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Pesticidal effects of *Clerodendron infortunatum* on the fat body of *Oryctes rhinoceros* (Linn.) male

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ABSTRACT

The *Clerodendron infortunatum* leaf powder [5,10 and 20% (w/w)] was thoroughly mixed with cow dung and fed to third instar grubs of *Oryctes rhinoceros* and grub and pupal mortatly, deformalities and fat body of adults in relation to reproduction was studied. Total mortality was 70%, 30% and 10% while *O. rhinoceros* reared with leaf powder and cowdung mixture 20%, 10% and 5%. At 5% leaf powder and cowdung, exhibited maximum survival rate, and this particular dosage was employed for rearing adult *O. rhinoceros* for studying the effect *of C. infortunatum* as a pesticide on the fat body of male coconut beetle *O. rhinoceros* in relation to reproduction. Observations made on fat body and testis of insect reared on plant powder and cow dung medium revealed certain morphological changes like enlarged lobes in parietal fat body, failure to form lobes in visceral fat body and reduction in size of the testis. Histological changes like disintegration of cell membrane and shrunken and scattered nucleus in the fat body cells, reduction in number and size of spermatids in testis was also observed.

Key words: *Clerodendron infortunatum*, *Oryctes rhinoceros*, fat body, testis, pesticide,morphology, histology.

INTRODUCTION

Oryctes rhinoceros or commonly known as rhinoceros beetle has caused major problems to plantations in many parts of Peninsula India and other Asian countries. The adult beetle bores into the unopened fronds and inflorescence of the palm. Decaying organic matter and cowdung are the breeding sites of adult beetle. Treatment of breeding sites with insecticides is one of the usually seen methods to control this pest. This control method is problematic not only because of resistance of insecticides by the grubs of this insect pest but also because of the negative effect of pesticides on the environment. Botanical products are useful tools in many pest management programmes because they are effective and specifically target plant's natural enemies (Isman, 2006). Plant extracts are capable of producing multiple effects in insects such as antifeedancy, growth regulation fecundity suppression, sterilization, ovipositional changes, repellency and change in the biological fitness like reduced life span, loss of flying ability,low absorption of nutrients, high mortality, immuno depression, enzyme inhibition and disruption of biological synthesis (Samidurai *et al.*, 2009).

Botanicals are reported to be safer than synthetic insecticides. They are easily degradable, environmentally safe , have broad spectrum in action, non -persistent and easily processed (Solsoloy and Solsoloy, 1995; Talukadar and Howse, 1995) The botanicals offer desirable alternatives to synthetic chemicals in the agro-ecosystem where protection of the environment and preservation of beneficial organism are important (Weathersbee and Tang , 2002). More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programme (Ahmed *et al.,* 1984; Emara *et al.,* 2002; Talukader, 2006). The present investigation aims to find out the possibility of eco friendly management of the pest *Oryctes rhinoceros* by incorporating a commonly seen weed *Clerodendron infortunatum* in its culture medium. Previously it was observed that *Clerodendron* spp. have insecticidal activity against various pests (Ahmed *et al*. 1981; Roychoudhary, 1994; Panday *et al*., 2005.). Furthermore, the impact of *Clerodendron infortunatum* against *Oryctes rhinoceros* (Chandrika and Nair, 2000; Sreelatha and Geetha, 2010), and *Helopeltis theivora* Waterhouse (Somnath Roy *et al.,* 2009) were also available in the literature. However, no one has studied the impact of *Clerodendron infortunatum* on adult fat body of *O. rhinoceros.* The investigator has made an attempt to study the pesticidal effect of *Clerodendron infortunatum* on the fat body and testis of *O. rhinoceros* .

MATERIALS AND METHODS

Third instar grubs of *O. rhinoceros* were collected from local manure pits and reared in the laboratory conditions (23 ºC – 33 ºC temperature and relative humidity 70% - 90%). Adult males emerged from the laboratory were used for the present study.

Preparation of culture medium

Tender stem and leaves of *C. infortunatum* were collected from our college campus. The plants were shade dried for one hour and chopped into small pieces and mixed with sun dried cow dung in three different proportions. Cow dung with out leaf powder served as control.

Bioassay

The plant powder [5%, 10% and 20 % (w/w)] was thoroughly mixed with cow dung. Late third instar grubs were introduced individually into small containers (10 x 5 cm) with 125g cow dung plant mixture. The containers were covered with plastic lids with holes for proper aeration. The culture medium was changed every week till the grubs became pupa. Twenty grubs were used for a treatment with each dose of leaf cow dung medium. Daily observations were made on the development of grubs. Larval and pupal mortality and formation of any abnormal adults were recorded. Adult eclosion date was noted on the container in order to identify the age. For adults, as 5% leaf powder cow dung medium shows minimum mortality, it was selected for rearing insects for morphological and histological studies. Adult beetles were kept in plastic containers (5 x 7 cm) covered with perforated lids and fed daily on slices of banana (30g/insect). The adults who attain sexual maturity in 35 days (Mini 1993, Sreelatha, 2008) were sacrificed to study the morphological and histological aspects of fat body and testis. Beetles reared in cow dung without leaf powder form the control group.

Morphological and Histological studies

Experimental and control insects were anaesthetized by cooling in a freezer (4 ºC) and dissected under binocular dissection micro scope. Thorax and abdomen were opened and the fat body lying beneath the body wall and around the gut was taken out, after severing it from reproductive organs. Similarly testis lobes were taken out. Microscopic observation of fresh unfixed fat body and testis was done. Photographs were taken using Kodak M1033 Digital camera. The fat body and testis surrounding the reproductive organ were removed from 35 day old beetles, fixed overnight in aqueous Bouin's fluid and processed for histological studies (Gurr, 1962). Paraffin blocks were made. Sections were cut at 5? and stained in Ehrlich's Hematoxylene and Eosin, mounted in DPX and examined under the light microscope and photographs were taken using digital camera.

RESULTS AND DISCUSSION

The experiment with grubs shows that the *C. infortunatum* can induce larval and pupal mortality and deformed adult formation in a dose dependent manner. The larval mortality was 20 % in 20 % leaf powder mixture and 5 % in 10% leaf powder mixture, while no mortality was observed in 5% leaf powder medium and control. The pupal mortality was 10 % in 20 % and 10% leaf powder mixture, while 5% in 5% leaf powder medium. However, no pupal mortality was observed in the control. The total mortality was 30 % in 20 % leaf powder medium and 15 % in 10 % leaf powder medium, but only 5 % in 5 % leaf powder medium. Percentage of deformed adult was 5% in 20% and 10 % leaf powder medium, while no deformed adults were formed in 5% leaf powder medium and control. In 20% leaf powder medium 35% of the beetles were affected either by mortality of larva or pupa or emergence of abnormal adults. The percentage of beetles thus affected was 20 % in 10 % leaf powder medium and 5% in 5% leaf powder medium. Neither mortality nor deformed adult formation was observed in control. Maximum mortality was observed in 20 % *C. infortunatum* cow dung medium followed by 10 % and 5 % mediums as observed by Yankanchi and Gadache (2010).

The fat body in an adult beetle is differentiated into a peripheral portion located underneath the body wall termed the parietal fat body and a central mass which exist as a compact mesh work of anastomotic mass in the space between body wall and gut referred to here as visceral fat body. The parietal fat body is seen composed of lobes attached by strands to main lateral trachea (Fig.1a). There are two types of lobes present in parietal fat body. One is short and restricted to the peripheral region of the body and the second type is long and multi lobular in young adult, which gradually turns to a single lobe as the reproductive organs undergo maturation. These are seen attached by one end to trachea and by the other end to the gut or reproductive organ through long strands. As soon as the reproductive organ attains maturity the posterior region of gut is seen surrounded by closely packed globular lobes connected by thin strands to gut and reproductive organ. These are visceral fat body lobes which are seen to be arising from the white colored ducts lying close to the gut wall (Fig. 1c). The new lobe formation of this type occurs in visceral fat body, but never occurs in parietal fat body after adult emergence. The lobes from both parietal and visceral fat body of a 35 day old beetle, are seen empty and porous, at which time the reproductive organ attains full development.

In the present experiment fat body in the control exhibited the same pattern as that of normal ones. Fat body of the treated beetle exhibited significant differences from the control. In the treated one the parietal fat body lobes were unusually enlarged and highly porous. There was also a considerable reduction in the number of fat body lobes and hence the lateral trachea was seen exposed among the lobes of fat body (Fig.1b).This abnormal enlargement of lobules of parietal fat body and reduction in the number of lobes in treated beetle may be due to the impact of leaf powder.

The visceral fat body of treated beetle showed a reduction in lobe formation from white colored duct lying close to the gut. Due to the partial failure of lobe formation, the gut was seen surrounded by poorly developed fat body (Fig. 1d). Lengthy white colored ducts remain as such, devoid of lobes. The visceral lobe of control was seen to be empty of their contents, while the one in treated, due to failure of lobe formation, was seen to be filled with secretion. In an adult beetle the essential parts of reproductive system include a pair of testis and accessory gland seminal vesicle bundle. Each testis is formed

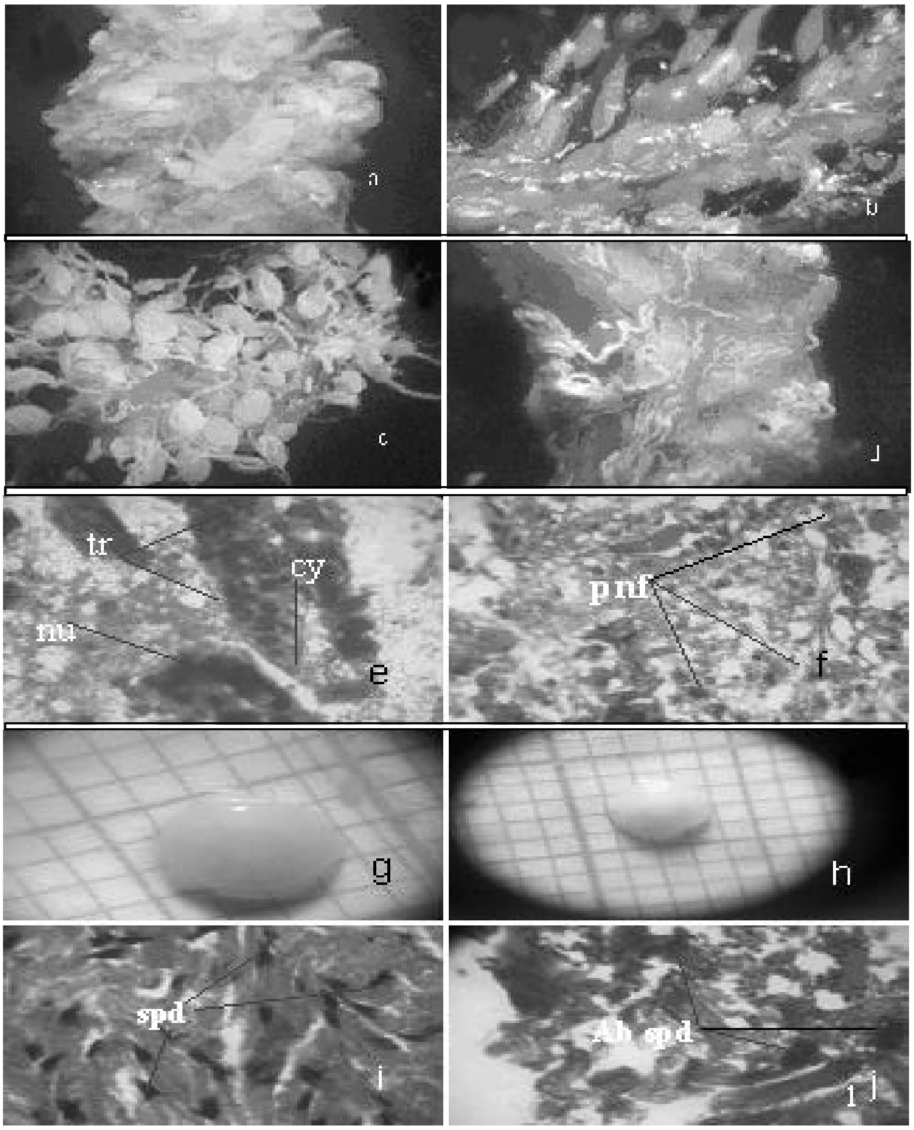

Figure 1. Morphology of partial fat body (a,b), visceral fat body (c,d), fat body lobe (e,f) testis (g,h), and L .S of testis (j, h) of *Oryctes rhinoceros*. Control partial fat body showing normal lobes (a), plant treated showing enlarged lobes (b), control visceral fat body showing fully developed lobes (c), plant treated visceral fat body showing underdeveloped lobes (d), section of fat body lobe (Hematoxylene Eosin - X450) showing definitely arranged large nucleated cells (e), treated fat body lobe showing scattered and shrunken nucleated cells. Control with normal size testis (Hematoxylene Eosin X450) (f), plant treatment reduced size (g), control showing normally developed spermatids (i) and plant treated showing reduced and shrunken spermatids (j). abspd - abnormal spermatid, cy- cytoplasm, nu – nucleus, pnfc – pycnotic and necrotic fat body cell, spd - spermatid, tr - trophocytes

of six bun shaped lobes of almost equal size having wavy margin. The reproductive organ is characterized by the rich supply of lobes from peripheral fat body and visceral fat body. The testis in the treated beetle was comparatively smaller than that in the control (Fig. 1g and 1h). The weight of the testis in control and treated insect was 115.88±1.84 and 63.75±2.05 mg/animal respectively. Thus a significant difference of ($t=18.93$, $P < 0.0001$) was observed in the testis weight of control and plant treated one. The fat body connections to the reproductive organ were found to be highly reduced. This may be due to the failure of lobule formation in the visceral fat body.

Histological observations showed that the parietal fat body and visceral fat body were charecterised by the presence of a single or double layer of trophocyte, the major component of fat body cells, which appear like a mass of cells arranged as lobular mesh. These cells have definite cell boundaries and large nucleus (Fig. 1e), while in treated beetle the boundaries of fat body cells, were found disintegrated and nuclei seen scattered and shrunken (Fig. 1f). A similar histology has been reported in species such as *Glossina messilatus, schistocerca gregaria*, *Deplotera punctata* and *Bombyx mori*. *(Centhilnayaki et al.,* 2003*)*. Disintegration of cell membranes and shrunken and pycnotic nucleus in treated insects suggest the effect of *clerodendron* as a pesticide.

The histology of the testis of control showed a definite arrangement of well developed spermatid bunches (Fig. 1i). But in treated beetle, the orderly arrangement of spermatids was lost and the number and the size were seen very much reduced in comparison with those in control (Fig. 1j). Reduction in the size of the testis and scattered and shrunken spermatids in the testis of treated insects again proved the efficiency of *Clerodendron* as a phytopesticide. Arrested spermatogenic meiosis in Azadiractin treated Desert locust *Schistocerca gregaria* observed by Linton *et al*. (1997) support the present study. The present investigation is further complimented by the results of Nazia and Pandey (2009) that the oral administration of alcoholic extract of *Clerodendron sithonanthus* leaf extract at different doses have shown a significant decrease in the weight of testis and accessory reproductive structures in albino rats. It is concluded that clerodendron powder causes drastic changes like reduction in the lobes of fat body and their derangement together with the disintegration of cell membrane, shrunken and scattered nucleus and anti androgenic effect like loss of weight of testis and reduction in the size as well. Reduction in the spermatid formation in *Oryctes rhinoceros* may be due to the toxic effects of *Clerodendron infortunatum* .

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