticida de óleos essenciais de *Cymbopogon citratus* (folha), *Zingiber officinale* (raiz) e *Mentha* sp. (folha), obtidos através do método de hidrodestilação, no controle de adultos do gorgulho-do-arroz, *S. oryzae*. A caracterização química dos compostos encontrados nos óleos essenciais foi realizada por cromatografia gasosa, sendo observados como principais componentes para *C. citratus*: geranial (48%) e neral (31%), para *Z. officinale*: 16% de geranial, 13% de α-zingibereno, 10% de neral e 5% de α-farneseno e para *Mentha* sp.: 92% de mentol. Os bioensaios foram realizados por aplicação tópica e fumigação. Os resultados da aplicação tópica mostraram que o óleo essencial de *C. citratus* apresenta maior toxicidade quando comparado à *Mentha* sp. e ao *Z. officinale*, pois apresentou uma CL_{50} de 0.027 μL mL^{-2} e um menor período de exposição, causando 70% e 100% de mortalidade de adultos de *S. oryzae*, respectivamente, 24 e 48 horas após dos tratamentos. Nos ensaios com fumigação, em adultos de *S. oryzae,* o óleo essencial de *Z. officinale*, apresentou a menor CL_{50} (1,18 μL cm^{-2}) e o menor período de exposição, causando 70% de mortalidade em 24 horas após a aplicação dos tratamentos. Desse modo conclui-se que os óleos de *C. citratus* e *Z. officinale* podem ser recomendados no controle de adultos do gorgulho-do-arroz *S. oryzae*.

PALAVRAS-CHAVE. Plantas medicinais; pragas de grãos armazenados; inseticidas botânicos.

Stored grains are subject to losses due to several causes, including physical, sanitary and nutritional degradation, from their maturation to the consumption. Grain degradation may be caused by fungi, insects, inadequate handling from harvest to storage and can result in important financial losses (Lazzari 1997; Lazzari & Lazzari 2002). Losses may reach 10% of the total product each year, which translates to 10,000,000 tons of grain lost per year (Smiderle 2007).

Among the stored grain pests, the rice weevil, *Sitophilus oryzae* (Linnaeus, 1763) (Coleoptera, Curculionidae), and the maize weevil, *Sitophilus zeamais* Motschulsky, 1855, are the most important pests of stored rice and maize, causing quantitative and qualitative losses (Amorim 1986; Sartori *et al.* 1990). In Brazil, these two species are found throughout warm, tropical regions of the world and they may infest grains in the field prior to storage (Pacheco & Paula 1995).

Despite the availability of modern technologies for grain storage and pest control, some farmers still use traditional methods of storage, such as corn in the cob in small cribs and rice stored in boxes or bags. Grain stored under such conditions is very susceptible to heavy losses (Amorim 1986). In warmer regions, natural aeration is not sufficient to control infestations, requiring the application of chemical control (Moreira 1993). The most effective and fast method to suppress stored grain pests is fumigation. Nowadays, phosphine is the most used fumigant, even though its use has several disadvantages. It corrodes non-ferric metals, requires a long aeration period, is inflammable in high concentrations and is acutely toxic and may cause depreciation of the value of the fumigated product (Santos 2006). The incorrect use of phosphine has selected resistant pest populations (Rosseto 1966; Calil 1995). The use of a mixture of insecticides also favors the development of resistant strains which then makes subsequent pest management difficult (Pereira *et al.* 1997).

Plants, due to their coevolutionary history with pests, are sources of natural insecticides and antimicrobial chemicals compounds that are produced to defend themselves against those organisms. Many plants are rich in secondary compounds with insecticidal activities, especially monoterpenes and their analogues, which are abundant in essential oils of higher plants. These compounds are typically lipophylic, with potential for toxic interference in basic biochemical processes with physiological and behavioral consequences for the insects (Prates & Santos 2002).

Monoterpenoid compounds have been tested as control agents for several insect pests in stored grains, working through contact, ingestion, as fumigant or repellant, showing ovicidal effects or acting on the life cycles of insects (Karr & Coats 1988; Rice & Coats 2003; Lee *et al.* 2003). In general, toxicity due to essential oils is related to the most abundant chemical component in the oils (Obeng-Ofori & Reichmuth 1997; Obeng-Ofori *et al.* 1997; Tapondjou *et al.* 2002; Singh *et al.* 2003; Aslan *et al.* 2004).

The lemon grass *Cymbopogon citratus* (DC.) Stapf (Poaceae), also known as bee balm, is a species commonly found in many tropical countries, its essential oil is composed mainly of citral, with 70–80%, which has insecticidal activity (Souza *et al.* 1991; Vartak *et al.* 1994).

The mint, *Mentha* sp. (Labiatae) produces an essential oil rich in menthone (14–32%) and menthol (30–50%) (Matos 1994; Cardoso *et al.* 2001). Menthol has wide application in the food and pharmaceutical industry; also exhibits insecticidal activity (Agarwal *et al.* 2001).

The essential oil of one of the oldest medicinal plants in the world, *Zingiber officinale* Roscoe (Zingiberaceae), also known as ginger, consists mainly of gingerol and gingerona (Chen *et al.* 1986; Balachandran *et al.* 2006). However, evaluation of these compounds with different aims, for example, insecticide, is recent, since there are few research papers published in this area.

Stored grain insect pests have unique characteristics that justify their importance and the need for specific control measures that should be used in their management. It can also be regarded as another reason for the growing interest in phytotoxins, the wide range of new sites of action in target organisms. This is important if we consider the speed with which the insects have developed resistance to chemicals. Thus, the insecticidal properties of plant essential oils of *Cymbopogon citratus*, *Zingiber officinale* and *Mentha* sp. were tested to control the rice weevil, *S. oryzae*.

MATERIAL AND METHODS

This study was carried out in the Microbiology laboratory at the Vale do Rio dos Sinos University, in São Leopoldo, Rio Grande do Sul, State, Brazil. Adults of *S. oryzae* were reared in environmental controlled chambers (25 ± 2ºC, relative humidity 70%, 12 h photophase). The insects were obtained from commercially available packed rice in Rio Grande do Sul, and reared for several generations on rice.

Fresh leaves of the lemon grass, *C. citratus* (DC.) Stapf. and spearmint, *Mentha* sp. were collected on campus in November 2007, while roots of ginger, *Z. officinale* were obtained at a market. Plants were hydrodistilled to extract the essential oils in an apparatus similar to a Clevenger, built according to Santos *et al.* (2004). Oils were stored in amber-colored glass jars hermetically sealed at ambient temperatures until use. Essential oils were analyzed by gas chromatography (HP-6890) in an HP-Innowax column at the Essential Oil Laboratory of the Caxias do Sul University.

For topical application bioassays, the weevils were first anesthetized at –4°C for 2 min to allow manipulation. The following quantities were used for topical application on the thorax of adult beetles using a micropipette in Petri dishes: *C. citratus* (0.01, 0.02, 0.04, 0.05 μL), *Mentha* sp. (0.01, 0.02, 0.04, 0.05, 0.09 μL), *Z. officinale* (0.05, 0.08, 0.10, 0.15, 0.20 μL), each one diluted in 1 μL of acetone. The same volumes were used for the control treatments, but only with acetone. Dosages were determined by a pilot study. Each treatment was applied to 120 adults of unknown sex. Dead and live beetles were counted 24 and 48 h after application.

Fumigation assays used 0.5, 1.0, 2.0, 4.0% *C. citratus* and *Mentha* sp. and 0.05, 0.1, 0.5, 0.8, 1.0% *Z. officinale* in 2 mL acetone; quantities were established in preliminary trials. These quantities were used to treat 20 g of rice, which were grounded with a mortar and pestle. After grinding, the resultant powder was dried, allowing the complete elimination of acetone. Similarly, control treatments used the equivalent volumes of acetone alone. After drying, 10 weevils were added to each quantity of 20 g, in 100 mL sealed jars. Each treatment included a total of 120 adults (12 jars with 10 beetles each). Live and dead beetles were counted 24 and 48 h after treatment. Mortality estimates were corrected following Abbott (1925). The 50% lethal concentration (LC_{50}) was determined by Probit analysis (Finney 1971).

RESULTS AND DISCUSSION

The principal constituents of the essential oil of *C. citratus* were geranial (48%) and neral (31%), which isomerical forms are citral (79%) and mirceno (11%) (Table I). Neral and geranial are the most important oils that are responsible for the citric aroma of the plant (Craveiro *et al.* 1981). Other studies have found slightly different concentrations of these two oils (32% to 40% neral, 41% to 32% geranial) (Sacchetti *et al.* 2005; Pereira *et al.* 2008).

The most important oils from ginger, *Z. officinale*, were geranial (16%), α-zingiberene (13%), neral (10%) and α-farnesene (5%). These values are similar to those obtained by Singh *et al.* (2008), for example, geranial (26%), α-zingiberene (10%), α-farneseno (8%), neral (8%). Differences may be due to the collect from different seasons of the year, soil type and climate, among others.

Table I. Components (%) of essential oils in the plants *Zingiber officinale*, *Cymbopogon citratus* and *Mentha* sp. identified by gas chromatography.

Componente	*Z. officinale*	*C. citratus*	*Mentha* sp.
α-pineno	2.38		
Canfeno	6.58		
β-pineno	0.29		0.18
Mirceno	1.48	11.10	0.17
Limonemo	1.44		0.52
Sabinemo	9.63		
a-tujona	1.11		
Linalol	0.77		
Neral	9.91	31.50	
Germacreno D	1.15		
α-zingibereno	13.27		
β-bisaboleno	3.94		
Geranial	15.95	47.53	
α-farneseno	5.41		
Acetato de geranila	1.14		
α-sesquifelandremo	7.05		
Ar-curcumeno	3.82		
Geraniol	1.27	2.40	
α-terpinolelo		1.03	
Citronelol		0.39	
Ácido gerânico		0.87	
Mentona			0.99
Mentofurano			1.57
Mentol			91.49
Total	86.59	94.82	94.92

The main essential oil of *Mentha* sp. is the monoterpene menthol (91%). Essential oils in *Mentha* sp. vary from 62% to 90% with an average of 70%, found commercially (Brilho 1963). The essential oil of *Mentha* sp. are rich in menthol, menthona, pinene, limonene, camphor, organic acids, flavonoids, heterosids of histeoline, apigenine (Martins *et al.* 2000) and 1,2-epoxipulegone (Matos 1994).

Mortality of *S. oryzae* varied from 10 to 100% after 48 h of topic application of *C. citratus*, *Mentha* sp. and *Z. officinale*, at 0.01 to 0.20 µL. These results demonstrate that the efficiency of the oils was directly related to concentration (Table II). Oil from *C. citratus* was the most efficient, causing 100% mortality at the lower lethal concentration (0.05 µL) in 48 h.

The oil from *C. citratus* comprises mostly α-citral (geranial) and β-citral (neral, 70% to 80% v/v), which are acyclic monoterpene aldehydes, usually called citral (Carlini *et al.* 1985; Ferreira & Fonteles 1989). Insecticide activity of *C. citratus* (Simões *et al.* 1986; Souza *et al.* 1991; Simões & Spitzer 2004) is primarily due to citral (Vartak *et al.* 1994) and is probably the cause of the insecticidal activity in this study.

Table II. Corrected Mortality (MC) (Abbott 1925) of essential oils of three plants as applied to adult rice weevil, *Sitophilus oryzae* (n = 120).

C. citratus		*Mentha* sp.		*Z. officinale*	
Topical application (µL mL^{-1})					
Concentration	MC (%)	Concentration	MC (%)	Concentration	MC (%)
0.05	100	0.09	95	0.20	95
0.04	80	0.05	70	0.15	65
0.02	40	0.04	45	0.10	30
0.01	10	0.01	10	0.05	10
Fumigation (µL cm^{-2})					
Concentration	MC (%)	Concentration	MC (%)	Concentration	MC (%)
4.0	90	4.0	85	1.0	85
2.0	50	2.0	45	0.8	65
1.0	30	1.0	15	0.5	45
0.5	10	0.5	0	0.1	10

The most toxic of the essential oils was that of *C. citratus*, when compared to the others, with an LD_{50} of 0.027 µL mL^{-1} (Table III), reaching 70% and 100% mortality 24 and 48 h after topical application, respectively (Table II). In comparison, mortality due to *Mentha* sp. and *Z. officinale* was 40% after 24 h, and 55% after 48 h. The greater mortality caused by *C. citratus* essential oil may have been because of higher concentration of citral (63%) in comparison with *Z. officinale* in which the most concentrated component was 41%. In *Mentha* sp., without citral, there was only menthol (91%). L-menthol has been shown to have insecticidal activies for *Tribolium castaneum* Herbst, 1797 (Coleoptera, Tenebrionidae), with a lethal dose (LD_{50}) of 108.4 ppm (Agarwal *et al.* 2001).

Table III. Corrected Mortality (MC) (Abbott 1925), Mean Lethal Concentration (LC_{50}) and Mean Lethal Dose (LD_{50}), their 95% confidence intervals (CI) of essential oils of three plants as applied to adult rice weevil, *Sitophilus oryzae* (n = 120).

	Topical application (µL mL^{-1})			Fumigation (µL cm^{-2})		
	LD_{50}	CI*	χ^2	LC_{50}	CI*	χ^2
C. citratus	0.027	0.0248–0.0298	1.57	4,15	3.20–6.03	0.84
Mentha sp.	0.044	0.0349–0.0586	5.10	3,51	2.20–17.42	4.63
Z. officinale	0.136	0.1142–0.1726	7.32	1,18	0.78–2.58	4.68

There are few studies that demonstrate the mechanisms of action of volatile oils. In general, the complexity of the chemical composition of most of the volatile oils gives them low specificity (Bakkali *et al.* 2008), because biological activity is not assigned to a single mechanism of action, since the wide variety of chemical groups allows for multiple targets in the cell (Burt 2004).

As in this study, others have shown that essential oils can be efficient insecticides for pest control in stored grain. Estrela *et al.* (2006) tested essential oils of *Piper hispidinervum* C. DC. and *P. aduncum* L. (Piperaceae) with topical application on the maize weevil, *S. zeamais*, and found from 90 to 100% mortality. Restello *et al.* (2009) tested the insecticidal effects of the essential oil from *Tagetes patula* L. (Asteraceae) on maize weevil and found it to be an effective control at concentrations of 10 µL. Also, oil from seeds of neem, *Azadirachta indica* A. Juss. (Meliaceae), were used at concentrations of 0.2 to 0.5% for controlling the beetles *S. oryzae, S. zeamais, Rhyzopertha dominica* (Fabricius, 1792) (Coleoptera, Bostrichidae)*, T. castaneum, T. confusum* Jaquelin Du Val, 1868 (Coleoptera, Tenebrionidae)*, Callosobruchus chinenses* (L., 1758) (Coleoptera, Bruchidae)*, C. maculatus* (Fabricius, 1775) and *Oryzaephilus surinamensis* (L., 1758) (Coleoptera, Cucujidae), being very effective with high toxicity (Rodríguez C, comm. pers.). Essential oils of *Evodia rutaecarpa* Evodiamine (Rutaecarpine) used to control *S. zeamais* and *T. castaneum* was effective to both insects when applied topically. As fumigants or repellent it worked best on *T. castaneum* (Liu & Ho 1999).

Using rice grounded with the essential oils, mortality ranged from 10 and 85% after 48 h. Oils of *Z. officinale* was the most efficient, causing 85% mortality at low concentration (1%) after 48 h. In fumigation, oils of *Z. officinale* had lower LC_{50} and were effective after a shorter exposure time, causing 70% mortality after 24 h. Mortality due to *C. citratus* and *Mentha* sp. Essential oils was between 60 and 80% in 24 h.

The use of ginger essential oils has increasing and its effectiveness is due to gingerol and gingerone (Chen *et al.* 1986; Balachandran *et al.* 2006). We have found those same components in this study; however, geranial, α-zingeberene and neral were found in higher concentrations. In many plants are found substances with potential insecticide or repellent, these substances are generally volatile and can be detected by the antennae or tarsi of insects. Among these are the monoterpenes (citronellal, linalool, menthol, α- and β-pinene, menthone, carvone and limonene), sesquiterpenes (farnesol, nerolidol), the phenylpropanoids (safrole, eugenol) and many other compounds (Panizzi & Parra 1991).

The potential use of essential oils as fumigants to control stored grain pests has been the subject of many studies (Klingauf *et al.* 1983; Su 1991; Shingh *et al.* 1989; El Nahal *et al.* 1989; Shaaya *et al.* 1991; Sarac & Tunc 1995; Ho *et al.* 1997; Shaaya *et al.* 1997; Tunc *et al.* 2000; Bouda *et al.* 2001; Park *et al.* 2003; Lee *et al.* 2004). The results obtained in this study demonstrate that the essential oils tested can be used to control stored grain pests and to support further studies.

The toxic effects of essential oils involve many factors, among which are the entry points of toxins (inhaled, ingested or absorbed) and which may have contact, fumigation and phagoinhibitory effects (Regnault-Roger 1997). We also found variation in toxicity depending upon the application method of the essential oils. The essential oils, especially *C. citratus* and *Z. officinale*, can be used as effective control for stored grain pests, and they may be applied either topically or by fumigation.

ACKNOWLEDGEMENT

To James J. Roper for translating this manuscript from the original Portuguese.

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Received 10/12/2009; accepted 141/1/2011
Editor: Sonia Maria Noemberg Lázzari