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Effects of plant extracts on developmental stages of the predator *Podisus nigrispinus* (Hemiptera: Pentatomidae)

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Podisus species (Hemiptera: Pentatomidae) are generalist predators that survive and reproduce in temporary agro-ecosystems. They have large numbers of offspring per generation (Oliveira et al. 2002) and adapt to various temperatures and prey (Torres et al. 1998; Lemos et al. 2003; Vivan et al. 2003). Such predators represent an alternative method of controlling insect pests in integrated management programs (Matos-Neto et al. 2002; Lemos et al. 2005).

The predator *Podisus nigrispinus* (Dallas) is one of the most common species of Asopinae in the Neotropics and has been reported in several countries in Central and South America (Matos-Neto et al. 2002; Silva et al. 2009). However, *P. nigrispinus* preys on insect defoliators and can be exposed to toxic compounds directly and indirectly via its prey (Torres et al. 2003, 2010; Torres & Ruberson 2004).

Synthetic insecticides can be harmful to the environment and natural enemies (Silva et al. 2005; Campiche et al. 2006; Rocha et al. 2006), making it necessary to select those which are effective, safe, and selective for pest control. Botanical extracts are a potentially valuable alternative method of controlling insect pests as they have lower persistence and toxicity than synthetic insecticides (Wiesbrook 2004; Isman 2006; Hossain & Poehling 2006). These natural insecticides possess secondary compounds such as terpenoids that protect plants by causing various effects in insects, including behavioral and physiological responses (Tedeschi et al. 2001), but may have adverse effects on natural enemies (Bottrell et al. 1998; Coley et al. 2006). These substances may be more harmful to generalist predators than to pests (Francis et al. 2000; Nishida 2002; Vivan et al. 2002; Coley et al. 2006).

The plant families containing the most promising botanical insecticides are Annonaceae, Asteraceae, Canellaceae, Lamiaceae, Meliaceae, and Rutaceae (Schmutterer 1990; Stein & Klingauf 1990; Mordue & Blackwell 1994; Isman 2006). The use of plant extracts in pest control is increasing due to consumer demand for pesticide-free products (Isman 2006). However, few studies have demonstrated the impact of botanical extracts on natural enemies, thus increasing the need to study their effects on beneficial organisms. The aim of this study was to evaluate the toxicity of aqueous extracts of *Annona squamosa* L. (Magnoliales: Annonaceae), *Azadirachta indica* A. Juss (Sapindales: Meliaceae), *Corymbia citriodora* Hook. (Myrtales: Myrtaceae), *Cymbopogon winterianus* Jowitt ex Bor (Poales: Poaceae), *Lippia sidoides* Cham. (Lamiales: Verbenaceae), *Mentha arvensis* L. (Lamiales: Lamiaceae), *Ricinus communis* L. (Malpighiales: Euphorbiaceae), and *Sapindus saponaria* L. (Sapindales: Sapindaceae) on the predator *P. nigrispinus*.

Tests were conducted and insects reared at the Laboratory of Agriculture and Forest Pests of the Department of Forestry, Federal University of Sergipe (UFS), Sergipe, Brazil, under the following conditions: temperature of 25 ± 2 °C, 60 ± 10% relative humidity, and a 12:12 h L:D photoperiod. Adults of *P. nigrispinus* were kept in screened cages measuring 60 × 40 × 40 cm and fed with pupae of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae); moistened cotton served as a water source. The egg masses in the cages were collected on the day of oviposition and transferred to Petri dishes (9.0 cm diameter × 1.5 cm height) with moistened cotton. After hatching, the nymphs were retained in those dishes and fed with pupae of *T. molitor* until adulthood. This rearing has been maintained for 1 yr, and new individuals are collected in the field and introduced in the rearing semi-annually.

Aqueous extracts were prepared from plants collected from the experimental plantation at the UFS. We used the leaves of *A. squamosa*, *C. winterianus*, *C. citriodora*, *L. sidoides*, *R. communis*, *S. saponaria*, and *M. arvensis*, and the flowers, leaves, and seeds of *A. indica*. These plants parts were dried at 40 °C for 48 h and ground in an electric mill to obtain a fine powder. Crude extracts were obtained by mixing 10 g of fine powder per 100 mL of distilled water and storing them for 48 h in hermetically sealed containers. The suspensions obtained were filtered using filter paper to obtain a 10% aqueous extract, and were diluted in distilled water to obtain 1, 3, 5, and 7% concentrations. Distilled water was used as negative control and considered to be 0%.

Eggs were collected from the rearing cages within 24 h of oviposition. Nine extracts were assessed at 6 concentrations (0, 1, 3, 5, 7, and 10%) using 5 replicates of 10 eggs, totaling 2,700 eggs. The same tests were conducted with 5th instars and 72-h-old unsexed adults. The eggs were immersed in the extracts or in distilled water (control) and then maintained in air to dry the excess liquid. They were then placed in Petri dishes, and viability (number of nymphs hatched) was evaluated. A similar method was used for nymphs and adults, except that 1 µL of each extract was applied with an automatic pipette to the pronotum of the insects. After application of the aqueous extract, insects were maintained in Petri dishes (9.0 cm diameter × 1.5 cm height) and provided with moistened cotton as a source of water and pupae of *T. molitor* as food. The number of surviving insects was assessed after 72 h.

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The mortality of insects in each test was corrected using Abbott's equation (Abbott 1925). The regression equations for LC50 and/or LC90 and confidence limits of 95% were calculated by probit analysis (Finney 1971) with SAS Proc PROBIT (SAS Institute 1997).

Results showed that extracts of *C. citriodora*, *C. winterianus*, and *M. arvensis* were not toxic to the eggs at the concentrations tested (Table 1). However, the eggs were susceptible to extracts of leaves from *A. squamosa* and *S. saponaria*, and to extracts of seeds and leaves from *A. indica* (Table 1). Those results were expected because the extracts of *A. squamosa* (Sonkamble et al. 2000; Dharmasena et al. 2001; Khalequzzaman & Sultana 2006, Kumar et al. 2010), *S. saponaria* (Santos et al. 2008; Porras & López-Ávila 2009), and *A. indica* (Isman 2006; Tavares et al. 2010) are used for controlling agricultural pests.

Fifth instars tolerated the botanical extracts, making it impossible to prepare dose-response curves. In this study, negative impact on the fifth instars exposed to the extracts was not observed, although their detoxification may affect nymphal duration and reproduction of adults (Tedeschi et al. 2001), which was not evaluated in our study. The mortality of adults (Table 1) was less than 50% with *A. squamosa*, *A. indica* (leaves), *C. citriodora*, *M. arvensis*, and *R. communis* extracts. By comparison, the adults were more susceptible to the extracts of *A. indica* flowers, *C. winterianus*, and *S. saponaria* (Table 1). Thus, the botanical extracts of *A. indica*, *C. winterianus*, and *S. saponaria* have potentially useful insecticidal properties (Schmutterer 1990; Stein & Klingauf 1990; Nawrot et al. 1991; Mordue & Blackwell 1993).

The extracts of *C. citriodora*, *L. sidoides*, and *M. arvensis* did not affect the predator *P. nigrispinus*, but are known to be effective in controlling *Anticarsia gemmatalis* Hübner, *Spodoptera frugiperda* Smith & Abbot (Lepidoptera: Nocytuidae) (Chrispeels & Raikhel 1991; Wilson et al. 1997; Tedeschi et al. 2001; Batish et al. 2008; Berthold Vargas et al. 2009; Mota et al. 2012), and other defoliating lepidopteran larvae. These extracts potentially could be used in conjunction with *P. nigrispinus* in integrated pest management programs. However, the extracts of *A. indica*, *A. squamosa*, *C. winterianus,* and *S. saponaria* reduced *P. nigrispinus*' egg viability and adult survival, indicating the need for care when used for pest control to avoid harming non-target insects. This study showed that botanical extracts vary in their potential suitability for use in integrated pest management programs—although some are selective, others can injure biological control organisms.

Summary

The aim of this study was to evaluate the toxicity of aqueous extracts of *Annona squamosa* L. (Magnoliales: Annonaceae), *Azadirachta indica* A. Juss (Sapindales: Meliaceae), *Corymbia citriodora* Hook. (Myrtales: Myrtaceae), *Cymbopogon winterianus* Jowitt ex Bor (Poales: Poaceae), *Lippia sidoides* Cham. (Lamiales: Verbenaceae), *Mentha arvensis* L. (Lamiales: Lamiaceae), *Ricinus communis* L. (Malpighiales: Euphorbiaceae), and *Sapindus saponaria* L. (Sapindales: Sapindaceae) on the developmental stages (eggs, 5th instars, and adults) of a generalist predator *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae). The eggs were dipped in extract concentrations of 0, 1, 3, 5, 7, and 10%, and the nymphs and adults received 1 µL of each extract on the dorsal thorax. The extracts affected hatching, with the extracts of *A. indica* (flowers), *A. squamosa*, and *R. communis* being the most toxic. None of the extracts affected the mortality in the nymphal stage, whereas *S. saponaria*, *A. indica* (flowers), and *L. sidoides* extracts caused relatively high mortality rates in adults. Due to the insecticidal effect of these extracts, they need to be used with care because they affect the life cycle of the predator *P. nigrispinus*.

Key Words: alternative control; natural insecticide; predatory bug

Table 1. LC50 and LC90 of botanical extracts against eggs and adults of *Podisus nigrispinus* (Hemiptera: Pentatomidae).

	Eggs					Adults				
Botanical extracts	LC50 (µL/mL)	LC90 (µL/mL)	χ^2	Slope	P	LC50 (µL/mL)	LC90 (µL/mL)	χ^2	Slope	*P*
Annona squamosa	4.6 (0.26–0.60)	14.8 (11.9–21.6)	20.60	1.25 ± 0.72	0.29	n/a	n/a	n/a	n/a	n/a
Azadirachta indica (flowers)	32.6 (1.49–8.2)	6,289 (74.2–55,720)	11.00	0.56 ± 0.17	0.97	32.5 (9.4–64.0)	2,468 (40.1–5,252)	10.86	0.68 ± 0.26	0.89
A. indica (green leaves)	102.9 (5.1–3554)	31.3 (1.9–18.6)	13.10	0.60 ± 0.26	0.78	n/a	n/a	n/a	n/a	n/a
A. indica (seeds)	10.1 (0.80–1.6)	2,383 (52.8–5,774)	18.15	0.94 ± 0.97	0.09	n/a	n/a	n/a	n/a	n/a
Corymbia citriodora	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Cymbopogon winterianus	n/a	n/a	n/a	n/a	n/a	43.0 (20.0–299)	296.1 (45.9–2,991)	16.08	0.70 ± 0.26	0.58
Ricinus communis	34.4 (1.29–6.91)	360.0 (129.1–1,169)	36.03	3.44 ± 1.29	0.05	n/a	n/a	n/a	n/a	n/a
Sapindus saponaria	12.5 (0.98–2.1)	3336 (528.2–5,774)	11.03	1.00 ± 0.30	0.09	62.2 (3.3–33.73)	466.7 (58.3–8,231)	12.96	0.68 ± 0.26	0.79
Mentha arvensis	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

n/a, not applicable

Sumário

O objetivo desse estudo foi avaliar a toxicidade dos extratos aquosos da *Annona squamosa* L. (Magnoliales: Annonaceae), *Azadirachta indica* A. Juss (Sapindales: Meliaceae), *Corymbia citriodora* Hook. (Myrtales: Myrtaceae), *Cymbopogon winterianus* Jowitt ex Bor (Poales: Poaceae), *Lippia sidoides* Cham. (Lamiales: Verbenaceae), *Mentha arvensis* L. (Lamiales: Lamiaceae), *Ricinus communis* L. (Malpighiales: Euphorbiaceae), e *Sapindus saponaria* L. (Sapindales: Sapindaceae) nos estágios de desenvolvimento (ovos, quinto instar e adultos) do predador generalista *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae). Os ovos foram imergidos em extratos com concentrações de 0, 1, 3, 5, 7 e 10%, ninfas e adultos do predador receberam 1 µl de cada extrato na área dorsal. Os extratos de *A. indica* (flores), *A. squamosa* e *R. communis* afetaram a eclosão de ninfas. Nenhum dos extratos avaliados afetou a mortalidade nas ninfas, porém os extratos de *S. saponaria*, *A. indica* (flores) e *L. sidoides* causaram alta mortalidade aos adultos. Apesar desses extratos possuirem ação inseticidas, eles devem ser utilizados com cuidado, pois podem afetar o ciclo de vida do predador *P. nigrispinus*.

Palavras Chave: controle alternativo; inseticidas naturais; percevejo predador

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