

Effects of neem (*Azadirachta indica*) extract on mitochondria  
of *Rubus fruticosus* cells

Paviani, V.<sup>1</sup>, Cesila, C.A.<sup>1</sup>, De Souza, W.R.<sup>1</sup>, Curti, C.<sup>1</sup>, Kinoshita, A.<sup>2</sup>, Baffa, O.<sup>2</sup>,  
Vargas-Rechia, C.G.<sup>1</sup>

<sup>1</sup> Dep de Física e Química, FCFRP-USP, Ribeirão Preto, Brasil;

<sup>2</sup> Dep de Física e Matemática, FFCLRP-USP, Ribeirão Preto, Brasil.

Neem has been used for centuries due to its medicinal and insecticide properties, but little is known about its impact on plants. Plants, similarly to other organisms, have the capacity to defend themselves from the attack of pathogens. These defenses include hypersensitive response (RH), which is characterized by necrosis of tissues in the local region surrounding the infection. Although several studies have demonstrated the involvement of mitochondria in this process, they are inconclusive; in addition, cell death is eventually associated with pro-oxidant action, which, in turn, may result from antioxidant activities themselves. Therefore, we evaluated here the antioxidant activity of the neem seed extract and its effect on mitochondria isolated from *R. fruticosus* cells. The powdered seeds was subjected to extraction with ethanol:water (1:1 v/v) for 20 min. The filtrated solution was evapored, lyophilized and resuspended in water. The antioxidant activity was assessed by Electron Spin Resonance Spectroscopy using DPPH (1,1-Diphenyl-2-Picrylhydrazyl) as probe; the IC<sub>50</sub> value was 14.85 mg/mL. In isolated mitochondria, it was monitored oxygen consumption and membrane potential by using NADH or succinate as oxidizable substrate, as well as reactive oxygen species (ROS) generation with 2,7-dichlorofluorescein diacetate. Neem decreased the rate of oxygen consumption by the respiratory chain, dissipated the mitochondrial membrane potential and inhibited ROS generation in a concentration-dependent manner. These results suggest that the extract possesses antioxidant activity, which, in principle, may be responsible for the well established medicinal effects, at the same time that, in plants, it causes cell death by a defense mechanism involving mitochondria.

Key words: *Azadirachta indica*, mitochondria and EROs.

Supported by Capes