Growth Inhibitory Effects of Azadirachtin Against *Pericalliaricini*  
(Lepidoptera: Arctiidae)

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**Abstract:** Laboratory studies were conducted to determine the Growth inhibitory effects of Azadirachtin against the 5th larvae of *Pericalliaricini* by oral and topical methods. Both methods revealed feeding deterrence in the *P. ricini* larvae and they decreased significantly the larval growth comparing with larvae in control group. The highest antifeedant activity and larval deformities were observed in 100ppm oral treatment and 300ppm topical treatment. In case of oral treatment, the 5th larvae were not successfully ecdysed and unable to develop into pupa. At topical application, larva-pupal intermediates were observed. No moult from larvae to pupa occurred and the body was still covered by the larval exuvium. This result was indicated that Azadirachtin has antifeedant properties and it disrupts the physiological events such as apolysis and ecdysis and finally it leads to death in the larval growth. The results showed that the Azadirachtin is promising as a biopesticide against *P. ricini*, it could be an alternative for chemical pesticides.

**Key words:** Oral and Topical application · Antifeedant · Larval Deformities · Biopesticide · Larval exuvium

**INTRODUCTION**

The larva of lepidopteramoth *Pericalliaricini* 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, *Cocciniagrandis*, Brinjal, Cow pea, Yam, Sweet, Potato, Radish, Arum which is one of the main vegetable crops of Tamilnadu [1-4]. During the initial stages, the larvae feed on the lower side of the leaves and when they reach 3rd instar begin to feed voraciously and defoliate the entire plant. When the larval population was controlled, the crop yield may extend up to 70 percent to 80 percent. [5].

Pacheco *et al.* [6] reported that the continuous use of chemical pesticides for control of stored grain pests has resulted insecticide resistance, sometimes the efficacy of insecticides against storage pests varies greatly after treatment [7]. The search for alternative and environmental friendly insecticides has resulted in the rediscovery of botanicals for plant protection.

Azadirachtin (AZ), a botanical pesticide derived from the neem tree, *Azadirachta indica* A. Juss., (Meliaceae) [8], is generally considered less harmful to the environment than other commonly used pesticides. Mordue and Blackwell [9] studied the natural chemical defence of AZ by plants, affecting feeding primarily through chemoreception (deterrence) and secondarily through toxic effects. It is a tetranortriterpenoid plant limonoid that is highly oxidized with many reactive functional groups in close proximity to each other [10].

The growth regulatory effects of AZ are due to the disruption of the hormonal control of metamorphosis and moulting [11]. These effects are manifested by changes in haemolymphecdysteroid and juvenile hormone titres due to a blockage and/or delay in the release of those hormones from neurohaemal organs [12]. Evaluation of AZ against numerous species of insect pest have demonstrated neem’s diverse biological effects repellence [13-15], feeding deterrence [16], reduced growth and abnormal development [17], ovipositor deterrence [18], reduced egg laying due to sterilizing effect [19] and also direct toxicity [20]. In the present investigation, attempts were made to observe the growth inhibitory effects of Azadirachtin against the larvae of *P. ricini*.

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MATERIALS AND METHODS

The eggs and the freshly emerged 1\textsuperscript{st} instar larvae of \textit{P. ricini} were collected from the castor plants cultivated in the vicinity of Madurai and kept in the laboratory at room temperature of 29 \textdegree C and 65\%-75\% 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 lay eggs. The egg hatching was completed on the leaf itself and the freshly emerged 1\textsuperscript{st} instar larvae were collected and separated. These individuals were maintained in the laboratory for the experimental purpose.

Neemazal F\textregistered (0.5\% Azadirachtin), was used for the assays. Azadirachtin was diluted in acetone and different concentrations were prepared for oral and topical application. Newly moulted 5\textsuperscript{th} instar larvae of \textit{P. ricini} were introduced in separate containers. For oral treatment, 5\textsuperscript{th} instar larvae were fed with \textit{Ricinuscommunis} leaves soaked in different concentrations of Azadirachtinsuch as 25ppm, 50ppm, 75ppm, 100ppm. Each larva was treated topically with 100ppm, 200ppm, 300ppm. In the control treatment, larvae were treated with 1 \textmu l of acetone. For each concentration three replicates were maintained. During the developmental period (5\textsuperscript{th} instar to pupa), deformed larvae, pupa and the mortality of larvae were recorded. Per cent mortality was calculated \cite{21}.

\[
\text{Per cent larval mortality} = \frac{\text{Number of Dead larvae}}{\text{Total number of treated larvae}} \times 100
\]

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Parameters & 25ppm & 50ppm & 75ppm & 100ppm & Control \\
\hline
Number of larvae tested & 60 & 60 & 60 & 60 & 60 \\
Number of Dead larvae & 50 & 54 & 58 & 60 & 5 \\
Mortality (%) & 83.3 & 90 & 96.6 & 100 & 8.3 \\
Abbott's corrected mortality & 81.7 & 89 & 96.2 & 100 & 0 \\
\hline
\end{tabular}
\caption{Per cent larval mortality of \textit{P. ricini} after the oral treatment of Azadirachtin}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Parameters & 100ppm & 200ppm & 300ppm & Control \\
\hline
Number of larvae tested & 60 & 60 & 60 & 60 \\
Number of Dead larvae & 37 & 43 & 50 & 5 \\
Mortality (%) & 61.6 & 71.6 & 83.3 & 8.3 \\
Abbott's corrected mortality & 58.12 & 69.02 & 81.7 & 0 \\
\hline
\end{tabular}
\caption{Per cent larval mortality of \textit{P. ricini} after the topical application of Azadirachtin}
\end{table}

RESULTS AND DISCUSSION

Insect growth regulation properties of Azadirachitin are very interesting and unique in nature, since insect growth regulator works on juvenile hormone. The enzyme ecdysone plays a major role in shedding of old skin and the phenomenon is called ecdysis or moulting. When the active plant compounds enter into the body of the larvae, the activity of ecdysone is suppressed and the larva fails to moult, remaining in the larval stage and ultimately dying \cite{22}. In the present study, percent of mortality, deformed development of larvae and pupae were noted both oral and topical application. Comparative account of the larvicidal effects of two methods was summarized in table 2 and table 3. The results revealed that 100ppm in the oral treatment produced complete larval mortality (Table 1) and in the topical application, 83.3\% of mortality was observed at 300ppm concentration (Table 2). Comparing with these two methods, complete mortality was observed in oral than topical treatment. Because Azadirachtin stimulates specific ‘deterrent’ cells in chemoreceptors and also blocks the firing of ‘sugar’ receptor cells, which normally stimulate feeding \cite{9}. This result in starvation and death of these species by feeding deterrence alone. Toxicity of Azadirachtin or different neem preparations had been reported by many authors against various insect species. El-Sayed \cite{23} observed complete mortality at 0.2-0.5\% of a neem extract in the majority of larval instars of \textit{Spodopteralittoralis}.

Abbott’s corrected mortality = \[
\frac{\% \text{ mortality in treatment-} \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} \times 100
\]
Osman [24] observed some different mortalities of *Pieris brassicae* after treatment of 1-day old 5th instar larvae with 5.0 and 2.5% Azadirachtin. The neem preparation caused complete larval mortality of the European leaf roller *Archips rosanum*, within 48 h of the treatment [25]. Similarly, Ahmed et al. [26] observed the mortality of *Tribolium castaneum* was 18, 30, 52, 66 and 86% at neem extract concentration of 649.35, 974.0, 1298.7, 1623.37 and 1948.05 µg/cm² respectively after 24 h. of treatment. Ulrichs and Mewis [27] observed that a single treatment with the neem product, in concentrations of 0.01, 0.1, 0.2 and 1.0 g azadirachtin/kg rice, increased the mortality rate. Neemazal-W (10% AZ) was evaluated for mortality and mortality values of *Tribolium castaneum* adults were 22.2, 97.8 and 100% at 50, 500 and 1000 ppm after 14 days posttreatment, respectively [28]. Among the different concentration, 100ppm produced maximum percentage of deformed larvae in oral treatment and 300ppm produced deformed pupae in topical application. In oral treatment, the results on insect development revealed that the extract of Azadirachtin disrupted developmental cycle of larvae after feeding. The 5th instar larvae were not successfully ecdysed and unable to develop into pupa. The malformed larvae were shorter in length, crumpled and the hairs were found to be lost. Body fluids were disentangled from the larvae in oral treatment (Fig 1). The vast majority of the larvae entered a state of arrested development in which they stopped feeding and growing and lived for up to two days as lingering fifth instar larvae. The insects die during ecdyses unable to swallow enough air to extricate themselves from the old cuticle (Fig 2). These results are consistent with the earlier reports on various Lepidoptera species [29-32].

Mesfin et al. [33] reported that Water extracts of neem seed and leaf have significant weight reduction effect on the larvae and pupae of *Helicoverpa armigera*. Kauser and Koolman [34] proposed that the compound binds directly to ecdysteroid receptors in target tissues and also it suppresses release of prothoracicotropic hormone interferes with the normal route of metabolism of
Fig. 3: Azadirachtin topical treatment on 5th instar larvae of *Pericalliaricini*

ecdysteroids[35], or inhibits the release of eclosion hormone. Schnutterer and Singh [36] had listed 413 insect pest species as sensitive to neem extracts. These extracts have wide ranging biological activities against insects [37, 38] including feeding and oviposition deterrence [39], impairing the development [40], as well as inhibiting growth, mimicking the juvenile hormone [41].

Larvae treated topically inhibited their normal development, most of these larvae moulted into defective or malformed pupae (Fig 3). The larvae might have failed to complete its development and they were unable to emerge into normal pupa. Larva-pupal intermediates had a pupal cuticle which is usually tanned on the abdomen and on the dorsal region of the head and thorax. The remaining parts became darker when the insect was close to death. However no moult occurred and the body was still covered by the larval exuvium. None was able to emerge.

To a great extent, Similar results were obtained when last instar larvae of *Spodopteralituras*, *Spodopteramauritia*, *Ephestiapuechhella* Zell. *Bombuxmorii* and *Manducasexta* were subjected to Azadirachtin [42]. Azadirachtin applied topically in acetone to *Oncopeltusfasciatus* 5th instar nymphs show a linear, concentration dependent relationship when the various IGR effects are totalled [9]. The morphological deformities at larval and pupal stages are due to the toxic effects of Azadirachtin on growth and development processes. Since morphogenetic hormones regu late these processes, it can be suggested that Azadirachtin interfere with the hormones of the insect. Such deranged or halted program of pupation in the present study may be attributed to the absence of necessary titer of ecdysteroids needed for achieving the larval-pupal transformation normally [43]. The appearance of deformed pupae by the action of Azadirachtin may be, also, due to the alterations in ecdysteroid and juvenoidtites [44] Also, the suggestion of hormonal influence by Azadirachtin was explained by the production of malformed pupae [45].This type of delayed development and appearance of malformations were reported from Azadirachtin treated *Spodopteralituras* [46], plumbagin treated *Helicoverpaarmigera* [47] and new benzyl derivative from plumbagin against *Aachaejanata*[48].*Ghoneim et al.*[49] observed tremendously depleted larval maximal weights and body weight gain of *Spodopteralititoralis* by the treatment of 2 or 4 instar larvae with NeemAzal.A variety of disruptive developmental phenomena have been observed following administration of Azadirachtin to lepidopteran larvae including *Manducacu*[44], *Heliothis*[12], *Bombyx*[42]and *Ephestia*[50]. That in the present report, we found the regulation in growth and development by Azadirachtin might be due to the inhibition of normal larval growth and in addition to having generalized anti-feedant properties, it can disrupt the normal sequence of physiological events associated with molting such as apolysis and ecdysis, culminating in death of pharate larvae within the exuvium. Present study showed that Azadirachtin is a promising botanical insecticide for the
control of the larvae of *P. ricini* not only for causing mortality but also for disrupting the development and for causing deformities and making the larvae vulnerable to several sorts of mortality agents or prevent them from causing damage to the crop.

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**REFERENCES**


