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Anti-moulting Activity of *Eremanthus erythropappus* (DC.) MacLeisch

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Abstract. Extracts and secondary metabolites from plants can act as feeding deterrents, toxic agents or developmental disruptors in insects. In this study, the experimental milkweed bug *Oncopeltus fasciatus* (Dallas), (Hemiptera) was used to evaluate the activities of crude ethanol extract and fractions, obtained from flowers or stems of *Eremanthus erythropappus* (DC.) MacLeisch (Asteraceae), on its mortality and development. Butanol fraction from ethanol extract showed toxicity against nymphs of *O. fasciatus* as well as growth inhibition activity. The results imply that *E. erythropappus* butanol fraction has active substances on this insect growth development physiology.

Keywords: Asteraceae; Candeia; Hemiptera; *Oncopeltus fasciatus*; Growth regulators.

Atividade Antimuda de *Eremanthus erythropappus* (DC.) MacLeisch

Resumo. Os extratos e metabólitos secundários de plantas podem agir como agentes tóxicos e inibidores do da alimentação e do desenvolvimento em insetos. Neste estudo foi utilizado *Oncopeltus fasciatus* (Dallas) (Hemiptera) como modelo experimental a fim de avaliar as atividades do extrato etanólico bruto e frações obtidos das flores ou caules de *Eremanthus erythropappus* (DC.) MacLeisch (Asteraceae), na mortalidade e sobre o desenvolvimento do inseto. A fração butanólica obtida do extrato etanólico mostrou toxidade sobre as ninfas de *O. fasciatus*, bem como atividade de inibição do crescimento. Os resultados sugerem que a fração butanólica de *E. erythropappus* possui substâncias ativas sobre a fisiologia, crescimento e desenvolvimento de insetos.

Palavras-chave: Asteraceae; Candeia; Hemiptera; *Oncopeltus fasciatus*; Reguladores do desenvolvimento.

Evolution has equipped plants with a diversity of chemical defenses against herbivorous and predator insects. Aware of these effects, plant parts or extracts to control insects since ancient times. More than 2000 plant species are already known to have metabolites with insecticide properties like pyrethrum, rotenone and nicotine (Balandrin 1985; Rawls 1986; Sukumar *et al.* 1991). Phytochemicals endowed with hormonal, anti-hormonal (Garcia *et al.* 1987) or toxic activities are potential agents for insect control (Bowers *et al.* 1976, Bowers 1984, Cabral *et al.* 2009, Nogueira *et al.* 2009). Alternatives may be found among natural sources, mainly higher plants that afford a number of repellent and secondary toxic metabolites (Subrahmanyam 1990). The discovery of phytotoxin insecticide activity in the Asteraceae species has stimulated interest in this plant family as part of the search for new plant derived insecticides (Rawls 1986; Jacobson 1989). Thus this bioration approach stimulates the interested to test crude extracts from the Brazilian Asteraceae species in the growth development control on insects. *Eremanthus erythropappus* (DC.) MacLeisch, known as the "Candeia" an Asteraceae species (Pio-Correa 1984), is found in Brazil from Bahia to Rio de Janeiro States, at altitudes ranging from 900 to 1700m (Teixeira *et al.* 1996; Longhi *et al.* 2009). This plant species produces essential oil in abundance from which the main component α-bisabolol, has antiflogistic, antibacterial and antimycotic properties (Teixeira *et al.* 1996; Silvério 2004;). Several plant extracts are under study with the aim toward insect control of disease vectors such as hematophagous mosquitoes, domestic flies, cockroaches and earwigs (Simas *et al.* 2004). Asteraceae species were tested on *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and their extracts activities for showed potential for integrated pest to management programs of this insect (Tavares *et al.* 2009). This paper shows the effects butanol fraction from ethanol flower or stems extract of *E. ehrythopappus* on the milkweed bug *Oncopeltus fasciatus* (Dallas).

MATERIAL AND METHODS

Material extraction. Flowers or stems of *E. erythropappus* were collected in Juiz de Fora, MG, Brazil, and a voucher specimen is deposited (CESJ No. 25363) in the Herbarium Leopoldo Kriger, Universidade Federal de Juiz de Fora (UFJF) (Silvério *et al.* 2008). Phytochemical studies were carried out at the Universidade Federal do Rio de Janeiro, RJ, Brazil. Flowers or stems of *E. erythropappus* were ground in an electric grinder and extracted with ethanol. The ethanol extract suspended in methanol/water (2:8) was submitted to liquid/liquid partition successively with hexane, dichloromethane, ethyl acetate and

butanol (Silvério *et al.* 2008) resulting four fractions: hexane fraction = F_1, dichloromethane fraction = F_2, ethyl acetate fraction = F_3 and butanol fraction = F_4.

Insect. *O. fasciatus* is kept in the Laboratory of Vectors Insect, Severino Sombra University, RJ. Milkweed seeds were used as insect food and the animals were kept in the incubator (BOD) at 28 ± 1°C. The fifth-instar nymphs of *O. fasciatus* were separated for the bioassays.

Bioassay. The insects were previously deprived of food and water for 24h before treatment and kept the incubator (BOD) under 28 ± 1°C. Plant material in acetone was diluted in NaCl 0.15M solution (0.8%) at 1 µg/µL – 100 µg/µL concentrations. The samples were applied in amounts of 1 µL to the abdominal ventral surface (µg/nymph) of each of 10-20 nymphs per group. The control groups consisted of acetone (without plant material) and untreated solution. Immediately after treatment, the insects received food and water and were maintained at room temperature during the remainder time of the experiment (Cabral *et al.* 1999). The insects of the experiments were observed over 18 days after treatment; death and ecdysis were recorded. The bioassays were realized in triplicate, and a statistical significance based on the Tukey test was established.

RESULTS AND DISCUSSION

Topical treatment of *O. fasciatus* with *E. ehrythopappus* fractions induced a delay in ecdysis. With hexane (F_1) and butanol (F_4) fractions at a dose of 10 µg/nymph, ecdysis delay in *O. fasciatus* 5st instar of 88 % ($P<0.01$) and 76%, respectively were observed (Table 1). A shorter interval between the fifth instar and the adult (2.9 ± 1.4) was observed with F_4 fraction compared to acetone control (4.4 ± 1.6) (1A). The fractions treatments caused 5-20% mortality in doses of 10 µg/nymph in *O. fasciatus* (1A). *O. fasciatus* 5st instar ecdysis delay induced by *E. erythropappus* butanol fraction (F_4) in doses of 1 µg/nymph, 10 µg/nymph and 100 µg/nymph was compared to the controls (Table 2). Treatment with the butanol fraction on fifth-instars resulted in 78 - 75% ecdysis and 10-20% mortality at 10 and 100 µg/nymph concentration, respectively (1B). The butanol fraction treatment caused 10% adult deformity. In no control experiments were any morphological alterations in adult insects reported. These deformities consisted of morphological adult alterations in dorsal abdomen with visible wing reduction.

This study described results from *E. erythropappus* biological activities focusing on toxicity and development inhibition of *O. fasciatus* that resulted in ecdysis inhibition and toxicity with butanol fraction treatment. Many studies confirm the importance of .*E. erythropappus* as highly effective schistosomicidal activity demonstrated by dichloromethane and hexane extracts (Almeida *et al.* 2011).

The results of this study clearly indicate that the phytophagous insects displayed sensitivity to *E. ehrythopappus* extracts. This also suggests a possible presence of special metabolites with potential insect disruption properties, such as the presence of anti-moulting principles.

This is the first report of biological activity of *E. erythropappus* (Asteraceae) fractions on the milkweed bug *O. fasciatus*.

Table 1. Duration in days of development, ecdysis and mortality of *Oncopeltus fasciatus* treated with *Eremanthus erythropappus* (Asteraceae) fractions (µg/nymph).

Treatment	Period (Days)		Ecdysis		Mortality
1A	X ± SD	VI	X ± SD	%	%
Control	4.1 ± 1.4 a,b	2-7	18.6 ± 0.5 a	100	0
Control 2	4.4 ± 1.6 a,b	2-7	20.0 ± 0 a	100	0
F_2-10 µg	4.6 ± 1.1 a	2-7	17.6 ± 0.6 b**	100	10
F_3-10 µg	4.3 ± 1.4 a,b	2-7	18.6 ± 0.63b	100	5
F_1-10 µg	4.4 ± 1.1 a,b	2-5	13.6 ± 0.6c***	88	20
F_4-10 µg	2.9 ± 1.4 b*	2-7	12.6 ± 0.6c***	76	20

Topical treatment with the fractions ($F_2 = CH_2Cl_2$, F_3 = AcOEt, F_1 = hexane, F_4 = butanol) from *E. erythropappus* (Asteraceae) treatment on *O. fasciatus* at 10µg/nymph concentration (1A). Values are mean ± standard deviation (X ± SD), average of three replicates of 20 nymphs (5st) per each group. VI= variation interval. Values followed by the same letter did not significantly different from each other, $P > 0.05$ when the Tukey test was used. Significance levels are represented as *** $P<0.001$, ** $P< 0.1$, * $P< 0.05$ vs. Control 2 = acetone.

Table 2. Duration in days of development, ecdysis and mortality of *O. fasciatus* treated with *Eremanthus erythropappus* butanol fraction (F_4) in three different concentrations (µg/nymph).

Treatment	Period (Days)		Ecdysis	Mortality
1B	X ± SD	VI	%	%
Control	7.8 ± 1.9 a	3-9	100	0
Control 2	6.5 ± 2.8 a	2-9	100	0
F_4 - 1 µg	7.8 ± 2.0 a	3-9	90	0
F_4 - 10 µg	8.4 ± 0.9 a	7-9	78	10
F_4 - 100 µg	8.0 ± 2.4 a	3-9	75	20

Butanol fraction (F_4) extract from *E. erythropappus* (Asteraceae) topical treatment on *O. fasciatus* at 1, 10 and 100µg/nymph concentrations (1B). Values are mean ± standard deviation (X ± SD), average of 10 nymphs (5st) per each group. VI= variation interval. Values followed by the same letter did not significantly different from each other, $P > 0.05$ when the Tukey test was used.

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