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A Potent Insect Growth Regulator Plumbagin from *Plumbago indica* Against *Pericallia ricini* (Lepidoptera: Aractidae)

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Abstract: Plumbagin, a 1, 4-Naphthalenedione isolated from the *Plumbago indica*, exhibited growth regulating activity in the larvae of *Pericallai ricini*. The fourth instar and fifth instar larvae were topically treated with 10, 20, 25, 35, 50, 75 and 100 mg mL^{-1} concentrations of Plumbagin, in solvent acetone. Interference in moulting process, ecdysial failure and blockage of adult emergence were the important morphogenetic abnormalities observed which resulted in the formation of larval-pupal intermediates, abnormal pupae and deformed non-viable adults. Results suggest that Plumbagin shows an effective insect growth regulating activity and exhibits great promise in suppressing the population of *P. ricini*.

Key words: Plumbagin, 1, 4-Naphthalenedione, *Plumbago indica*, *Pericallai ricini*, morphogenetic abnormalities, ecdysial failure

INTRODUCTION

The larva of lepidopteron moth *Pericallia ricini* is a serious polyphagous pest of agricultural crops. The black hairy caterpillar is a voracious leaf feeder of country bean *Dolichos lablab*, Elephant Foot, Drumstick, *Coccinia grandis*, Brinjal, Cow pea, Yam, Sweet, Potato, Radish, Arum which is one of the main vegetable crops of Tamilnadu (Fletcher, 1914; Nair, 1970; Bustani and Damo, 1984; David, 2001). During the initial stages, the larvae feed on the lower side of the leaves and when they reach third instar they begin to feed voraciously and defoliate the entire plant. When the larval population was not controlled, the crop yield loss may extend up to 70-80% (Pandey *et al*., 1983).

Use of botanical pesticides for protecting crops from insect pests has assumed greater importance in recent years owing to the growing awareness of indiscriminate use and consequent harmful effects of the chemical pesticides (Mordue *et al*., 1995). Natural plant products are comparatively less toxic, easily biodegradable and have made them to be the best alternate to the synthetic pesticides. Identification of phytochemicals which mimic insect morphogenetic hormones or have growth regulating activity and synthesis of potent hormone agonists and antagonists in the recent past have led to their consideration as components of biorational approach to pest management. The well known nature product, Plumbagin from *Plumbago capensi* cause an anti-ecdyson effect and interfere with insect ecdysis (Rembold and Sieber, 1981; Gujar and Mehorotra, 1998).

Plumbago species are reported in the literature for its biological activities such as: Antiparasitic (Chan-Bacab and Pena-Rodriguez, 2001), insect antifeedant (Villavicencio and Perez-Escandon, 1992), antitumoral (Devi *et al*., 1994), antimalarial (Likhitwitayawuid *et al*., 1998), antimicrobial (Didry *et al*., 1994), anticancer (Parimala and Sachdanandam, 1993), cardiotonic (Itoigawa *et al*., 1991) and antifertility action (Bhargava, 1984) and others, some of them attributed to the presence of special chemical compounds, such as naphthoquinones. Plumbagin is a naphthoquinone well distributed among *Plumbago* species, specially found in their roots (Van der Vijver, 1972). This compound has been described in the literature and showed to possess a wide variety of bioactivities.

In the present investigation, Plumbagin is screened for its growth-regulating activity against the larvae of *P. ricini* reared on the leaves of *Ricinus communis*.

MATERIALS AND METHODS

Collection of larvae: The eggs and the freshly emerged Ist instar larvae of *P. ricini* were collected from the castor plants cultivated in the vicinity of Madurai and kept in the laboratory at room temperature of 29°C R.H throughout the period of study. The larvae were fed with fresh castor leaves and allowed to hatch in to moths. These moths were fed with 10% sucrose solution soaked in a small piece of cotton. Male and female moths were kept in specially designed cages for mating. Castor leaves were introduced for egg laying and the moths were allowed to

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lay eggs. The egg hatching was completed on the leaf itself and the freshly emerged first instar larvae were collected and separated. These individuals were maintained in the laboratory for the experimental purpose.

Growth regulation activity: Plumbagin was obtained from Sigma Company and it was used for the assays. Plumbagin was diluted in acetone and different concentrations were prepared for topical application. Growth regulation activities of Plumbagin were studied at four different concentrations against fourth and fifth instar larvae of *P. ricini*. Three larvae were introduced in a petri plate and each larva was treated topically with 10, 20, 25, 35, 50, 75 and 100 mg for three days. In the control treatment, larvae were treated with 1 μL of acetone. For each concentration three replicates were maintained. During the developmental period (fifth instar to pupa), deformed larvae, pupa and the mortality of larvae were recorded. Percent mortality was calculated (Abbott, 1925):

$$\text{Percent larval mortality} = \frac{\text{No. of dead larvae}}{\text{Total No. of treated larvae}} \times 100$$

$$\text{Abbortt's corrected mortality} = \frac{\text{\% mortality in treatment} - \text{\% mortality in control}}{100 - \text{\% mortality in control}} \times 100$$

RESULTS AND DISCUSSION

Severe morphogenetic abnormalities were observed in plumbagin treated resultant insects, at various concentrations (Table 1). The degree of effectiveness was varying with the concentration. There is 76.6% of mortality was observed at 100 mg mL^{-1} concentration of plumbagin and 63.3, 41.6 and 30% of mortality was occurred at 75, 35 and 25 mg mL^{-1}. The fourth instar larva which is undergone the treatment of 50 mg mL^{-1} of plumbagin was ecdyses into fifth instar. But the larvae could not able to moult completely. It was partially entangled in the larval exuvium. At the concentration of 75 and 100 mg mL^{-1}, the treated larvae could not ecdyses and the malformed larvae were shorter in length and crumpled. Such larvae were inactive and their life cycle was terminated (Fig. 1). The mode of action of plumbagin is being a chitin synthesis inhibitor, affects the mechanical properties of the insect cuticle and produces abnormalities in the skin and resists moulting. Inhibition of moulting results in increase of the internal body pressure in the larvae. Due to plumbagin activity insect cuticle often becomes stiff (Fox, 1990). Consequently feeding is often hampered, the starved larvae were reduced in body size. Mondal (1984) and Rahman (1992) reported to avoid feeding on the treated medium *Tribolium* species and as a result adults lose weight and length. Kuwano *et al.* (2008) suggested that this malformation may be due to the persistence of juvenile hormone in the haemolymph where it is only in the absence of juvenile hormone that ecdysone could be activated and lead to the formation of the next stage. Gujar and Mehorotra (1998) reported that plumbagin might be acting on the neuro endocrine system thereby it halts the moulting process.

Larval-pupal intermediate stages were also observed in the fifth instar larvae treated with 100, 35 and 25 mg mL^{-1} concentrations of plumbagin. Pupal case was formed normally but exhibited serious disturbances during pupal formation. The larva has failed to complete its development and they were unable to emerge into normal

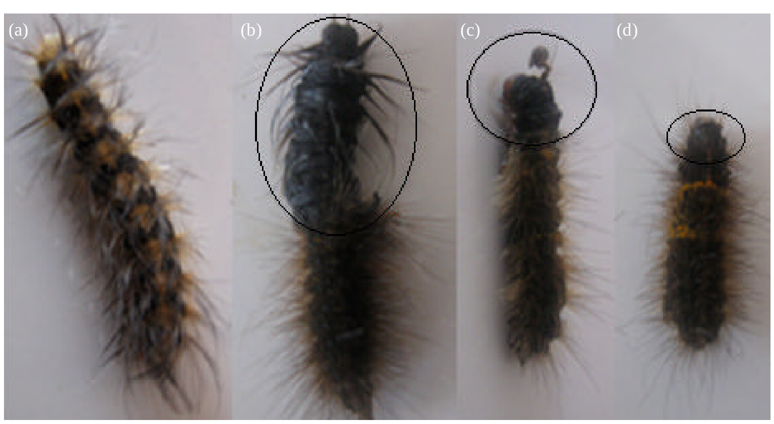

Fig. 1: Molting deformities in Ivth instar larvae of *Pericallia ricini* treated with Plumbagin (Topical treatment) (a) Control, (b) 50, (c) 75 and (d) 100 mg

Table 1: Percent larval mortality of *P. ricini* after the topical application of Plumbagin

	Concentration of Plumbagin (mg mL^{-1})				
Parameters	Control	25	35	75	100
No. of larvae tested	60.0	60.0	60.0	60.0	60.0
No. of dead larvae	5.0	18.0	25.0	38.0	46.0
Mortality (%)	8.3	30.0	41.6	63.3	76.6
Abbott's corrected mortality	0	23.6	36.31	59.97	74.48

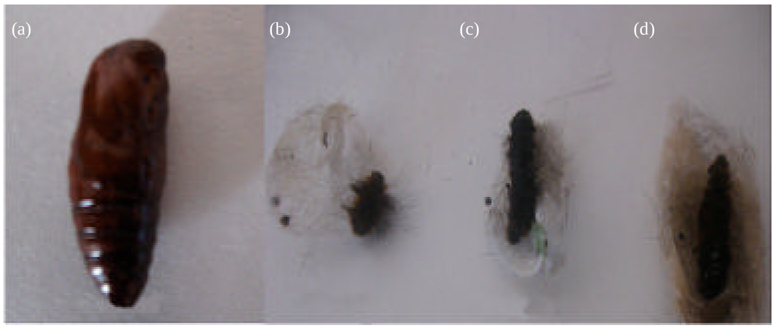

Fig. 2(a-d): Effect of Plumbagin (topical application) on pupae of *Pericallia ricini* (a) Control, (b) 50, (c) 35 and (d) 25 mg mL^{-1}

Fig. 3(a-c): Effect of Plumbagin (Topical application) on adult of *Pericallia ricini* (a) Control, (b) 10 and (c) 20 mg mL^{-1}

pupa. The larva died within the pupal case. Imperfect metamorphosis was observed (Fig. 2). Abdel Fattah A. Khalaf *et al*. (2009) observed that larval-pupal intermediates in the larvae of *Synthesiomyia nudiseta* treated with botanical volatile oils. This may be due to an imbalance in the hormone titers at critical times of moulting. This view was supported by Retnakaran *et al.* (1985). Similar observation was reported by Martinez and Van Embden (2001) in *Spodoptera littoralis* [Boisduval] treated with azadirachtin. Gandhi *et al.* (2010) reported that the extracts of plants like *Annona squamosa* [L.], *Lantana camara*, *Cleodendrum inerme*, *Cassia fistula*, *Azadirachta indica* and *Calotrophis procera* interfere with moulting process.

At lower concentrations [10 and 20 mg mL^{-1}] the larvae pupated normally and developed into apparently normal adults. But these adults had deformed wings and it was wrinkled. The front legs and antennae were not observed. The size of adult moth was smaller compared with control and also these moths were died within a few hours after moulting (Fig. 3). Application of lower concentrations resulted in the formation of adults that survived only for a few hours and therefore were not able to mate or oviposite. Similar observations were noticed with other IGRs reported by Koul *et al.* (1987). Increase in the dosage of plumbagin resulted in an interference in ecdysis and the formation of deformed larvae. These larvae were arrested from further development and reproduction. The present study suggests that topical application of plumbagin prevented normal development and metamorphosis of *P. ricini* which was manifested at different stages of the life cycle. It disturbs the endocrine mechanisms especially it affects the concentration of the hormone secretion from corpus allatum and prothoracic gland thereby it forms abnormal larvae and non-viable adults. Hence, plumbagin shows effective Insect Growth Regulative activity and exhibits great promise in suppressing field populations of *P. ricini*.

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