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Bioactivity of latex from *Euphorbia splendens* var. *hislopii* (Euphorbiaceae) on post-embryonic development of *Megaselia scalaris* (Phoridae)

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ABSTRACT

Larvae of *Megaselia scalaris* (Loew, 1866) feed on a wide range of decomposing organic matter and present a great importance to public health. This study evaluated the effect of crude latex extract from *Euphorbia splendens* var. *hislopii* (Euphorbiaceae) on post-embryonic development time of *M. scalaris* under laboratory conditions. The latex was used in its crude lyophilized form, dissolved in distilled water and tested in concentrations of 5 μg/mL, 10 μg/mL and 20 μg/mL. The latex was applied with the aid of an automatic pipette (1 μL/larva) on the newly-hatched larvae. Each group (the three concentrations of latex and the control group) was composed of 50 larvae and fed with 25 mg of decomposing horse flesh. The experiment was made in quadruplicate. The observations were recorded daily. The data were submitted to analysis of variance (ANOVA) and Tukey's post hoc-test with a 5% significance level. The post-embryonic development time for all stages (larval, pupal and newly-hatched larvae to adult) tested with all three latex concentrations was significantly shorter than for the control group, but without any significant difference among the different concentrations. The more sensitive stages to the substance were pupal and newly-hatched larvae to adult. The viability was less than 51.5% in the three concentrations of latex in these stages and they were lower than for the control group (67.4% for pupal stage and 64% for newly-hatched larvae to adult). Therefore, it is likely that this substance has influence on the development and viability of these flies and can become a promising agent for insect pest management.

The larvae of *Megaselia scalaris* (Loew, 1866) are able to feed on an exceptionally wide range of decaying organic matter and consequently the larvae are easily reared in the laboratory (Disney, 2008), but they can also be considered as plague insects infesting laboratory insect colonies (Costa et al., 2007). Larvae of *M. scalaris* can become facultative predators, parasitoids or parasites (Disney, 2008). Some cases of myiasis in human beings have already been reported as a result of larvae infestations by *M. scalaris* such as nasopharyngeal, urogenital and intestinal myiasis and in leg wounds. However, most of these cases were considered facultative and accidental (Singh et al., 1988; Meinhardt and Disney, 1989; Singh and Rana, 1989; Hira

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et al., 2004). In Brazil, Silva et al. (1999) registered a case of animal myiasis in *Crotalus durissus terrificus* (Laurenti, 1768). Zwart et al. (2005) observed a myiasis case in anurans of the Dendrobatidae and Hylidae families. Broderick and Hancock (1997) found turtle eggs infested with *M. scalaris* larvae.

These flies are also vectors of several microorganisms that cause intestinal infections, since they use a wide range of decomposing organic matter as a substrate for feeding and oviposition (Prawirodisastro and Benjamin, 1979). Therefore, the scuttle fly is a cause for great epidemiological concern due to its efficient adaptive capacity, dispersion, high population density and diversity of feeding habits (Gomes, 2000).

The control of synanthropic flies of public health importance is generally carried out using insecticides; however, the residual products of these compounds are harmful to the environment and can cause toxic damage to the human population and domestic animals (Rahuman and Venkatesan, 2008). The coevolution of insects and plants has promoted the development of a powerful and sophisticated plant defense strategy based on certain secondary phytochemical metabolites which are able to directly disrupt specific physiological routes related with neuroendocrine and feeding systems, metamorphosis, reproduction, diapausis and behavior of arthropods, constituting vulnerable points for the population control based on the life cycle of insects vectors (Stoka, 1987; Garcia and Azambuja, 2004).

Some studies revealed that *Euphorbia splendens* var. *hislopii* (Syn. *Euphorbia milii*—Zani et al., 1993) (Euphorbiaceae), in natura or lyophilized, is an important plant molluscicide (De-Carvalho et al., 1998; Schall et al., 1998; Vasconcellos and Amorim, 2003). Previous phytochemical studies of this species demonstrated the presence of triterpenes, flavonoids, antileukemic macrolide, ingenol, phorbol sters, lasiodiplodin (Lee et al., 1982) and eight types of milliamines (milliamines A, D and E and five new compounds of class milliamine L—a dianthraniloyl peptide ester of ingenol) (Zani et al., 1993). Diterpene esters of phorbol and ingenol types, which typically occur in plants belonging to Euphorbiaceae family, are known to be highly active tumor promoting agents (Delgado et al., 2003).

The hypothesis of this study is that the topical use of different concentrations of crude extract of latex applied on newly-hatched larvae of *M. scalaris* can alter the post-embryonic development time and decrease the viability of these flies. Therefore the purpose of this study was to test and to evaluate the toxic effects of crude latex extract from *E. splendens* var. *hislopii* on the post-embryonic development period and viability of *M. scalaris*.

1. Material and methods

The establishment and maintenance of *M. scalaris* colony followed the recognized methodology of Queiroz and Milward-de-Azevedo (1991). The latex samples of *E. splendens* var. *hislopii* were extracted from various plants at the Universidade Federal Rural do Rio de Janeiro (UFRRJ) campus. The raw latex that exuded on transversal sectioning, around 10 cm below the apical meristem of each branch, collected in a glass test tube closed with a screw cap and transported to the laboratory. For the experiment, the latex was used in its crude lyophilized form, dissolved in distilled water and tested in concentrations of 5 μg/mL, 10 μg/mL and 20 μg/mL. The control group was tested only with distilled water.

Fifty newly-hatched larvae of *M. scalaris* were tested for each concentration of latex and the experiments were performed in quadruplicate. These larvae were grouped together on Petri dishes containing 5 mg of a diet based on decomposing horse flesh and different concentrations of latex were inoculated directly onto the bodies of the newly-hatched larvae with an automatic pipette (1 μL/larva). After inoculation the larvae were transferred to a recipient containing 20 mg of the same diet, these recipients (100 mL) were then placed in larger recipients (500 mL) containing vermiculite as a substratum for pupation and were covered with a nylon fabric held down with rubber band. The control group was tested with distilled water instead of the latex solution and the dose response was verified according to the bioactivity found. The bioassays were carried out in a climatic chamber at $27 \pm 1\,^{\circ}C$, $60 \pm 10\%$ RH and 12 h photoperiod and the observations were recorded daily.

The post-embryonic development time of *M. scalaris* (larval, pupal and newly-hatched larvae to adult periods) and the viability of each period of development for all concentrations of latex and control group were recorded. The results were confirmed with analysis of variance (ANOVA): development time was used as a dependent variable and the latex concentrations as an independent variable. For posterior comparisons the Tukey's post hoc-test was used. Viability differences in the different stages of the fly were performed with Chi-squared test, in this analysis it was used presence (1) and absence data (0). All the statistics analyses were carried out with a 5% significance level (Zar, 1999) and it was used Systat 8.0 program.

2. Results and discussion

The larvae of *M. scalaris* treated with different concentrations of the crude latex extracted from *E. splendens* var. *hislopii* did not present any significant differences in their developmental time among the different concentrations, however all concentrations showed a significant difference when compared to the control group, in which the larvae had a slower development time ($F_{3702} = 11.66$; $p < 0.01$) (Table 1). The larvae pupated, in most cases, on the fourth day after inoculation with latex in all treatments, finalizing this process on the fifth day (Fig. 1). Disney (2008) observed a pupation interval for *M. scalaris* that varied from four to six days under the same abiotic conditions ($27 \pm 1\,^{\circ}C$, $60 \pm 10\%$ RH and 12 h photoperiod) without the presence of latex in the diet. Also the pupal development time and newly-hatched larvae to adult did not present any significant difference among the three concentrations tested; however, when compared to the control group, which also presented a slower development, there was a significant difference ($F_{3306} = 18.04$; $F_{3365} = 17.26$ $p < 0.001$, respectively) (Table 1).

The reason for the accelerated development time in the presence of this substance is not yet clearly under-

Table 1
Duration of post-embryonic development of *Megaselia scalaris*, treated with different concentrations of latex from *Euphorbia splendens* var. *hislopii* (5 μg/mL, 10 μg/mL and 20 μg/mL) and control group (without inoculation of the substance), under laboratory conditions.

Concentrations of latex	Larval stage (days)		Pupal stage (days)		Newly-hatched larvae to adult (days)	
	(Mean ± S.D.)#	Range	(Mean ± S.D.)#	Range	(Mean ± S.D.)#	Range
5 μg/mL	4.33 ± 0.47a	4–5	8.18 ± 0.42a	8–10	12.18 ± 0.42a	12–14
10 μg/mL	4.27 ± 0.45a	4–5	8.16 ± 0.43a	8–10	12.23 ± 0.48a	12–14
20 μg/mL	4.22 ± 0.42a	4–5	8.21 ± 0.44a	8–10	12.21 ± 0.44a	12–14
Control	4.48 ± 0.51b	4–5	8.86 ± 0.81b	8–10	12.86 ± 0.81b	12–14

Values within a column followed by the same letter are not significantly different at the 5% level according to Tukey's HSD.

stood; however some authors have suggested that some compounds extracted from plants may be capable of modulating the insect's endocrine system and consequently influencing the post-embryonic development time (Cabral et al., 2007a,b).

Also the viability of all developmental stages of *M. scalaris* including the newly-hatched larvae period of the samples exposed to or not exposed to the latex concentrations was recorded. The results presented in Fig. 2 show the percentage of viability of different development stages of the fly in the different concentration of latex. It was recorded by Chi-squared test that viability presented significant differences in all development stages among the treatments ($\chi^2 = 27.6$, $df = 3$, $p < 0.01$ for the larval stage; $\chi^2 = 20$, $df = 3$, $p < 0.01$ for the pupal stage and $\chi^2 = 33.6$, $df = 3$, $p < 0.01$ for the newly-hatched larvae to adult period). Pupal viability was 46.5% and newly-hatched larvae to adult was 36.5% in 5 μg/mL of latex; at 10 μg/mL of latex the pupal viability and newly-hatched larvae to adult were 48.3% and 42.5%, respectively, while at the 20 μg/mL of latex the pupal viability was 51.4% and newly-hatched larvae to adult viability was 46.5% (Fig. 2). The control group showed a pupal viability of 67.4% and the newly-hatched larvae to adult viability was 64% (Fig. 2).

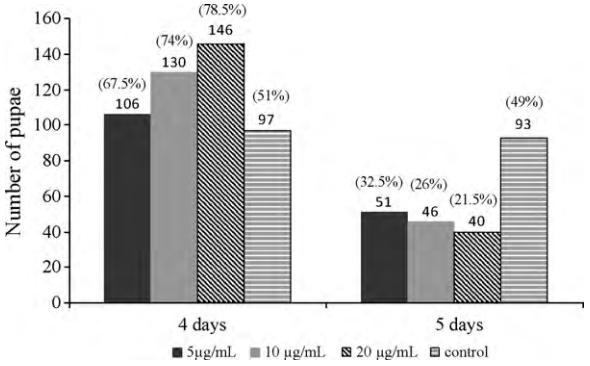

Fig. 1. Number of pupae formed in four and five days after exposure of the newly-hatched larvae to the latex in different concentrations (5 μg/mL, 10 μg/mL and 20 μg/mL) and in the control group, under laboratory conditions.

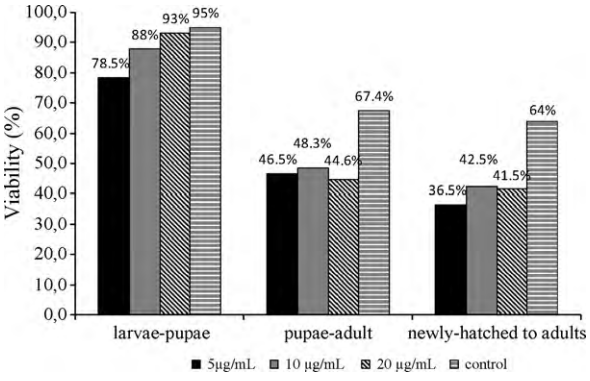

Fig. 2. Viability of the larval period (newly-hatched larvae to pupae), pupal period (pupae to adult) and newly-hatched larvae to adult of *Megaselia scalaris* after exposure to different concentrations of latex from *Euphorbia splendes* var. *hislopii* and the control group, under laboratory conditions.

Gomes et al. (2003) tested the effect of lyophilized latex of *E. splendens* var. *hislopii* on the post-embryonic development of *Peckia chrysostoma* (Wiedemann, 1830) (Sarcophagidae) with concentrations of 0.1%, 0.2% and 0.3%, that equaled 100 mg, 200 mg and 300 mg of latex in 100 g of meat, i.e., not applied the latex concentrations topically on the larvae as performed in the present study. These authors observed that the larval, pupal and newly-hatched larvae to adult development time were faster in the presence of this substance when compared with control group, as was observed in the present study. But, these authors, also, observed significant differences in development time among the concentrations of latex, mainly in the newly-hatched larvae to adult period, which presented a faster development in the highest concentration of latex (0.3%) when compared with less latex concentration. It is important to detach that methodology above employed was different of the present study, the lyophilized latex was placed on the diet, and in the present study the latex solution was placed topically on the larvae. So, this may have caused differences between these studies.

Gomes et al. (2003) also evaluated the larval, pupal and newly-hatched larvae to adult viability and observed that the newly-hatched larvae to adult period was the most sensitive to the latex concentrations, having a gradual reduction of viability with the increase of latex concentration, so the lowest viability was found in 0.3% of latex (53.66%) and the highest was observed in control group (82.66%). This result was the inverse of the observed in the present study, in other words, there was a direct relationship in this study, increasing the latex concentration there was an increase in the viability. However, the viability of all latex concentrations for the all development stage was lower than control group viability. Gomes et al. (2003) suggested that latex from *E. splendens* var. *hislopii* could have contributed, perhaps, in causing the surviving

larvae to have difficulties in feeding and consequently in absorbing the necessary nutrients, thus abandoning the substratum prematurely. In this way, possibly many larvae did not reach the ideal weight and size to pupate, which in this present study could have caused the reduced viability of these flies and accelerated the development time in treatment with latex.

The majority of studies testing the action of different compounds of plants on fly development and viability are carried out with structural variations of lignoids that are units of phenylpropanoids dimers (C6–C3) connected by the central carbon of their side chains (lignans) or that show other types of connections (neolignans). Cabral et al. (2007a) observed the effect of several lignoids from different plants such as yangambin (*Ocotea duckei* Vattimo, lauraceae), burchelin (*Aniba burchelli* Kostern, Lauraceae), licarin A (*Nectandra amazonum* C.G.D. Nees, and *N. glabrescens* Benth, Lauraceae) and grandisin (Piper solmsianum C.D.C., Piperaceae) on the development and viability of *Chrysomya megacephala* (Fabricius, 1794) (Calliphoridae). They observed that flies treated with yangambin presented a development time significantly slower for all developmental periods (larval, pupal and newly-hatched larvae to adult) in relation to those treated with the other substances and the control group. However, the treatments with other types of lignoids did not present any differences in the development time among them and in relation with the control group. Besides, the viability of the larval and newly-hatched larvae to adult period appeared to be sensitive to yangambin, showing a viability of 44% and 39%, respectively, with similar results for burchelin (53% and 41%) when compared to the control group (82% and 68%).

In another study, Cabral et al. (2007b) observed that yangambin from (*O. duckei* Vattimo, lauraceae) influenced the *C. megacephala* viability in the egg to adult period reducing by up to 20% the emergence of adults in relation to the control group. Thus, can be observed that both crude latex extract from *E. splendens* var. *hislopii* and some types of compounds present in other plants had an action on the development of flies and can influence the development time and/or reducing the viability of the different stages of development. However, it is important says further studies are needed, particularly with the purpose of finding the active compounds of the latex for a more effective and direct control.

The study of different concentrations of crude latex extract from *E. splendens* var. *hislopii* on the *M. scalaris* development and viability is unprecedented; most of the studies carried out with latex from this plant were aimed at evaluating the moluscicide effects against the intermediate hosts of schistosomiasis which have indicated a high potential for control. Vasconcellos and Amorim (2003) observed in *Lymnaea columella* (Say, 1817) (Pulmonata: Lymnaeidae), the intermediary host of *Fasciola hepatica* (Linnaeus, 1758), a mortality rate of 97.4% when 5 mg/L of latex from *E. splendens* var. *hislopii* was applied and a 100% viability without the latex application. Schall et al. (1998) observed differences in susceptibility to latex for three species of the intermediary host of schistosomiasis: *Biomphalaria glabrata* was the most susceptible requiring the lowest lethal dose (LD_{90} 1.0 ppm = 1 mg/mL), while for *B. straminea* and *B. pfeifferi* the LD_{90} was 4.0 ppm (4 mg/mL), these concentrations were obtained by dilutions made from a stock solution of 1000 ppm (1 g of lyophilized material diluted in 1000 mL of distilled water).

From the data presented in this study, it is possible inferred that crude latex of *E. splendens* var. *hislopii* is an important alternative method of flies control. Because it reduced the viability, primarily of newly-hatched larvae to adult period as confirmed by Chi-squared test and, also, accelerated the *M. scalaris* development time, which may have contributed in reducing the viability in these concentrations due to the abandonment of the larvae from the diet have been earlier. This control type is interesting mainly because it is performed with natural substances that cause no adverse effects to the environment.

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