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Toxicity of extracts of *Cyperus rotundus* on *Diabrotica speciosa*

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ABSTRACT. The objective of this work was to evaluate the insecticidal effect or repellency of *Cyperus rotundus*, an important weed plant, through alternative methods of extraction, on *Diabrotica speciosa*, a pest that affects several plant species. The experimental design was completely casual, and consisted of five repetitions. The *C. rotundus* extracts were prepared using leaves and roots by alcoholic extraction, aqueous (hot water) extraction and aqueous (cold water) extraction and diluted to four different concentrations (0, 5, 10, and 15% of the volume of each extract). These dilutions were then tested and compared with a control. The higher mortality of *D. speciosa* adults as well as a smaller leaf consumption area were observed after treatments with increasing dosages of different *C. rotundus* extracts generated by alcoholic extraction (55% of mortality and 28% leaf consumption). Therefore, the alcoholic extract of the *C. rotundus* foliage is an option for the control of *D. speciosa* in agroecologic systems.

Keywords: rootworm, nutgrass, Chrysomelidae, alternative control.

RESUMO. Toxicidade de extratos de *Cyperus rotundus* sobre *Diabrotica speciosa*. O objetivo deste trabalho foi avaliar o efeito inseticida ou repelência de *Cyperus rotundus*, importante planta infestante, por meio de métodos alternativos de extração sobre *Diabrotica speciosa*. O delineamento experimental foi inteiramente casualizado, com cinco repetições. Os extratos foram elaborados utilizando raízes e folhas de *C. rotundus* mediante extração alcoólica, aquosa (água quente) e aquosa (água fria) diluindo-se em quatro diferentes concentrações (0, 5, 10 e 15%) em comparação com o controle (água). A maior mortalidade de adultos de *D. speciosa*, bem como, menor consumo de área foliar foram observadas com o aumento da dose nos diferentes extratos das folhas e das raízes de *C. rotundus*, destacando-se a extração alcoólica das folhas (55% da mortalidade e de 28% do consumo de folha). Portanto, o extrato alcoólico de folhas de *C. rotundus* é uma opção para o controle de *D. speciosa* em sistemas agroecológicos.

Palavras-chave: vaquinha, tiririca, Chrysomelidae, controle alternativo.

Introduction

Cyperus rotundus L. (Cyperaceae) is an herbaceous perennial plant. Its stem is herbaceous and rhizome ramified (ARANTES et al., 2005). It is considered one of the most persistent species in the world (ARRUDA et al., 2005) and is the main weed species in cultivated soils in tropical areas (JAKELAITIS et al., 2003a and b). According to Costa (2000), *C. rotundus* is rich in alkaloids, anthraquinone, coumarins, steroids, triterpenes, flavonoids, saponins, tannins, and resins. In addition, it has possible insecticidal properties and could be a repellent against arthropods.

Control of polyphagous pests is difficult because of their easy adaptation to different cultures. *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae) affects diverse cultures in Brazil (GALLO et al., 2002) and usually occurs in large population densities (LEITE et al., 2008; PICANÇO et al., 2004). Young pests feed on the root system, while the adults feed on leaves, new budburst, husks or fruits, thus reducing productivity or depreciating the product. The pest population has considerably increased in bean and soy crops, resulting in systematic application of organosynthetic insecticides (MARQUES et al., 1999).

Pest control in agriculture has usually been done using chemical insecticides, causing harm to the farmers who apply the chemicals and to the environment, and decimating populations of beneficial species. In addition to these negative factors, these products are expensive, burdening the family producers who make them (VENZON et al., 2006).

Therefore, there is a need to control pests without causing imbalance and harm to the environment. This can be accomplished by alternative methods, such as the use of plant extracts (BARBOSA et al., 2011; VIEGAS JÚNIOR, 2003) that favor their natural enemies. These compounds are required to establish the biological balance and reduce production costs. Thus, organic cultivation, since it uses environmentally safe technologies, constitutes a promising alternative (VENZON et al., 2006).

However, there are few studies using plant extracts for pest control, and even when they are used as pesticides, they are extracted with expensive and toxic substances such as methanol (BENEVIDES et al., 2001) and hexane. Other studies using plants for insect control use plant essential oils, which require more sophisticated extraction equipment, making their use incompatible with the reality of family producers.

The objective of this study was to determine the insecticidal effect of roots and leaves of *C. rotundus* on *D. speciosa* through alternative methods of extraction.

Material and methods

This study was carried out in the "Instituto de Ciências Agrárias da Universidade Federal de Minas Gerais" between December 2006 and February 2007. The experimental design was completely casual, with five repetitions. Each repetition consisted of one Petri dish (10 x 2 cm) with 10 *D. speciosa* adults (sex and age unknown) collected in the organic bean crop, incubated at 25ºC, and evaluated for mortality after 24 and 48 hours.

Three extraction methods were tested for each part of the plant (leaves and roots): 1) For the alcoholic extraction, we used 25% of the fresh weight of leaves or roots (25 g), both picked separately, in commercial 100% hydrated ethyl alcohol (100 mL). This mixture was left for 15 days at room temperature in glass amber and was agitated twice a day. 2) For the aqueous extraction, we used 25% of the fresh weight of leaves or cut roots, which were softened separately (25 g), in 100% of distilled water (100 mL). The extract was left in glass amber for 24 hours at room temperature. 3) For the aqueous extraction for infusion (tea), we used 25% fresh weight of the leaves or separately picked roots (25 g) in 100% distilled water (100 mL). The mixture was boiled (100ºC) then conditioned in glass amber until it was cool. All of the obtained extracts were filtered and conditioned in glass amber flasks until their use.

After each plant extract was obtained, four dosages of each extract were tested: 0, 5, 10 and 15% of the volume. An apical bean leaflet (*Phaseolus vulgare* L.) was immersed for five seconds in each concentration of the appropriate extract. This leaflet was maintained in the shade and free air for two hours until all excess water had evaporated. After this, 10 *D. speciosa* adults were conditioned in Petri dishes (10 x 2 cm), and their mortality after 24 and 48h as well as their consumption of the foliage (visual inspection) was recorded. The control plant (dosage = 0) was immersed in the solvent used in the extraction process and dried similarly to the sample. The data were submitted to variance analysis and regression analysis, all to a 5% significance level.

Results and discussion

A higher mortality of *D. speciosa* adults as well as a smaller foliage consumption area was observed with increased dosage of the different extracts of leaves and roots of *C. rotundus* (Figures 1 and 2). Among the different extraction types, higher concentrations of alcohol extract caused the greatest mortality of *D. speciosa*. After 24 or 48h of treatment, the highest concentration of root extract caused 35 and 45% mortality, respectively, while the highest concentration of leaf extract caused 50 and 55% mortality, respectively (Figure 1). In addition, the alcohol extract of leaves had a higher straight line inclination than the alcohol root extract, indicating greater insecticidal capacity (Figure 1). This effect was similar when area of foliage consumption was evaluated; alcohol extracts were more effective at reducing this consumption area. After 24 or 48h of treatment, the greatest concentration of root extract decreased consumption by 13.75 and 46.25%, respectively, and the greatest concentration of leaf extract decreased consumption by 22.75 and 28%, respectively. The leaf extract again had a larger straight line inclination (Figure 2).

Sharma and Gupta (2007) observed that methanolic extract of *C. rotundus* tubers strongly inhibited the activity of acetylcholinesterases (AChE). These authors suggested that the AChE inhibitors in nutgrass could possibly act as the plants' defense against herbivores. Raja et al. (2001) reported that aqueous extracts from *C. rotundus* tubers effectively protected pulses stored post-harvest without any infestation by *Callosobruchus maculatus* (Coleoptera : Bruchidae) for up to 6 months. The insecticidal effect of *C. rotundus* is probably a result of compounds in the plant such as alkaloids, anthraquinones, coumarins, steroids and triterpenes, sesquiterpenoid, flavonoids, saponins, tannins, and resins (COSTA, 2000; JEONG et al., 2000; KILANI et al., 2005). These compounds have an insecticidal effect against other insects. Examples include the effect of flavonoids against *S. zeamais* Mots. and *Aedes aegypti* L. larvae (Diptera: Culicidae) (SILVA et al., 2005; TREVISAN et al., 2006), coumarins against *A aegypti* (CHAITHONG et al., 2006), or triterpenes against mites such as *Oligonychus ilicis* McGregor (Acari: Tetranychidae) and *Iphiseiodes zuluagai* Denmark & Muma (Acari: Phytoseiidae) (MARTINEZ, 2002; MOURÃO et al., 2004). Saponins, tannins and alkaloids are generally toxic and are deterrents for most herbivores (CAVALCANTE et al., 2006; MARTINS et al., 2005). *C. rotundus* extract is effective against wood, agricultural, hygiene, cereals, and domestic insect pests and is nontoxic to fish and animals due to alpha-cyperone, cyperotundone, and rotundone (KUMIAI CHEM. IND. CO. LTD, 2008). Their sesquiterpenes are effective against domestic and agricultural insect pests (e.g., flies, cockroaches, mosquitoes, slugs, wolf moths, and rice weevils) and are used to make conventional repellents as well as wide-spectrum and long-lasting insect repellents without fear of toxicity and environmental pollution (YUGAKI YAKUHIN KOGYO KK, 2008).

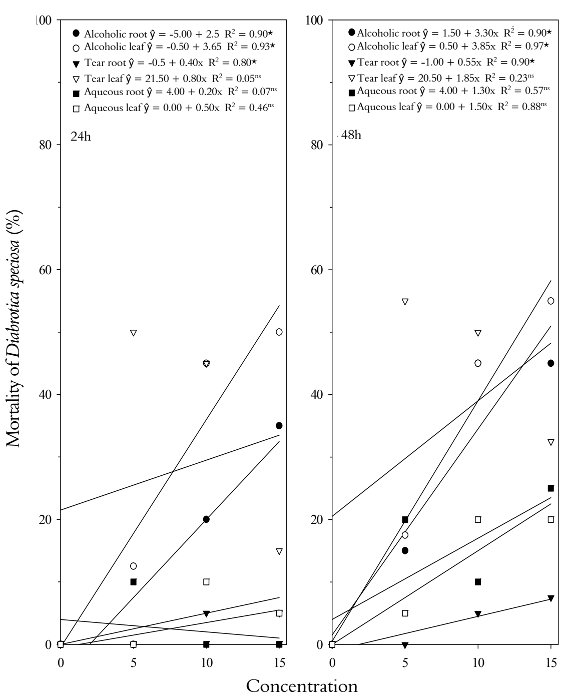

Figure 1. Mortality of adults of *Diabrotica speciosa* (%) under differents extracts of *Cyperus rotundus*. *Significative to 5% by F test. ns = no significative.

This plant has an allelopathic effect on other vegetable species, probably due to the presence of these secondary compounds, reducing the growth of the aerial part and root system of *Brassica oleraceae* L. and *B. rapa* L. (Brassicaceae) (YAMAGUSHI et al., 2007), as well as the germination of *B. oleraceae*, *B. rapa*, *Zea mays* L. (Gramineae), *Phaseolus vulgaris* L. and *Glyicine max* (L.) (Fabaceae), and *Lactuca sativa* L. (Asteraceae) (MUNIZ et al., 2007; YAMAGUSHI et al., 2007).

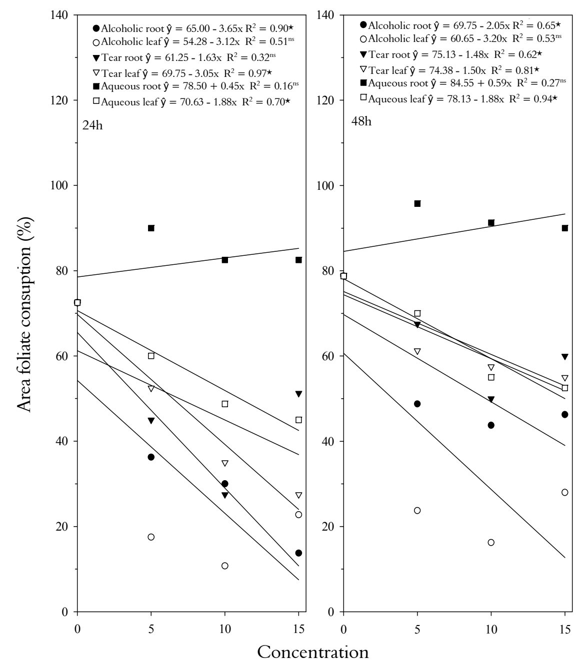

Figure 2. Area foliate consumpion (%) dor *Diabrotica speciosa* under differents extracts of *Cyperus rotundus*.*Significative to 5% by F test. ns = no significative.

The antibacterial effects of ethyl acetate extracts of *C. rotundus* observed by KILANI et al. (2008) and anti-malarial action of *C. rotundus* of the *Plasmodium falciparum* verified by Thebtaranonth et al. (1995) are strong indicators of their possible action on insects.

On the other hand, similar mortality values to those seen in this work were also observed in other natural plant extracts, such as piretro against *Argyrotaenia sphaleropa* (Meyrick) (Lepidoptera: Tortricidae) (MORANDI FILHO et al., 2006), *Ruta graveolens* L. (Rutaceae) against *Sitophilus* spp. (Coleoptera: Curculionidae), and *Ctenocephalides canis* (Curtis) (Siphonaptera: Pulicidae) (ALMEIDA et al., 1999; LEITE et al., 2006), *Prosopis juliflora* Sw. (D.C.) (Fabaceae) on *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae) (CAVALCANTE et al., 2006), as well as the synthetic organic insecticide thiamethoxam against *D. speciosa* (CALAFIORI; BARBIERI, 2001).

It is worth emphasizing that the studied plant is easily generated by treating a spontaneous plant, thus generating an alcoholic extract, a good option for use as an alternative for pest control in agroecologic systems.

Conclusion

We conclude that extracts of *C. rotundus* generated by alcoholic extraction have more insecticides effect on *D. speciosa* well as demonstrated a smaller area of leaf consumption that extracts prepared with aqueous (hot water) and aqueous extraction (cold water) with (55% of mortality and 28% leaf consumption).

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