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ORIGINAL ARTICLE

Bioefficacy of violacein against Asian armyworm *Spodoptera litura* Fab. (Lepidoptera: Noctuidae)

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Abstract The aim of the study was to assess the bioefficacy of violacein isolated from *Chromobacterium violaceum* against *Spodoptera litura*, an important field pest. Antifeedant, larvicidal and pupicidal activities of violacein were evaluated for its bioefficacy against the third instar larvae of *Spodoptera litura* at 125, 250, 500 and 1000 ppm concentrations. After 24 h of treatment, violacein treated larvae showed maximum antifeedant activity of 72.46% at 1000 ppm concentration with LC_{50} of 392.25 ppm. Four days after treatment, violacein treated larvae showed a mortality of 77.10% at 1000 ppm concentration with a LC_{50} value of 255.06 ppm. Violacein treated larvae recorded 20–24% pupicidal activity at 125–1000 ppm concentration. The antifeedant and larvicidal activities were increased with increasing concentration. Violacein could also be considered for use in the management of pests.

1. Introduction

Spodoptera litura Fab. is a polyphagous pest attacking more than 150 host species (Rao et al., 1993). It is one of the most economically important insect pests in many countries including India, Japan, China and Southeast Asia. Use of insecticides to control insects has resulted in resurgence and pesticide resistance (Armes et al., 1997). Due to this reason, many workers have concentrated on alternative control methods. Botanical and microbial pesticides are highly effective, safe, and ecologically acceptable. Biologically active substances from plants and microbes affect the growth and development of insects and provide protection against herbivores including lepidopteran pests (Sukumar, 1993, Senthil Nathan et al., 2005; Ignacimuthu et al. 2006; Baskar et al., 2009, 2010, 2011a,b).

Natural products derived from plants and microorganisms have been used for insect control (Hu et al., 2007). Azadirachtin, a natural compound isolated from neem *Azadirachta indica*, is considered superior over other compounds since it has wide range of biological activities. In the past many workers have used azadirachtin as positive control (Koul et al., 2005; Tewary et al., 2006; Tang et al., 2009; Jeyasankar et al., 2011). Bacterial and viral-based insecticides controlled different pests (Nagarkatti, 1982). Anti-diarrhoeal and ulcer-protective effects of violacein isolated from *Chromobacterium*

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violaceum (Antonisamy et al., 2009). *C. violaceum* is a soil bacterium which produces a violet coloured pigment violacein. Violacein acts as a growth inhibitor of Gram-positive and Gram-negative bacteria (De Moss, 1967). Previously, Martin et al. (2007) studied the supernatants and extracts from *Chromobacterium sp*. against leaf feeding insects like colorado potato beetle, corn rootworm, diamondback and gypsy moths larvae. They also reported that violacein was toxic to insects. No reports are available on the effect of violacein against the polyphagous pest *S. litura*. Hence the present study was aimed to assess the efficacy of violacein isolated from *C. violaceum* against *S. litura*.

2. Materials and methods

2.1. Bacterium and violacein

2.1.1. Isolation of bacterium

Chromobacterium violaceum ESBV 4400 was isolated from the forest water body soil sample of Kolli Hills situated in Namakkal district of Tamil Nadu, India. The bacterium was identified using standard biochemical methods (Cappuccino and Sherman, 2004) and confirmed by 16S rRNA gene identification (Hao et al., 2007).

2.1.2. Production and extraction of violacein

Chromobacterium violaceum was inoculated in 50 mL of LB broth and incubated at room temperature (28 ± 2 °C) at 150 rpm for 12 h. This mother inoculum (5%) was introduced into the sterile production broth containing 200 mL of liquid medium (0.5% D-glucose; 0.5% peptone; 0.2% yeast extract) in a 1000-mL Erlenmeyer flask with sponge (10 × 6 × 3 cm) and was incubated at 28 °C for 48 h in a rotary shaker at 150 rpm following a slightly modified method of Rettori and Duran (1998). After the incubation period, the sponge attained a bright violet colour because of intracellular production of violacein by the bacteria which was accumulated or trapped in the sponge.

The sponges were removed from the flask and squeezed together to eliminate excess medium and then washed twice with distilled water. Violacein was extracted twice with 500 mL of commercial ethanol. The ethanolic solution was filtered and evaporated under reduced pressure. This step was repeated many times.

2.1.3. Purification of violacein

The crude mass (3 g) was packed in a soxhlet apparatus and was washed with chloroform followed by diethyl ether (for 3–4 h). The final extraction of violacein was carried out using ethanol. Ethanol was evaporated under reduced pressure; this semi-purified violacein was further purified by crystallization with the solvent pair methanol:water (1:1). The crystals were harvested by centrifugation and vacuum-dried (Rettori and Duran, 1998). Finally, violacein was purified by preparative thin-layer chromatography. Violacein was characterized by 1H-nuclear magnetic resonance (NMR) (AL-300 MHz JEOL using DMSO: JEOL, Tokyo, Japan) (Nakamura et al., 2002), infrared (IR), mass and ultraviolet (UV) spectra (Riveros et al., 1998). It was compared with the previous report and the structure was elucidated (Fig. 1).

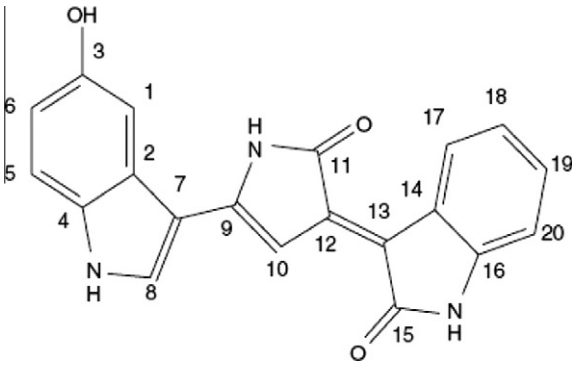

Figure 1 Structure of violacein.

2.2. Insect culture

Egg masses of *Spodoptera litura* were collected from groundnut field at Eagattur in Thiruvallur District of Tamil Nadu, India. The eggs were surface sterilized with 0.02% sodium hypochlorite solution, dried and allowed to hatch. After hatching, the neonate larvae were reared on castor leaves (*Ricinus communis)* till the prepupal stage and sterilized soil was provided for pupation. After pupation, the pupae were collected from soil and placed inside the oviposition chambers (40 × 25 × 25 cm). After adult emergence, cotton soaked with 10% (w/v) sugar solution with few drops of multivitamin was provided for adult feeding to increase the fecundity. Potted groundnut plant was kept inside adult emergence cage for egg laying. After hatching the larvae were provided tender castor leaves for feeding. These laboratory reared larvae were used for bioassay.

2.3. Antifeedant activity

Antifeedant activity of violacein was studied using leaf disc no-choice method. Fresh castor leaf discs of 4 cm diameter were punched using cork borer. They were dipped in 125, 250, 500 and 1000 ppm individually. The leaf discs dipped in acetone were used as negative control since acetone was used to dissolve the compound. Azadirachtin (40.86% purity, obtained from EID-Parry India Ltd., Chennai) was used as positive control. In each plastic petridish (1.5 × 9 cm) wet filter paper was placed to avoid early drying of the leaf discs. Single third instar larva was introduced into each petridish for 24 h. The test was terminated at the end of the 24 h. Progressive consumption of treated and control leaves by the larvae for 24 h was recorded using Leaf Area Metre (Delta-T Devices, Serial No. 15736 F 96, UK). Leaf area eaten by larvae in treatment was corrected from the negative control. Five replicates were maintained for each treatment with 10 larvae per replicate (total, $n = 50$). The experiment was conducted at laboratory conditions (27 ± 2 °C) with 14:10 light and dark photoperiod and 75 ± 5% relative humidity. Antifeedant activity was calculated according to the formula of Isman et al. (1990);

$$\text{Antifeedant activity} = \frac{\text{Leaf area consumed in control} - \text{Leaf area consumed in treatment}}{\text{Leaf area consumed in control} + \text{Leaf area consumed in treatment}} \times 100$$