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Toxicity and repellency of nine medicinal plants against *Tribolium castaneum* in stored wheat

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Abstract

Aqueous and methanolic plant extracts of *Ambrosia tenuifolia* Spreng., *Baccharis trimera* (Less.) DC, *Brassica campestris* L., *Jacaranda mimosifolia* D. Don, *Matricaria chamomilla* L., *Schinus molle* (L.) var. *areira* (L.) DC., *Solanum sisymbriifolium* Lam., *Tagetes minuta* L. and *Viola arvensis* Murray were tested in the laboratory for their insecticidal and repellent effectiveness against the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae). The adult beetles were exposed to plant extracts by topical application and grain treatment. Mortality was recorded after 1, 2 and 7 days after exposure. The repellent action of these plant extracts was also studied. Only methanolic extracts showed activity. The highest mortality (68%) of *T. castaneum* was caused by *V. arvensis* on grain, followed by *M. chamomilla* (57%), *B. campestris* (56%) and *J. mimosifolia* (49%) after 7 days. Moreover, *J. mimosifolia*, *M. chamomilla* and *T. minuta* exhibited high repellency (IR = 0.04) against insects. The application of these botanicals may be promising in protecting of stored grains against coleopteran pests.

Key words: *Tribolium castaneum*, plant extracts, stored grain, repellent activity, toxicity effect.

Introduction

There is a continuous need to protect the stored products against deterioration, especially loss of quality and weight during storage, mainly due to insects and fungi, which usually work in concert. Cereal grains make up the majority of commodities maintained in storage, and represent an important component of the world food supply. After harvest, the grain is usually stored on-farm or in large commercial elevators, where it can be infested by a variety of beetles. Among them, *Tribolium castaneum* Herbst (Coleoptera Tenebrionidae) is one of the most widespread and destructive pests of stored products, feeding on different stored-grain and grain products (Weston and Rattlingourd, 2000; Mishra *et al.*, 2012a; 2012b).

Grain managers tend to look only at chemical alternatives to control stored-grain insect pests but interest in non-chemical methods of controlling insects in stored grain is increasing, as consumers become less tolerant of pesticide residues in their food (Flinn and Hagstrum, 2001). Consequently, the choice of pesticides for storage pest control is very limited because of the strict requirements imposed for the safe use of synthetic insecticides on or near food. Furthermore, the continuous use of chemical pesticides for control of stored-grain pests has resulted in serious problems such as insecticide resistance (Mohan *et al.*, 2010). Current research and the increasing knowledge about the harm derived from the indiscriminate use of synthetic insecticides have encouraged studies related to novel tactics of pest control like the use of botanical insecticides. Plant materials with insecticidal properties, are one of the most important locally available, biodegradable, and inexpensive methods for control of stored-grain pests (Mishra *et al.,* 2012b). The main advantage of botanicals is that they are easily produced by farmers, small-scale industries and are potentially less expensive (Nikkon *et al*., 2009). The utilization of botanical insecticides to protect stored products is promising, mainly due to the possibility of controlling environmental conditions inside the storage units, maximizing the insecticidal effect; in these places the natural product can be used as powder, extract and oil (Guzzo *et al*., 2006). Moreover, the use of plants materials for storage protection is sustainable; they can be continuously propagated year after year; biodegradable; and do not have any negative impact on the environment as long as care is taken to avoid the propagation of plants from foreign ecosystems which might, therefore, become established as weeds (Golob *et al*., 1999). Nevertheless, many plants commonly regarded as safe contain noxious compounds, which may render them unsafe for both animals and humans to consume (Golob *et al*., 1999; Suthisut *et al*., 2011). Among the medicinal plants, several locally available species has been reported to be repellent and toxic to *T. castaneum* (Sighamony *et al*., 1984; Obeng-Ofori *et al*., 1998; Golob *et al*., 1999; Mareggiani *et al*., 2000; Nikkon *et al*., 2009; Suthisut *et al*., 2011). For example, *Ambrosia tenuifolia* Spreng. (Asteraceae), *Baccharis trimera* (Less.) (Asteraceae), *Brassica campestris* L. (Brassicaceae), *Jacaranda mimosifolia* D.Don (Bignoniaceae), *Matricaria chamomilla* L. (Asteraceae), *Schinus molle* (L.) var. *areira* (L.) DC (Anacardeaceae), *Solanum sisymbriifolium* Lam. (Solanaceae), *Tagetes minuta* L. (Asteraceae) and *Viola arvensis* Murray (Violaceae) have demonstrated insecticidal activities against coleopteran pests of stored grain including *T. castaneum* (Padín *et al*., 2000; Tsao *et al*., 2002; Al-Jabr, 2006; Juan Hikawczuk *et al*., 2008; Benzi *et al*., 2009; Arora *et al*., 2011). These species were chosen because in addition to being scarcely attacked by insects, they are easily available to farmers either as ornamental and medicinal plants or weeds. Therefore, the present study was conducted to investigate the insecticidal potential of aqueous and methanolic extracts of leaves from *A. tenuifolia*, *B. trimera*, *B. campestris*, *J. mimosifolia*, *M. chamomilla*, *S. molle* var. *areira*, *S. sisymbriifolium*, *T. minuta* and *V. arvensis* against the red flour beetle, *T. castaneum*.

Materials and methods

T. castaneum was cultured in a controlled environment room at 28 °C and 70% R.H. in the dark. The food media was broken wheat grain. Adult insects used for all bioassays were of mixed sex (50% of each sex) and 10-20 days old and all experiments were carried out in the rearing incubator. The upper leaves of *A. tenuifolia*, *B. trimera*, *B. campestris*, *J. mimosifolia*, *M. chamomilla*, *S. molle* var. *areira*, *S. sisymbriifolium*, *T. minuta* and *V. arvensis* were dried at room temperature for 7 days and finally at 45 °C in a hot air oven for 48 h before grinding into powder by a grinding machine. Dried powder (100 g) was then extracted by maceration with 1000 mL of methanol (Biopack, Argentina, chemical purity 99.98%) 3 times for 24 h per batch. After 3 days, the solvent was evaporated by rotary evaporator at 40 ºC under reduced pressure which produced the crude methanol extract. Test solutions were prepared by dissolving the concentrate in methanol in order to get a 1000 ppm concentration of each extract. The test dose (1000 ppm) was selected based on our preliminary tests where using 500 ppm, the mortality was very low and 1000 ppm equaled to 2000 ppm (data not shown). Likewise, in the present study we follow the protocol from other authors (Clemente *et al*., 2003; Fekete *et al*., 2004; Sivagnaname and Kalyanasundaram, 2004; Liu *et al*., 2007; Passero *et al*., 2007; Boussaada *et al*., 2008; Auamcharoen *et al*., 2012) who used a single dose of extracts and/or methanol as solvent in trials related to the insecticidal effect both on *T. castaneum* as in other insect pests.

To obtain aqueous extracts, 100 g of the plant powders were separately soaked in 1000 mL of water for 24 h and the mixtures were filtered using a muslin cloth. The filtrates were then transferred to rotary evaporator at 60 ºC to separate the solvent from the extract. For each extract, a concentration of 1000 ppm using distilled water as diluent was tested. All crude extracts were used within 2 h post filtration. Aliquots of 1 μL of the dilutions were topically applied to the dorsal surface of the thorax of each insect using a microsyringe. Before treatments, insects were transferred into Petri dishes and chilled for 3 min to reduce their activity to enable topical treatment to be carried out. Fifty beetles in 5 replicates of 10 insects each were treated with each extract. The same number of insects was treated with solvent only as control. After treatment, insects were put in a 250 mL glass jar (10 insects/jar) together with 50 g of food. The jars were capped with a nylon mesh held with rubber bands. Insect mortality was recorded at 24 and 48 h after treatments.

In order to evaluate the effect of extract-treated grains on adult mortality of *T. castaneum*, broken grains of wheat were treated separately with the individual extracts at a dosage of 10 mL at 1000 ppm/kg of grain (Obeng-Ofori and Reichmuth, 1997; Obeng-Ofori *et al*., 1998). An appropriate amount of methanol or water alone was used as the negative control. Test solutions were mixed with 50 g samples of grain in 250 mL glass jars and stirred continuously for 1 min to ensure even spread of the material over the surface of the grains and kept for 3 h to allow the solvent to evaporate completely. Adult beetles (10 beetles per jar) were placed on the treated grain and each jar was covered with a nylon mesh. Five replicates were performed. Insect mortality was recorded 7 days after treatments. Data were analysed using ANOVA after transformation into arcsine $\sqrt{x/100}$ values. Instat 3.05 (GraphPad Software Inc., San Diego, CA) was used for all statistical analysis.

Repellency was assessed using the area preference method (Obeng-Ofori *et al*., 1998). Test areas consisted of 10 cm Whatman Nº 1 filter papers cut in half. Each solution (1 mL) was applied to a half-filter-paper disc as uniformly as possible with a pipette. The other filter paper half was either treated with methanol or water alone. Extract-treated and control half-discs were air-dried to evaporate the solvent completely. Full discs were then re-made by attaching treated halves to untreated halves of the same dimensions with sellotape. Each filter paper was placed in a Petri dish and ten adult beetles were released at the centre of each filter paper disc and then covered. Each treatment was replicated five times and the percentages of insects present on treated (G) and control (P) areas were recorded after 30 min. Index of repellency (IR) was calculated by following formula: $IR = 2 G / G + P$ (Mazzonetto, 2002). The repellency index was classified as: values <1 repellency; 1 neutral; >1 attractant.

Results

None of the aqueous extracts either killed or repelled more adult beetles than the solvent control (data not shown). For the contact toxity at 24 h, only *A. tenuifolia*, *S. sisymbriifolium* and *T. minuta* were not different that the control. Within 48 h of treatment, all methanolic extracts tested by topical application were significantly ($F = 10.8$, $P \leq 0.01$) more toxic to the beetles compared with the controls, producing between 18-37% mortality. The largest percentage kill by contact (37%) corresponded to *B. campestris* and *B. trimera* (table 1). In grain treated with different methanolic extracts, *B. campestris*, *J. mimosifolia*, *M. chamomilla* and *V. arvensis* offered the best protection against *T. castaneum* adults (49-68% mortality) against the beetles ($F = 25.04$, $P \leq 0.01$). *V. arvensis* tended to be the most effective causing 68% mortality in *T. castaneum*, after 7 days of storage. In contrast, the other plants were not toxic. All the plant extracts tested had a repellent effect on *T. castaneum* in the choice arena (table 1). At 30 min, *J. mimosifolia*, *M. chamomilla* and *T. minuta* produced the highest repellency to the beetles bioassayed (IR = 0.04).

Table 1. Effect of the methanolic extracts on mortality and repellency of *T. castaneum* adults.

Botanical name	Application directly on insect (% mortality)		Application to grain (% mortality)	Repellency (Index of repellency)
	24 h *	48 h *	7 days *	30 min **
Control	0 ± 0 a	0 ± 0 a	0 ± 0 a	1.00
Ambrosia tenuifolia	6 ± 3 ab	18 ± 5 b	0 ± 0 a	0.28
Brassica campestris	21 ± 1 c	37 ± 1 c	56 ± 4 b	0.12
Baccharis trimera	15 ± 3 bc	37 ± 3 c	14 ± 6 a	0.12
Jacaranda mimosifolia	15 ± 2 bc	33 ± 2 bc	49 ± 8 b	0.04
Matricaria chamomilla	16 ± 2 bc	32 ± 2 bc	57 ± 2 b	0.04
Schinus areira	15 ± 3 bc	31 ± 4 bc	14 ± 6 a	0.20
Solanum sisymbriifolium	8 ± 1 ab	22 ± 1 bc	14 ± 6 a	0.28
Tagetes minuta	7 ± 3 ab	21 ± 5 bc	11 ± 4 a	0.04
Viola arvensis	14 ± 1 bc	33 ± 2 bc	68 ± 6 b	0.20

Mean (± SE) of five replicates of 10 insects each. $P \leq 0.01$.
*Values followed by the same letter in a column do not differ significantly Tukey´s multiple range test ($P \leq 0.05$).
**IR: <1 repellency; 1 neutral; >1 attractant.

Discussion

There is a renewed interest amongst scientists to study the bioactivity of plant extracts against stored-grain insect pests (Dubey *et al*., 2008; Benzi *et al*., 2009). In our study, some botanicals were highly effective when they were applied on the grains, while others caused more mortality by topical application. Obeng-Ofori and Reichmuth (1997) demonstrated that higher doses of eugenol applied topically were required to achieve 100% kill in *T. castaneum* compared to grain treatment. On the other hand, the high insecticidal effect of different extracts against *T. castaneum* in topical application method was also reported (Tripathi *et al*., 2003; Suthisut *et al*., 2011). In this investigation *B. campestris*, *J. mimosifolia*, *M. chamomilla* and *V. arvensis* preparations showed good potential as repellent and toxicant agents to adults of *T. castaneum*. This confirms the findings of several studies which demonstrated the highly lethal/repellent effect of some of these species against stored-product beetles (Alok-Krishna *et al*., 2005, Zia *et al*., 2011). Toxicity and protectant potential of different terpenoids and quinones isolated from *Jacaranda* species, including *J. mimosifolia*, against insects had been reported (Varanda *et al*., 1992; Castillo and Rossini 2010). These compounds are present in leaf waxes of woody plants and, among other functions, are associated with the inhibition of fungi proliferation and attack by insects (Varanda *et al*., 1992; Bichuette *et al*., 1998). The fact that these compounds are insoluble in water could explain the failure of the aqueous extracts. Zia *et al.* (2011) studied the effect of aqueous extracts from ten plant species on mortality of chickpea beetle, *Callosobruchus chinensis* L. Results revealed that *J. mimosifolia* was little effective in comparison with control. Regarding the anti-insect activity of *Jacaranda* extract, the diet treatment exhibited a higher mortality when compared with the topical application, so demonstrating a relatively stronger efficacy. It has been reported that the jacaranone, a quinone isolated from *Jacaranda* species, showed activity against insects and also exhibited an effect depending upon the application mode: when topically treated houseflies were not affected, however, when offered as part of a sugar diet, it was toxic after ingestion (Xu *et al*., 2003; Castillo and Rossini, 2010).

Plants in the family Cruciferae, such as *Brassica* species produce glucosinolates as secondary metabolites that have shown anti-insect activity against coleopterans, including weevils and beetles. The economic impact on stored grain insect management of the glucosinolate breakdown products was reported by Tsao *et al*., (2002). In the case of *M. chamomilla*, data on their biological activity against insects are scarce. Further, to our knowledge there have been no reports regarding the insecticidal effects of *M. chamomilla* extracts on *T. castaneum*. In the present study, extracts of *V. arvensis*, *M. chamomilla*, *J. mimosifolia* and *B. campestris* caused the highest mortality of insects (49-68%). Plants of the Violaceae family are known for producing large quantities of cyclotides (macrocyclic polypeptides) that have been first isolated from aerial parts of *V. arvensis*. These metabolites have a role in plant defence against insect predation and display a range of biological properties including insecticidal activities, which can be useful in crop protection (Göransson, 2002). As far as we know, there are no records of these compounds to control stored grain insects. In the repellence tests it was verified that the highest rates of repellency corresponded to *J. mimosifolia*, *M. chamomilla* and *T. minuta*. It has been reported that essential oils of *M. chamomilla* exhibited strong repellent action against *Oryzaephilus surinamensis* (L) and *T. castaneum* (Al-Jabr, 2006). Repellency of oils of *Tagetes* spp. have also been recorded against insects in the laboratory and field. *Tagetes erecta* L. is a potential plant whose essential oil from flowers has been effective repellent against insects (Ray *et al*., 2000). Accordingly, ocimene from *T. minuta* has also repellent properties which need to be exploited in detail (Koul *et al*., 2008). Moreover, were proved the repellent effects of the essential oil isolated from *Tagetes terniflora* Kunth against *T. castaneum* larvae and adults (Stefanazzi *et al*., 2006). References on the repellency of *J. mimosifolia* were not found but Gachet and Schühly (2009) reported that people of the French

Guinea and Brazil, use the burned leaves of *Jacaranda copaia* (Aubl.) D. Don as black-fly and mosquito repellent. Interestingly, all plant products of the present study were repellent at 30 minutes which reduces the possibilities of contamination of grains by the insect pests. Although other authors studied the repellency of various botanicals in longer periods (Jayasekara *et al*., 2005; Mkolo *et al*., 2011; Wekesa *et al*., 2011), we can consider that speed in the assessment allows to reveal the best anti-insect capacity of a product. The findings of this investigation revealed that *B. campestris*, *J. mimosifolia*, *M. chamomilla* and *V. arvensis* have good insecticidal activity. They can be used in the control of *T. castaneum* population with integrated pest management system which seems to be economically feasible and ecologically sound. However, more research should be directed towards isolation of bioactive compounds as well as field trials must be conducted before these extracts are used in grain storages. This task is further complicated by the possibility that some of the compounds in these mixtures may act as synergists (Fields *et al*., 2010) or as antagonists (Kordali *et al*., 2006). Ideally, insecticides should be very toxic to target insects, yet should be not toxic to non-target organisms such as plants, insects (e.g., parasites, predators, and pollinators) and other animals, such as fish and birds (Tomlin, 2003). Above all, the risk to workers and consumers must be very low. Since the use of any plant materials with insecticidal activity is likely to involve some unwanted exposure of human and domestic animals to toxic substances, toxicity studies on the effects of the plant materials and their extracts on non-target organisms will need to be undertaken. Therefore, the toxicity of plant materials either directly admixed into foodstuffs or of extracts which are applied onto the produce, may be unacceptable and even long-standing use does not guarantee a level of safety which can be recommended unconditionally (Golob *et al*., 1999).

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