

**NIGERIAN JOURNAL OF ENTOMOLOGY (1998) 15: 90 - 98****Influence of Azadirachtin on the Ecdysome - Controlled Developmental Processes in the Stored grain Pest, *Tribolium confusum* (Duval)****DEENA VARDHINI<sup>1</sup>, A. MARY<sup>2</sup>, S.RAJA<sup>1</sup> and P. RENUKA<sup>3</sup>**<sup>1</sup> Department of Zoology, Osmania University, Hyderabad - 7 A. P. INDIA.<sup>2</sup> St. Ann's college for Women, Hyderabad - 7 A.P. INDIA.<sup>3</sup> Department of Zoology, Government college for women, Wanaparthy A.P. INDIA.**(Accepted 17 March,1998)****ABSTRACT**

Azadirachtin, a potent antifeedant and growth regulator isolated from the neem tree *Azadirachta indica* A. Juss was tested against the stored grain pest *Tribolium confusum* (duval). Optimum growth regulating effect was observed at 1000 ppm of azadirachtin. The larval development to adults was greatly reduced. Pupal mortality and abnormally developed adults were also observed. The strong influence of azadirachtin on larval - pupa and pupal - adult moults is interpreted as an interference with moulting hormone pools, thus signifying azadirachtin's role as a potential tool in controlling the stored grain pest *T. confusum* (Duval).

**INTRODUCTION**

Azadirachtin, a tetranortriterpenoid compound isolated from seed kernels of the tropical neem tree, *Azadirachta indica* (A. Juss) (rembold, 1980) is known to act as a potent insect feeding deterrent (Pradhan et al., 1962; Butterworth and Morgan, 1968; Nakanishi, 1975), phagorepellent (Zanno et al. 1975) and as a disruptor of growth and moulting (sieber and Rembold, 1983; Gaaboud and Hayes, 1984; Garcia and Rembold, 1984; Dorn et al, 1986; Meisner et al, 1992).

The biological effect of azadirachtin, especially those interfering with moulting and metamorphosis, suggest a negative interaction of azadirachtin with the neuroendocrine system and hormones regulating these processes. It seems to have a direct

effect on the cells of the prothoracic gland in addition to those indirect effects brought about by the perturbations in the hormone levels themselves. Application of azadirachtin suppresses ecdysone titres, a major developmental hormone (Mordue Luntz) et al, 1986; Smith and Mitchell, 1988; Barnby and Klocke, 1990). These ecdysteroids are significant for the initiation of moulting, larval growth and cell differentiation during metamorphosis. Therefore any disruption of ecdysteroid titre would induce profound developmental changes.

In view of the developmental derangements caused by azadirachtin, its effect on the morphogenesis of the stored grain pest, *Tribolium confusum* Duval (Coleoptera : Tenebrionidae) was investigated.

## **MATERIALS AND METHODS**

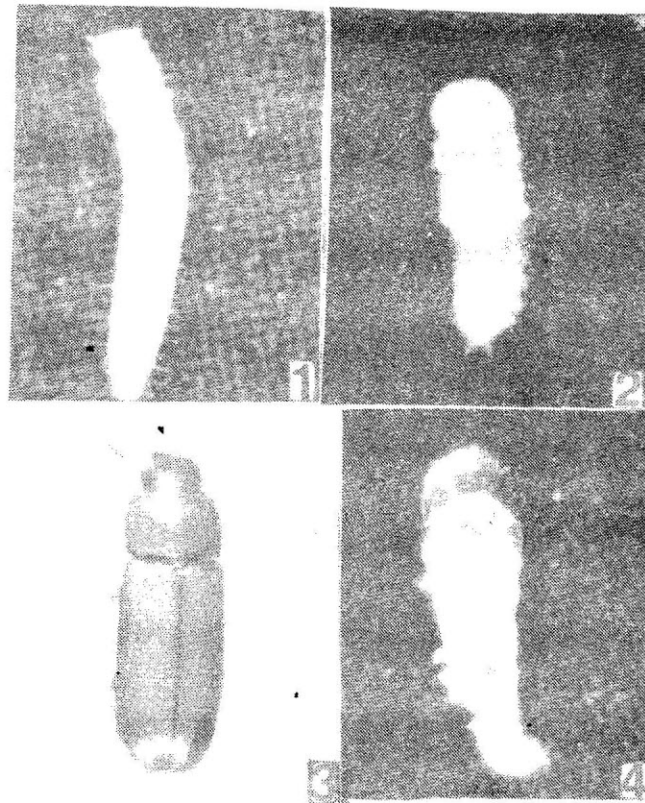
*T. confusum* was reared on mixed flour of wheat and sorghum (1 : 1) at a temperature of 27 +/- 1 deg. C and RH 65 +/- 5%. Freshly moulted 3rd, 4th, 5th and 6th instars were treated topically on the abdominal region with 1 ul of different concentrations of azadirachtin ( 100 ppm 500 ppm, 2000 ppm, 5000 ppm) dissolved in acetone, using the Hamilton microsyringe. Thirty larvae were treated each time and the experiments were replicated five times. Controls were treated with equivalent volume of acetone. A period of 2-3 minutes was given, for the complete absorption of azadirachtin before transferring the treated insects into the diet. The treated insects were observed daily for morphogenetic changes and the abnormal insects were collected from the die media.

## **RESULTS**

At 1000 ppm, azadirachtin showed optimum effect on the 6th instar larvae and on the zero hour pupae of *Tribolium confusum*. At 100 ppm and 200 ppm concentrations, azadirachtin did not show any drastic morphogenetic changes. At higher concentrations i.e., 2000 ppm and 5000 ppm the larvae died within few hours of treatment.

**Effect of azadirachtin on the 6th instars:**

(1) Forty percent of the treated larvae failed to moult. The treated larvae attempted to shed the skin which led to a rupture along the mid-line of the head capsule and pronotum and over the meso and metathorax. They tried to cast off their exuviae, but succeeded only partially (Fig. 4).



(2) Forty percent of the treated 6th instar larvae metamorphosed into Larval - pupal intermediates with deformities such as malformed wing Pads and contracted posterior body (Fig. 5 and 6). All the treated resultant forms exhibited cuticular melanization characterized by black spots and darkening of the cuticle.

(3) Twenty percent of the treated larvae remained as over - aged larvae for an extended period of time. They are known as "permanent larvae". They survived for several weeks and eventually died. (table 1.)

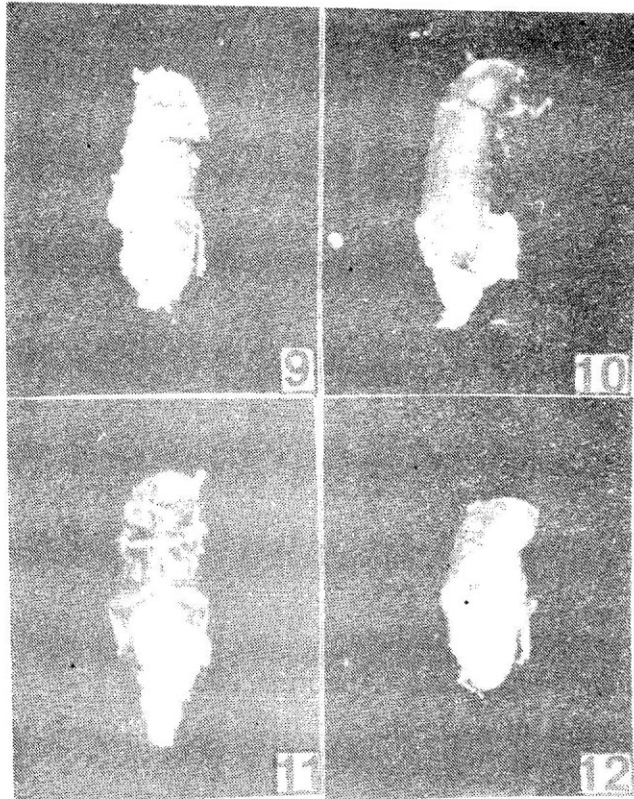
Table 1 : Effect of azadirachtin on the vi instar larvae and pupae of *Tribolium confusum* (Duval)

Instar and Age in days	No. of Insects	Dose in ppm	Days after treatment	Mortality occurring As: Ecdysis inhibition
VI	30	Control	3	0
VI	30	1000	2-3	12
			7	12 -L.P.I. 6 - P.L.
Pupae	30	Control	6-7	0
Pupae	30	1000	8	9
				12-A.A 9-P.A.I.

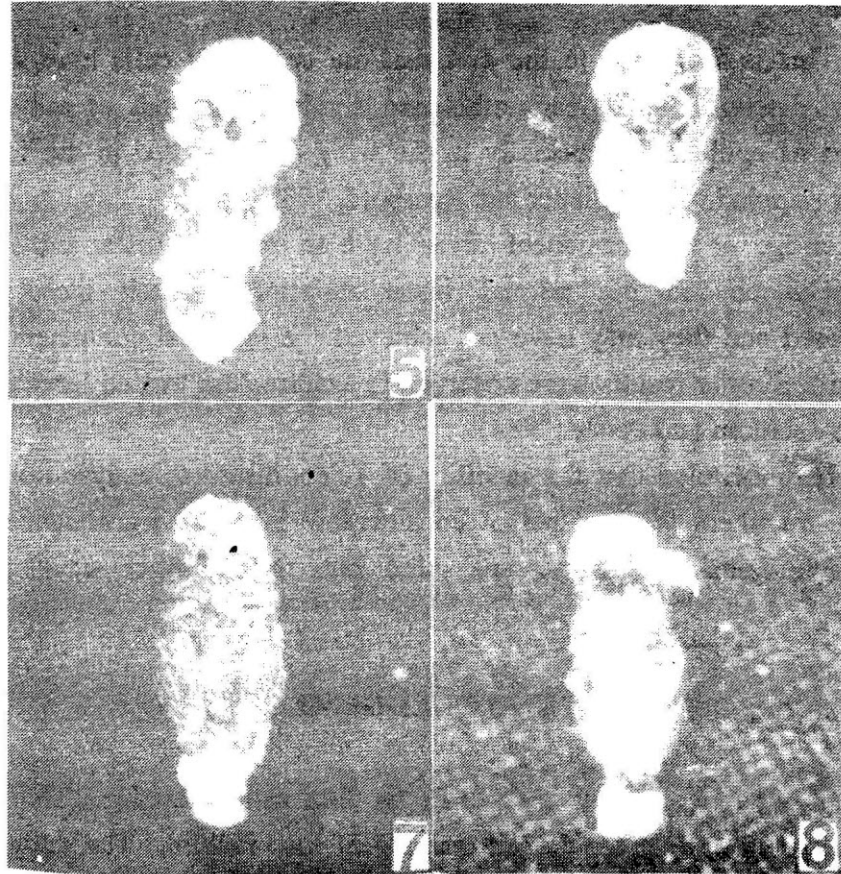
- L.P.I. - Larval - pupal Intermediate;
- P.L. - Permanent larvae; S.A. - Abnormal adults;
- P.A.L. - Pupal - adult Intermediate

Effect of azadirachtin on the pupae:

- 1) Thirty percent of the pupae did not eclose into adults, but died within the exuviae.
- 2) Twenty percent of the treated pupae moulted into abnormal adults which showed deformed wings and asymmetric bodies. These forms died within few hours (fig. 7).
- 3) Thirty percent of the treated pupae developed into pupal - adult Intermediates and were characterized by specimens which developed within the pupal case, but could not shed it completely. In some cases, these forms were able to free their legs and Mouthparts partly so that feeding and walking were possible to a Limited extent. These crippled forms lives only for a few days (Fig.8).



(4) In twenty percent of the treated pupa the resultant adults were deformed. The exuvium was seen attached to the anal region preventing the insect from defaecation, mating and oviposition. The exuvium was also seen attached to the anterior region, Preventing the insect from feeding. In their attempts to shed off the exuvium, the mouth parts were lost (Figs. 9, 10, 11 and 12). Table 1.)



### DISCUSSION

The results suggest azadirachtin's role as a potent growth regulator which can effect the endocrine mechanisms that regulate moulting and metamorphosis. Inhibition of moult occurred as a major consequence after treatment with azadirachtin, in various stages of *T. confusum*. The treated insects failed to cast off their exuviae. These individuals are not viable since they were prevented from the feeding defaecation. The observations confirm reports by Redfern et al. (1982). And Schluter et al (1985).

Cuticular melanization of the treated 6th instar larvae of *T. confusum* was noticed which resulted in black spots. This may be due to the low levels of ecdysone (Hori et al. 1984). Some of the treated 6th instar larvae failed to pupate because the epidermis is a major target organ for the moulting hormone, ecdysone (Riddiford, 1976). This regeneration of the epidermis leads to the inability of the insect to secrete a new cuticle, thereby preventing moulting. In a few of the azadirachtin treated 6th instar larvae, cuticle was attached to several regions of the body, severely disturbing

moulting. This is attributed to the fact that the epidermal cells may start mitosis normally but disconnect from each other and divide due to lack of ecdysone. This degeneration of epithelium especially in the wing disc resulted in the formation of deformed or crippled wings (Sieber and Rembold, 1983).

The 'over-age' or the 'permanent larvae' have an extended instar and survived for several weeks beyond the normal instar duration. Such insect have not metamorphosed and they may have not achieved the imaginal competence for adult development. Similar results were observed in azadirachtin treated larvae of *Locusta migratoria* by shalom and Pener (1984).

It can be concluded that the inability of *T. confusum* to ecdyse normally after azadirachtin treatment is an effect of considerable practical significance, since the insects which failed to ecdyse are ruled out from further development and reproduction.

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**Legend to figures**

- FIG. 1 : Control Larva
- FIG. 2 : Control Pupa
- FIG. 3 : Control Adult
- FIG. 4 : Treated Larva Partially pupated
- FIGS. 5 & 6 : Larval - pupal intermediates
- FIG. 7 : Abnormal adults with deformed wings and assymmetric bodies.
- FIG. 8 : Pupal - adult intermediates
- FIGS. 9,10,11,12 : Abnormal adults with exuviae attached to the cephalic and abdominal regions.