TOXICITY OF *Prasiola crispa* EXTRACT FRACTIONS IN THE ADULT FRUIT FLY *Drosophila melanogaster*: AN UPDATE

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Ana Paula Pegoraro Zemolin, Mauro Eugenio Medina Nunes, Nathane Rosa Rodrigues, Cháriston André Dal Belo, Antonio Batista Pereira, Thais Posser, Jeferson Luis Franco*

Centro Interdisciplinar de Pesquisa Biotecnológica, Universidade Federal do Pampa, Avenida Antonio Trilha, 1847, CEP 97300-000, São Gabriel, RS, Brazil

*e-mail: jefersonfranco@unipampa.edu.br

Abstract: In a previous report we tested the insecticidal effect of a crude extract of *Prasiola crispa*. In the present study, we obtained three different fractions and tested which of them presents higher toxicity on a fruit fly (*Drosophila melanogaster*) model. Fraction 1 (ethanol), Fraction 2 (methanol) and Fraction 3 (ethyl acetate) we administered to flies for 48 hours at concentrations of 0, 1, 2, 5, 10 and 50 µg/ml in a 1% sucrose solution. Sucrose alone was used as control. Toxicity was assessed as % mortality and negative geotaxis behavior. A significant decrease in survivability of flies exposed to Fraction 1 and 2 at the higher concentrations tested was observed, while Fraction 3 posed a significant increase in mortality also at intermediate concentrations. The comparison between the three fractions showed that Fraction 3 was significantly most prominent in inducing mortality. Similar results were observed for the climbing performance, indicating an alteration in negative geotaxis behavior. In our previous report we showed that *Prasiola crispa* crude extract presents high toxicity to *Drosophila melanogaster*. Our present results show that Fraction 3 may contain high concentrations of toxic metabolites compared to other two fractions. Further studies are necessary to elucidate the exact mechanisms of toxicity of this Antarctic alga to *Drosophila melanogaster* and to identify which components of Fraction 3 are responsible for such an effect.

Keywords: *Prasiola crispa*, Toxicity, *Drosophila melanogaster*, Antarctica

Introduction

The bioinsecticide ability of a number of plants has been investigated for thousands of years, and some of the plants can substitute many synthetic means of control. In this respect, it is important to emphasize that natural agents are environmentally less harmful than synthetic pesticides. Moreover, natural agents can act in many insects in different ways (Sujatha, 2010). In this respect, in this study we intended to evaluate the potential insecticidal properties of the Antarctic alga *Prasiola crispa*.

Prasiola crispa is a terrestrial eukaryotic green alga from Antarctic continent. Although there are no studies targeting the biological effects of this algae, interesting characteristics, like adhesive properties have been described for the plant of the genus *Prasiola* sp. (Mostaert *et al.*, 2006), highlighting the biotechnological importance of this organism. Other

studies carried out with other Antarctic plant species, have demonstrated low toxic effects for mammalian and non-mammalian cells, associated with protective effects against UV-induced damage (Pereira *et al.*, 2009).

It has been recognized that organisms living in Polar Regions, are subject to extreme environmental conditions. This fact has lead to the developing of natural strategies that enable these organisms to survive under the most extreme environmental conditions on Earth. Among these adaptation strategies is the production of photoprotective compounds, such as mycosporine-like amino acids, scytonemim secreted by cyanobacteria and flavonoids secreted by plants (Pereira *et al.*, 2009). This fact emphasizes the importance of studies concerning the biological effects of these organisms, which may present in its constitution a

combination of chemical compounds normally not found in other organisms.

In a previous report we tested the insecticidal effect of a crude extract of *Prasiola crispa* (Posser *et al.*, 2011). In the present study, we obtained three different fractions and tested which of them presents higher toxicity on a fruit fly (*Drosophila melanogaster*) model.

Materials and Methods

Plant material: *Prasiola crispa* (Lightfoot) Kützing 1843 was collected in the ice-free areas near Arctowski Polish Station Region, Admiralty Bay, King George Island (61°50' - 62°15' S and 57°30' - 59°00' W), Antarctica. The extract was fractionalized into three different fractions and an ethanolic (Fraction 1), methanolic (Fraction 2) and ethyl acetate (Fraction 3) was obtained.

***Drosophila* stock and culture:** *Drosophila melanogaster* (Harwich strain) was obtained from the National Species Stock Center, Bowling Green, OH, USA. The flies were reared in 2.5 cm × 6.5 cm glass bottles containing 5 mL of standard medium (1% w/v brewer's yeast; 2% w/v sucrose; 1% w/v powdered milk; 1% w/v agar; 0.08% v/w Nepagin) at constant temperature and humidity (25°C ± 1°C; 60% relative humidity, respectively). All experiments were performed with the same strain.

***Prasiola crispa* Fractions exposure:** Adult flies, 1- to 4- days post eclosion, were exposed to alga fractions using

the following protocol. 180 adult flies (males) were starved in empty vials for 3 hrs at 25°C. Following starvation, groups of 30 flies were placed for 48 hours in vials containing a filter paper saturated with 500 µl of Fractions 1, 2 or 3 (0, 1, 2, 5, 10 and 50 µg/ml) solution diluted in 1% sucrose. Sucrose only, served as control.

Survival rate analysis: To quantify fly survival rates, five replicates of groups of 30 adult flies were exposed to Fractions for 48 hours and the number of live, and dead, flies counted every 24 hours. Results were analyzed and plotted as total "number of dead flies".

Locomotor assay: Locomotor ability was determined using the negative geotaxis assay as described previously with some modifications. Briefly, for each assay, 20 flies (1-4 days old; both genders) were immobilized on ice for 1-2 min and placed separately in vertical glass columns (length, 25 cm; diameter, 1.5 cm). After 30 min recovery, flies were gently tapped to the bottom of the column and the number of flies that climbed up 6 cm in the columns during 6 seconds was registered and plotted as the "number of flies on top".

Results

Treatment of flies with *Prasiola crispa* extract fractions resulted in a substantial increase in mortality after 48 hours (Figure 1A). Fractions 1 and 2 presented toxicity towards

Figure 1. *Drosophila melanogaster* mortality and behavior after treatment with *Prasiola crispa* extract Fractions. (A) mortality (B) negative geotaxis behavior. Statistical significance was assessed by One Way ANOVA followed by Duncan's *post hoc* test. * $p < 0.05$, ** $p < 0.01$, comparing to control (sucrose only). # $p < 0.05$ comparing different Fractions.

mortality rate at concentrations of 10 and 50 µg/ml, while Fraction 3 showed increased mortality at concentrations of 5, 10 and 50 µg/ml. The comparison between the three different fractions showed that Fraction 3 caused a significantly ($p < 0.05$) increased mortality when compared to the other two fractions. Similar results were observed for the locomotor behavior (Figure 2B), being Fraction three significantly more effective in decreasing locomotor activity of flies.

Discussion and Conclusion

In a previous report, our group presented for the first time the potential bioinsecticide action of an Antarctic alga, *Prasiola crispa*. The present results point that the ethyl acetate fraction (Fraction 3) may contain increased

concentrations of toxic metabolites, which are responsible for the observed effects in *Drosophila melanogaster*.

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