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ARTICLE

Insecticidal effects of selected plant extracts on the neuroendocrine system of the red cotton bug *Dysdercus cingulatus* Fabr. (Heteroptera: Pyrrhocoridae).

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Abstract

Application of plant extracts (Eupatorium odoratum EOE, and Vitex negundo VNE) caused substantial histological changes in the neuroendocrine system of the red cotton bug Dysdercus cingulatus.The remarkable change occured in EOE treated insects is the formation of vacuoles around the A type median neurosecеretory cells. Significant increase in the size of the 'A' cells has been noticed after EOE and VNE treatment. Heavy accumulation of neurosecretory material in the median neurosecretory cells resulted after the application of EOE and VNE.The neurosecretory index showed an increase in treated insects. The present study thus implicates the inhibitory role of EOE and VNE on the neuroedocrine system of D .cingulatus.

Keywords: Dysdercus cingulatus, Eupatorium odoratum, Vitex negundo, neurosecretory cells, Corpus cardiacum, Corpus allatum.

Introduction

Insect plant interactions had begun since the Devonian period of the paleozoic era. This intimate insect plant association led to the development of phytochemicals in plants which could have a vast array of biological effects on different insect species. Hence plant kingdom offers an excellent source of secondary metabolites which affect the behaviour ,physiology, growth, reproduction and development of insects (Koul 1996, Nakatani et al 1999,Rawi et al 2011). These naturally occurring secondary metabolites stored and used by plants for their defense against pests have galvanized scientists throughout the world and ever so many of research efforts were made not only to discover the types of bioactive compounds but also their isolation , identification and synthesis (Rembold 1984, Rao et al 1995). Many scientists demonstrated that plants are considered as one the richest sources that can be used as pest control agent (Dilawari et al 1994, Jannet et al 2000).The red cotton bug Dysdercus cingulatus are thought to be the serious pests of cotton (Shonouda & Osmam 2000). Nymphs and adults suck the sap from immature seeds which do not ripe remain light weight. The aim of the present study is to investigate the influence imparted by two plant extracts namely Eupatorium odoratum (Compositae) and Vitex negundo (Verbenaceae) on the neuroendocrine system of the red cotton bug Dysdercus cingulatus .

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Materials and Methods

The red cotton bug Dysdercus cingulatus Fabr were obtained from our laboratory colony maintained under controlled conditions (temp 28±4°C, r.h 75-80) by feeding them on soaked cotton seeds. Rearing techniques adopted and described by Jalaja (1975). Newly emerged fourth instar nymphs separated from the stock colony were used for the experiments. Extracts of two plants viz. Eupatorium odoratum (Compositae) (EOE) and Vitex negundo (Verbenacea) (VNE) were applied on experimental nymphs. After treatment with each extract the treated and control fourth instar nymphs were allowed to feed on soaked cotton seeds . The newly emerged four day old female insects (both treated and control) were mildly anaesthetised and brain along with corpus cardiacum, corpus allatum complex were dissected out in insect Ringer.The tissue dissected out were fixed in Bouin's fluid. Paraffin sections of 5μ thickness were stained with chrome-haematoxylin phloxine (CHP) for brain,and sections of corpus cardiacum and corpus allatum were stained with Erlich's haematoxylin-eosin. For calculating neurosecretory index arbitrary rank was given to neurosecretory cells as per their secretory content. For each category of cell (+)rank indicated some content, (++) rank medium secretory content and (+++)rank given to cells which were full of neurosecretory content . The number of neurosecretory cells in each category was assessed and the number of (+) marks were added and then multiplied by the number of cells in each category . The three products were added which gave the 'index' of the neurosecretory material of each animal.

Preparation of Extract

Leaves of both the plants were repeatedly extracted with methanol. After evaporation of the solvent known quantity of the residue was dissolved in methanol (1mg/ml). Ten microlitre of each of the extract containing ten micrograms of residue was topically applied on the dorsal side of the newly emerged fourth instar nymphs using a microlitre syringe (Hamilton). Controls were treated similarly only with methanol. On adult emergence treated and control insects of four day old were used for experiments.

Results and Discussion

In D. cingulatus the neuroendocrine system consists of neurosecretory cells (NSC), the corpora cardiaca (CC) and the corpus allatum(CA). The present experimental studies are limited to investigate the effect of plant extracts on 'A' type median neurosecretory cells of the brain, corpora cardiaca and corpus allatum. A cells stained blueblack in chromealum haematoxylin phloxine. These are 13-16 in number and seen as two groups or in a single group and are rounded or oblong measuring about 21µm in diameter. Secretory material is seen in the cytoplasm. The nuclei of the cells are often unstained and occur as clear spaces.

Effect of EOE ON 'A' Type NSC

EOE has got a profound influence on the 'A' type neurosecretory cells of parsintercerebralis. Significant increase in the size of the A cells has been noticed after EOE treatment. The size of A cells increased to 28.5µm when compared with the respective controls of 15.32µm. (Fig 1&3) (Table 1). The nuclear diameter varied from 9.1µm to 6.4µm. One

Table 1. Proximal composition of leaf of Hyptis capitata Jacq.

AGE (Days)	DOSE	NAME OF EXTRACT	CELL SIZE(µm)		NUCLEAR SIZE(µm)		NEUROSECRETORY INDEX	
			Control	Experiment	Control	Experiment	Control	Experiment
4	10µg	EOE	15.326±0.277	28.5±0.255***	6.41±0.106	9.11±0.240	52.6±0.184	74.16±0.413***
4	10µg	VNE	15.326±0.277	29.7±0.395***	6.41±0.106	9.11±0.240	52.6±0.184	72.6±0.1828***

***P<0.001

(Mean of 8 observations ±SEM)

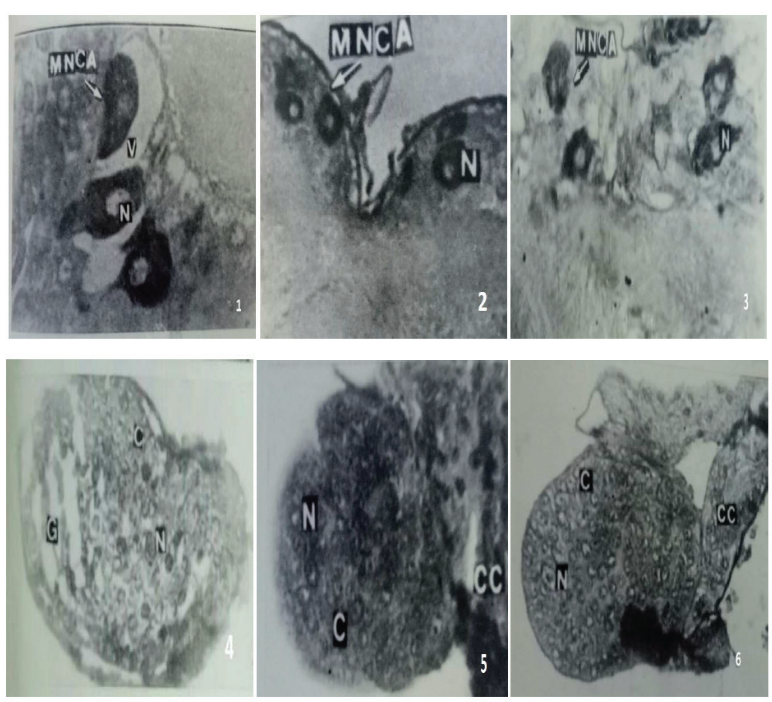

Figure 1-6- 1.Section of the brain through the parsintercerebralis (day 4 after the applicaton of EOE) x400; 2. Section of the brain through the pars intercerebralis (day 4 after the application of VNE) x400; 3. Section of the brain through the pars intercerebralis (day 4 control) x 400); 4. Section of the Corpus allatum (day 4 after the applicaton of EOE) x100; 5. Section of the Corpus allatum (day 4 after the applicaton of VNE) x100; 6 .Section of the Corpus allatum (day 4 control)x100. MNC-median neurosecretory cell, C-cell, CC-corpora cardiaca, G- intercellular space, N- nucleus,V- vacuole.

noticeable change occured in EOE treated insects is the formation of vacuoles around A type cells (Fig1). EOE application enhanced the accumulation of neurosecretory material in the perikarya of A type neurosecretory cells (Fig 1). The difference in neurosecretory index in treated and control insects is found to be statistically significant (Table 1).The neurosecretory index rises upto 72 in treated insects while in control insects the neurosecretory index is only 52. From these studies it becomes clear that EOE has a profound influence on the physiological parameter of neurosecretory index, cell diameter and nuclear diameter.

Effect of VNE on 'A' Type NSC

The neurosecretory index of 'A' type cells of parsintercerebralis of VNE treated insects increased to 75, while it is only 52 in control insects. In treated insects the neurosecretory material found to be increased when compared with the control insects.(Fig 2 & 3). Remarkable changes have aiso been noticed in the size of the A type cells as a result of VNE treatment.In treated insects the cell size is only 15.32μm(Table 1). The size of the nucleus increases to 8.2μm whereas in controls the size is only 6.4μm.These differences were also found to be statistically significant (Table1).

Effect of EOE and VNE on Corpora cardiaca and Corpus allatum

The CC lie behind the brain closely attached to the either side of the ventral region of aorta. CA is usually an unpaired gland associated with the ventral wall of the aorta; its anterior portion fused with the posterior part of the corpus cardiacum and with the aortal wall . EOE application reduces the cell size of the corpus allatum. The cells of treated insects measure 3.3μm whereas in controls the cells measure only 4.9μm. The nuclear diameter reduced to 1.6μm in treated insects as against in controls the nuclear diameter is 2.2μm. The cells are loosely packed in treated ones whereas in controls the cells are packed closely (Fig 4 &6). One striking feature notice in EOE treated CA is the formation of intercellular spaces (Fig 4). VNE application does not cause any profound influence on CA. (Fig 5) The cells of treated insects measure 3.9μm while in control the cells measure 4.6μm. The CA of treated insects show diffused cells whereas in controls the cells are closely packed (Fig 6). EOE and VNE both found to cause no histological changes in CC.

Intensive research work is being done throughout the world to elaborate control procedures which can replace the present synthetic insecticides. To achieve this goal ,development of alternate plant protection technologies became imperative and as a result botanical pest control materials have come under investigation.The plant derived products are considered to be attractive in pest control programmes as they are species specific ,easily biodegradable, non toxic to humans and are environmentally compatible. Topical application of plant extracts (EOE and VNE) resulted in the accumulation of neurosecretory material in the A type neurosecretory cells of brain of D. cingulatus. Neurosecretory cell size, nuclear size also show an increase in treated insects when compared to the control ones. These results are in conformity with the findings of Subramanyan and Rao (1996) that application of azadirachtin caused an increase in the amountof neurosecretory material in the A type neurosecretory cells of brain of Spodoptera gregaria. Schulz and Shluter (1983) studied the effect of neem seed kernel extract on the neurosecretory axons of Epilachna varivestis and reported a remarkable concentration of neurosecretory material in the neurosecretory axons. These findings support the present observations of heavily loaded NSC due to plant extract application in D. cingulatus. It seems from the present studies that application of plant extract stimulated the synthesis of neurosecretory material , but it inhibits the release of neurosecretoy material. The material accumulates in the median NSC. Accumulation of neurosecretory material in the NSC of the parsintercerebralis region of brain of treated insects may indicate that the effect of plant extract is mediated through changes caused in the NSC. Sabesan and Ramalingam (1979) have observed increased synthetic activity and accumulation of neurosecretory material in the NSC and vacoulation of motorneurons of endosulphan treated Odontopus varicornis. Omprasad and Srivastava (1982) reported vacoulization of NSC following DDT treatment in Poekilocerus pictus.

Similar results were obtained in the present studies with plant extract application in D.cingulatus.From the present studies it can be noticed that the application of plant extracts acts in the same way as does other synthetic insecticides act on the brain cells of insects. EOE and VNE treated insects also showed some changes in the corpus allatum also.Formation of intercellular spaces and loose arrangement of cells of corpus allatum are the noted characteristics of insects after treatment of plant extracts. The formation of intercellular spaces and increased vacoulation of corpus allatum cells after treatment of neem seed kernel extract in Epilachna varivestis was reported by Schulz and Shluter (1983). Further in Epilachna, the neemseed kernel breaks the integrity of cells of corpus allatum and corpus allatum themselves disintegrate. Thus it seems that the results of present investigations corroborates the findings of Schulz and Shluter (1983). Unnithan et al (1997) reported the decreased volume and progressive degeneration of corpus allatum in Oncopeltus fasciatus after the application ofprecocene. Krishnayya and Rao (1995) pointed out the decrease in volume of corpora allata of *Helicoverpa armigera* as a result of plumbagin application. Present studies on the corpus allatum of D. cingulatus after application of plant extracts show the loosely packed cells and the formation of intercellular spaces.These results seem to be due to the partial degenerating action of corpus allatum as a result of the application of plant extracts.Thus the present observation imply that the plant extracts application caused several disturbances in the neuro endocrine system of D. cingulatus. The studies clearly demonstrate the potency of using locally available plant materials to combat the heteropteran pest

D. cingulatus, without affecting the ecological balance and also the contamination caused by the use of synthetic pesticides to the biosphere which could be eliminated by the use of bioinsecticidesThe present histological destruction caused by the investigated plant extracts may suggest that any of these extracts are capable of causing death of an insect when entering into tissues in adequate amounts.

Conclusion

The results of the present study show that ethanolic extract of Eupatorium odoratum and Vitex negundo has high toxicity on Dysdercus cingulatus .Various inhibitory effects of plant extracts on the neuroendocrine system of D.cingulatus throw light to the prospects for control of this serious pest of cotton with these biopesticides.

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