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Laboratory evaluation of insecticidal activity of *Adathoda vasica* (Acanthaceae) and *Glyricidia maculata* (Leguminosae) on the third instar larvae of *Oryctes rhinoceros* L.(Coleoptera: Scarabaeidae)

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ABSTRACT

The study evaluates the effect of feeding the third instar larvae of the coconut rhinoceros beetle, *Oryctes rhinoceros* L.(Coleoptera: Scarabaeidae) with leaf powders of *Adathoda vasica* (Acanthaceae) and *Glyricidia maculata* (Leguminosae).Control larvae were fed on sterilized and powdered cow dung while the experimental larvae were fed on a mixture of cow dung and leaf powder. Concentration of the leaf powder in the mixture was 1%. Both plants induced mortality in the larvae, prepupae, pupae and adults. Higher rate of mortality of the larvae and prepupae was induced by *G.maculata* while higher pupal and adult mortality rate was noticed in *A.vasica* fed group. Adults emerged from the larvae fed on the experimental diets revealed morphological deformities. Deformities were greater in the Gly*ricidia* fed group. Malformations were more in the wings and legs. Exoskeletal deformities, distorted and prolapsed wings, vertically split elytra, melanization of the cuticle and wings and oozing out of haemolymph are the major deformities observed in the study. Fat body and ovarioles of a few experimental female beetles showed some abnormalities. Both plants are promising for the control of *O. rhinoceros* by causing mortality, disrupting development and also by causing deformities. The deformities observed in the study can result in abnormalities in vital activities like walking, flying and reproduction. The results direct towards the potential of the experimental diets to induce developmental deformities, metabolic abnormalities and reproductive aberrations in *O.rhinoceros*

Key words: *Oryctes rhinoceros, Adathoda vasica, Glyricidia maculata* , mortality, morphological deformity, ovary, fat body

INTRODUCTION

Adult rhinoceros beetle, *Oryctes rhinoceros* L. is an important pest of the coconut trees. Coconut palms are grown in more than 80 countries of the world; traditional areas of coconut cultivation in India are the states of Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Orissa, West Bengal, Maharashtra and the islands of Lakshadweep , Andaman and Nikobar. Adult *Oryctes rhinoceros* burrows and attacks the deeper, soft parts of the crown of coconut trees and results in stunted growth of the plants. The affected leaves when fully open exhibit characteristic geometric cuts. Nut production decreases leading to low yield. Decreased nut production brings about great economic loss to the farmers. Hence this pest has an important status in Economic Entomology.

Adathoda vasica belongs to the family Acanthaceae. Medicinal properties of *A.vasica* have been known in India and several other countries for thousands of years. *A. vasica* is an ayurvedic medicine which is mostly recommended for a variety of ailments such as bronchitis, asthma, fever, jaundice and leprosy in traditional healthcare and Ayurvedic systems (Seema *et al*., 2010). In addition it has been used for centuries in India as an insecticide and has proved itself as insect repellent (Kokate *et al*., 1985). It is vernacularly known as Malabar Nut, Adulsa, Arusha, Vasaka, Justicia adathoda, Adathodai, Bakash, Adathoda, Adalodakam, Adusoge, Addasaramu, Lion's Muzzle, and Stallion's Tooth. It is known as Adathoda in Tamil and Adalodakam in Malayalam and is a small evergreen and sub-herbaceous bush which is commonly found in India, Sri Lanka, Myanmar and Malaysia.

Glyricidia maculata is a tree from Mexico and South America that belongs to the Leguminosae family. The plant has the common name Mexican lilac, in Tamil Seemai agathi, in Malayalam Seema konna, in Kannada Gobbarda Mara and in Telugu Seema kanuga.It is a small to medium-sized, thorn less tree. *G. maculata* is often cultivated as an intercrop in coconut gardens and its leaves are used as green manure. It grows well in marginal and sub-marginal soils and successfully thrives as an intercrop in sandy soils where no green manure can thrive. It is a quick growing; hardy, resistant plant that can be grown along the borders and between rows of coconut palms. Addition of Glyricidia leaves in the soil helps in meeting a major portion of nitrogen, and a part of phosphorus and potassium needs of the coconut trees. The leaves decompose easily within 2-3 weeks after mixing with the soil thereby providing nutrients relatively quicker.

Botanical insecticides are one of the best alternatives for the hazardous chemical insecticides. Phytochemicals are able to induce different types of abnormalities in insects which could safely be used for insect pest control. Aqueous extracts of *Clerodendrum viscosum* reduced the population as well as infestation of two major pests of tea, *Camellia sinensis* (L) and *Helopeltis theivora* (Roy *et al.*, 2010). Prolongation of larval and pupal period and reduction in the percentage of adult emergence were observed in the larvae of *Culex quinquefasciatus* which were treated with *Momordica tuberose* leaf extract (Sethuraman *et al.*, 2009). *Spodoptera litura* larvae fed on castor leaves treated with *Porteresia coarctata* leaf extract showed significant larval mortality and reduction in protein and DNA content in the fat body and midgut tissues (Ulrichs *et al.*, 2000). Leaf extracts of *Vernonia cinerea* cause reduction in population and larval mortality in *Culex quinquefasciatus* (Arivoli *et al.*, 2011). Sahayaraj and Kombiah (2010) suggested that neem gold could be used as insecticide against the banana rhizome weevil, *Cosmopolites sordidus* because neem gold reduced the total and differential counts of haemocytes and induced the appearance of five new polypeptide bands among the total body protein profile of *S.litura* in SDS PAGE studies.

Research for assessing the insecticidal properties possessed by plants is going on all over the world. It is realized that disruption of growth and reproduction are the main characteristics of pest control rather than antifeedancy and repellency (Mordue (Luntz) and Nisbet, 2000). The current study was undertaken to test the insecticidal property of two common plants, *Adathoda vasica* and *Glyricidia maculata* with a view to control the coconut pest, *Oryctes rhinoceros* using these plants. Hence in the study the effects of feeding the third instar larvae of *O.rhinoceros* with the leaves of *A. vasica* and *G. maculata* were evaluated.

MATERIALS AND METHODS

Preparation of leaf powder

Fresh and mature leaves of *A.vasica* and *G.maculata* were dried under shade separately. The dried leaves were finely powdered separately using domestic grinder. The leaf powder was used to prepare the experimental feed.

Rearing

Advanced third instar larvae of *Oryctes rhinoceros* used in the study were collected from dung pits in the locality. Colony of larvae, prepupae, pupae and adults were maintained in the laboratory at an ambient temperature of 23 to 33 ^{o}C and 75 to 95% relative humidity. A single larva was kept in a glass jar (18 cm high 9 cm diameter) closed with a lid with perforations for adequate aeration. Before introducing the larva, the container was filled ¾ its volume with the larval feed. Experimental larvae were fed on a mixture of the corresponding leaf powder mixed with sterilized (steamed in a pressure cooker for 1 hour), cooled and powdered cow dung. Concentration of the leaf powder in the mixture was 1% (2.5 g leaf powder mixed with 247.5 g sterilized cow dung). To test the effect of each plant feed, three sets of larvae, each set consisting of ten larvae were used. All controls were reared in sterilized cow dung which does not contain the leaf powder. The larval feed was renewed every seven days until prepupation after which the cow dung was not renewed. Experiment and control insects were maintained in similar laboratory conditions. Beetles emerged in the laboratory were kept individually in glass jars and were fed on slices of ripe banana (40 g/beetle); the feed was renewed on alternate days. Date of adult emergence was noted on the glass jar to identify the age. Observations on the control and experimental insects were done on a daily basis to monitor survival. Laboratory emerged adults were observed for 35 days to record mortality or deformities.

Studies on survival and morphology

Effect of feeding the experimental diets on the survival and morphology of the insects were studied until the adults that emerged from the treated larvae became 35 days old. Larval, pre pupal, pupal and adult mortality was recorded. Percentage of mortality was calculated and presented as mean ± SD. Details of morphological abnormalities were recorded and relevant photographs were taken using a digital camera (Canon-A 3100).

Studies on the ovary

Female *O. rhinoceros* reaches sexual maturity in 35 days (Sreelatha, 2008). Thirty five day old control and experimental adult females were dissected in cold insect Ringer solution under binocular dissection microscope. Studies on the morphology of ovaries and the nature of fat body covering the ovaries were made; photographs were taken using digital camera.

RESULTS AND DISCUSSION

Survival of the experimental larvae, pre pupae, pupae and adults was decreased as compared to the corresponding controls. Mortality resulted in all the four life stages by both the experimental diets.*Glyricidia maculata* produced 33.3±5.77% and *A.vasica* induced 23.33 ± 5.7 % larval mortality. Higher pre pupal mortality of 30.14 ± 2.73 % was resulted by *G.maculata* against 21.4 ± 5.09 % in the pre pupae developed from *A.vasica* fed larvae. 27.73 ± 7.87 % pupae and 38.33 ± 12 % adults emerged from *A.vasica* fed larvae died. Comparatively lower pupal mortality of 21.66 ± 2.88 % and adult mortality of 27.76 ± 4.79 % was resulted in the *G.maculata* group.Comparing the two experimental diets, *G.maculata* was more effective in inducing larval and pre pupal mortality. On the other hand, higher rate of mortality of pupae and adults was induced by *A.vasica*. Mortality observed in the present study in *O. rhinoceros* can be connected with abnormal and imbalanced action of moulting hormones competent enough to mess up normal metamorphosis due to the consumption of the experimental diet.

Insecticidal and defective metamorphic efficiency of plants had been confirmed in *Epilachna varivestis* by *Azadirachta indica* (Rembold *et al.*, 1980); in *Spodoptera littoralis by Adathoda vasica* (Sadek, 2003); in *Trogoderma granarium* by *Annona squamosa* (Rao *et al*., 2005)and in *Schistocera gregaria* by *Solanum sodomaeum* (Zouiten *et al.*, 2006). Arivoli and Tennyson (2011) reported arrested larval and pupal development, decreased pupal transformation, prolonged larval and pupal periods,delayed adult emergence in addition to larval-pupal and pupal-adult intermediates in *Aedes aegypti*, *Anopheles stephensi* and*Culex quinquefasciatus* treated with leaf extracts of *Abutilon indicum*. Jbilou *et al.* (2008) observed high rate of mortality and retarded growth in the larvae of *Tribolium castaneum* reared on feed treated with the extracts of *Centaurium erythraea* and *Peganum harmala*.

In the current study morphology of the experimental larvae, prepupae and pupae was not affected. But compared to the controls (Fig.1) adults emerged from the experimental larvae showed diverse morphological deformities which indicate abnormal pupal-adult transformations (Fig. 2, 3). Beetles that emerged from *G. maculata* fed larvae showed vestigial wings with disproportionate size. The present results are supported by similar findings. *Spodoptera litura* larvae fed on *Adathoda vasica* interfered with normal development and metamorphosis, caused larval and pupal mortality, produced moulting abnormalities and resulted in nymph-adult intermediates and adult deformities (Sujatha *et al*.,2010). Jagajothi and Martin (2010) report pupal mortality, deformities in adults, disruption of metamorphosis and pupal adult intermediates in *Corcyra cephalonica* pupae applied with andrographolide (a commercial terpenoid extracted from *Andrographis paniculata)*. According to them the application of andrographolide mimics the juvenile hormone which appears to cause disruption of metamorphic processes leading to mortality of pupae, delayed pupal – adult transformation and structural abnormalities. The mortality and developmental deformities observed in the present study in *O. rhinoceros* can be connected with disrupted endocrine events as suggested by Martinez and van Emden (2001) in *S. littoralis* by azadirachtin. Azadirachtin affects the neurosecretory system and is more likely to kill the insect by disturbing the ecdysteroid regulation.According to Nijhout (1994) during pupation and the pupa-adult moult, insects undergo transformations that involve complex neuroendocrine processes that do not occur at the same intensity during larva to larva moults. This can be the reason for the appearance of malformations of various degrees in the adult stage of *O.rhinoceros* as compared to larval, pre pupal and pupal stages.

Fig. 1-4. Control (1) and experimental beetle, *G.maculata* (2,3,4)

In the control beetles, surface of the elytra had a smooth appearance (Fig.1) while in the experimental beetles of the *Glyricidia* group, the elytra were rough, had several small depressions, had a crumbled appearance and were split vertically so that the abdomen was not completely covered. Wings prolapsed out from the wing case (Fig. 3,4) and many of them showed different patterns of cuticle colour and spots (Fig.4) which can be suggested as a result of low levels of moulting hormones. Such cuticular spots were also formed in *Spodoptera litura* larvae treated with azadirachtin (Koul *et al.*, 1995) and were associated with low levels of ecdysteroids and juvenile hormone (Hori *et al.*, 1984).

Wings in the control are light coloured, delicate and with the characteristic pattern of venation (Fig.5) while in the experimental beetles the wings were distorted, shriveled, strongly melanized and the venation was vague (Fig.6).

Compared to the controls the distensibility of the wings was also less. Most of the experimental beetles had a lethargic

Fig. 5-9. Control *G.maculata* showing normal wings (5), experimental beetle with abnormal wings, legs, ovary and fat body (6-7), experimental beetle, *A.vasica* (8,9). Arrows indicate membranous cuticle (Fig. 8) *vasica* (8,9). Arrowndicate membranous cuticle (Fig. 8)

appearance and exhibited sluggish movements denoting abnormalities in vital activities. A few experimental beetles had nonfunctional and immobile legs (Fig.6,7). Malformations observed in the experimental beetles were more pronounced in the wings and legs.The reason for this can be due to impaired physiological activity as advocated by Martinez and van Emden (2001) in *Spodoptera littoralis* fed on azadirachtin. According to them, the most common pupal defect, in S.*littoralis*, was deformed or swollen wings. Koul (1984) observed prolonged development, wing deformities, unplasticisation of wing lobes, development of wingless adults and larval mortality in the red cotton bug, *Dysdercus koenigii* by azadirachtin. Similarly deformities of the front legs, head appendages and deformed wings produced by azadirachtin in *Spodoptera litura* (Rao and Subrahmanyam, 1987), *Manduca sexta* (Schluter *et al.*,1985) and in *Schistocera gregaria* (Nicol *et al.*, 1995) were also discussed as physiological impairment.

Beetles formed from the larvae fed on *A.vasica* in the current study had incomplete inter segmental membranes in the thoracic and abdominal regions. On the ventral side of the abdomen of many experimental beetles, the cuticle was not completely differentiated and hence these regions appear membraneous. Membrane between the leg segments also showed pores. As a result, haemolymph oozed out from the legs, thorax and abdomen (Fig.8, 9). Martinez and van Emden (2001) observed very thin and ruptured cuticle which allowed the leakage of haemolymph from the body of the pupae of *S. littoralis* emerged from larvae reared on azadirachtin.Topical application of apholate caused haematological changes and reduced haemolymph volume in the adults of *Dysdercus koenigi* (Bhargawa and Pillai, 1976).According to Keeley (1978),haemolymph is the metabolic centre of insects. Hence leakage of haemolymph observed in the current study could result in serious metabolic disorders which had a major role in the developmental difficulties noticed in *O. rhinoceros.*

In *O. rhinoceros* there are two ovaries one on each side of the posterior part of the abdomen. Each ovary is formed of six ovarioles and each ovariole of 30-35 day beetle has 5-7 oocytes. Successive oocytes are connected together by interfollicular tissue (Fig.10) as reported by Sreelatha and Geetha (2008, 2010). In some experimental beetles (of both groups), the interfollicular tissue was not developed and so the eggs were arranged one above the other without any tissue in between (Fig.12).Reproductive organs of adult male (Sreelatha and Geetha, 2011) and female *O.rhinoceros* are richly supplied with fat body.In female *O. rhinoceros* the ovarioles were completely hidden from view so that and the oocytes were seen only when the fat body mass was removed. The fat body makes very strong and intimate connections with the ovarioles through very fine tubules (Sreelatha, 2008). Similar condition was seen in the control female beetles of the present study (Fig.11) while in both experimental groups fat body mass was very much reduced so that many oocytes were exposed (Fig.6, Fig.12).Reduced fat body supply to the testis of *O.rhinoceros* emerged from larvae fed on leaves of *Clerodendron infortunatum* was observed by Sreelatha and Geetha (2011).Insect fat body is a multifunctional tissue and is the centre of metabolic activities. It is the principal tissue for intermediary metabolism and is comparable to the vertebrate liver since both tissues store excess nutrients, detoxify foreign chemicals and acts as the biosynthetic sources for circulating metabolites (Keeley, 1978).Hence the reduction in the fat body observed in the present study can hamper normal metabolism.

Abnormal wings and legs seen in many experimental adults are competent enough to affect the normal movement and flight. Hence these beetles did not have the normal power of flight and walking as shown by their sluggish movements and lethargic appearance.Such developmental deformities can be due to the mimicking action of the phytochemicals with the insect hormones regulating normal metamorphosis; thus causing confusion in metamorphic events.

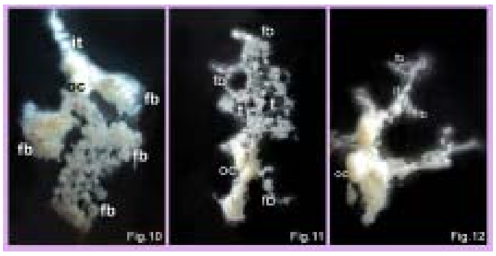

Fig.10. Posterior part of the ovariole of the control (10), with attached fat body (11) and experimental insect ovariole (12). ab – abdomen, al – abnormal legs, cd- cuticular depression, cs – cuticular spots, d- depressions, fb – fat body, h- haemolymph , if –inter follicular region, it – inter follicular tissue, mw –melanized wing, oc – oocytes, pw- protruded wings, se- smooth elytra, t- tubules of fat body, W- abnormal wings

It is suggested that mortality and morphological deformities observed in the present study can be a result of abnormal metabolism induced by the ingestion of the leaf powder by the larvae. Mixing the leaves of these plants along with cow dung in manure pits could be expected to reduce the population of this pest. Apart from this, this will probably induce developmental deformities, metabolic abnormalities and reproductive aberrations in *O.rhinoceros* which are competent enough to distort normal growth and reproduction. Both plants tested are promising for the control of the species not only by causing mortality but also by disrupting the development and by causing deformities involved in vital activities like walking, flying and reproduction. In addition, it can be suggested that the abnormalities observed in the study can make the insects exposed to various mortality agents or prevent them from attacking the host plants.

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