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Medicinal Plants and its Impact of Ecology, Nutritional Effluents and Incentive of Digestive Enzymes on *Spodoptera litura* (Fabricious)

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Abstract: This study having following objectives such as to study the biology, nutritional indices and mid gut enzymes response with three different botanical insecticides and to determined the nature of insecticidal action was examined by dermal, oral and contact treatments. As an alternative to synthetic ones, plants have been received much attention as a source of pesticidal active secondary metabolites such as phenolics, terpenoids and alkaloids. The effect of water extracts prepared from the leaves of *Ipomea carnea* (Linn.) Convolvulacea and *Pedaliium murex* (Linn.) Pedaliacea and *Adhatoda vasica* (Linn.) Acanthacea on the biology, nutritional indices and digestive enzymes of the polyphagous pest *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) was investigated. Increased larval and pupal mortality and drastically decreased larval growth were recorded in the experimental extracts treated caterpillars. Feeding efficiency and fecal excrement were also greatly affected. The digestive enzymes profiles were decreased by the plant extract treatments. Furthermore, they were facilitating the active and easy proliferation of the chemicals for the subsequent pathogens of larvae. Among the three application methods tested, oral treatment caused the highest toxicity, followed by topical application and contact treatment. From this study clearly revealed about the active secondary metabolites were arrested or restricted the ecdyseal process during the moulting period of experimental pest. These results suggest that the three plant species tested may have potential for use as natural bio-pesticides.

Key words: Lepidopteron pest, *Spodoptera litura*, medicinal plants, digestive enzymes

INTRODUCTION

The use of conventional insecticides has raised some concern about their threat to the environment and development of insecticide resistance in insects (Huang *et al.*, 1998), there is an imperative need for the development of safer, alternative crop protectants such as botanical insecticides and antifeedants. Plants are rich sources of natural substances that can be utilized in the development of environmentally safe methods for insect control (Sedek, 2003). Recently, Briska and Sahayaraj (2009) reported the botanical plant extracts often consist of complex mixtures of active compounds, they may show greater overall bioactivity compared to the individual constituents. The deleterious effects of crude plant extracts on insects are manifested in several ways, including toxicity (Hiremath *et al.*, 1997)

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feeding inhibition (Wheeler and Isman, 2001). The search for plant-derived chemicals that have potential use as crop protectants (insecticides, antifeedants and growth inhibitors) often begins with the screening of plant extracts (Kanat and Alma, 2003; Martin and Gobalakrishnan, 2005). It has a wide range of host feeding on 12 species world wide of which 40 species are known from India (Paulraj, 2001). Traditional farmers have been practicing synthetic pesticides to eliminate the *S. litura* and hence, it has developed resistance against almost all the commonly using pesticides in this area. Certain plant families, particularly Meliaceae, Rutaceae, Asteraceae, Labiateae, Convolvulaceae and Pedaliaceae are viewed as exceptionally promising sources of plant-based insecticides (Schutterer, 1990; Isman, 1995). In the past Sahayaraj *et al.* (2003) reported bio-pesticidal property of *Chrystella parasitica* and *Ipomoea carnea* on *Achaea janata*. The activity of these extracts also suggests that future exploitation of the materials in to potential insect management chemicals with a minimum environmental in pest. It interestingly previous Devanand and Rani (2008) employed with the present study possess antifeedent and toxicity in the lepidopteran pest. Since the bioactive compounds which are often active against a limited number of species including specific target insects are biodegradable to non-toxic products and potentially suitable for use in integrated pest management programs, they could lead to the development of new classes of safer insect control agents (Ahmad *et al.*, 2009) The *S. litura* (Fab.) (Lepidoptera: Noctuidae) is a cosmopolitan and polyphagous pest affecting several crops worldwide causing extensive loss of agricultural production. Consequently, intensive efforts have been made to find alternatives, especially insecticides and plant origin which are safe, effective and environmentally acceptable. Hence, the objectives of this study are: (1) understand how the biology, nutritional indices and mid gut enzymes varied in response to different botanical insecticides and (2) to determine the nature of insecticidal action was examined by dermal, oral and contact treatments.

MATERIALS AND METHODS

Three hundred of Caterpillars and various life stages were collected from the infested groundnut field and subsequently reared in the laboratory on fresh groundnut leaves at $30 \pm 1.56^\circ\text{C}$, $70 \pm 10\%$ relative humidity and 12L: 12D photoperiod. Laboratory emerging moths, five males and five females were placed in to oviposition cages and fed with a 10% sucrose solution fortified with a drop of vitamin mixture to enhance the oviposition. The egg batches were removed and kept in Petridishes for hatching. This study was carried out during the year of 2009.

Preparation of Plant Extract

Crude extracts were made from the leaves of four plants: *Ipomea carnea* Linn. *Pedaliium murex* (Linn.) and *Adhatoda vasica* (Linn.). The aqueous extracts of these plants were made by macerating 100 g of the dry leaves partially on a mortar and pestle then heating with 250 mL water in sox let apparatus at 100°C for 24 h. The dissolves solute gave 100 to 150 mL of crude aqueous extract. The un-dissolved portion was removed and dried in an oven at 80°C for 6 h to get a constant weight (20, 29, 31, 36 g) for *I. carnea*, *P. murex* and *A. vasica*, respectively. The weighed aqueous extract was calculated from the initial weight of the leaves and final weight of the undissolved portion. The LD_{50} concentration of *I. carnea*, *P. murex* and *A. vasica* (w/v) were 3.9, 0.7, 4.5 and 1.3%. The effect of the botanical extracts were tested using three application methods namely topical application, oral and contact toxicities.

Steamy Application

Five grams of each fresh groundnut leaves were dipped in the LD₅₀ concentration *I. carnea*, *P. aquilinum* and *A. vasica* were prepared and treated on ground nut leaves for half an hour and air dried thoroughly. Ten fourth instar larvae of *S. litura* from laboratory culture were starved for 6 h and then placed in plastic containers (250 mL capacity) containing plant extracts treated groundnut leaves. Control larvae were treated with water. The unconsumed leaves and faecal pellets with in each container were subsequently weighed after 24 h and then the larvae were provided with fresh untreated groundnut leaves until pupation.

Dermal Application

Whatman (No. 1) filter paper discs (2 cm²) were dipped in the LD₅₀ concentrations of the plant extracts separately for 15 min and shade dried for 20 min. Fourth instars larvae of *S. litura* ten in number (uniform age and size) were placed in plastic containers (500 mL capacity) containing plant extract. After 24 h the larvae were removed and placed into plastic containers containing untreated groundnut shoots and maintained until pupation. Each find was replicated six times. The impacts of the treatments were measured by recording larval, pupal and adult mortality larval and pupal developmental periods and adult longevity. Deformities in the larvae, pupae and adults were also recorded.

Food utilization experiment was conducted in the newly moulted 7 h starved fourth instar larvae of *S. litura*. A gravimetric technique was used to determine weight gain, food consumption and faeces produced. Pre-weighed larvae (10 larvae/category with ten replications) were introduced in to the separate plastic containers (1 L capacity) and allowed to feed on known 5 g quantity of groundnut leaves treated with water extracts of IC, PM and AV. Three containers were similarly set up, but without larvae in order to estimate the leaf dessication factor. The weight gain was assessed till the pre-pupation for every 24 h on fresh containers i.e., after subtraction of the leaf dessication factor. The unfed leaves and faecal pellets were separated each day, oven-dried and the percentage dry weight conversion value was established. From these observations, growth indices such as Consumption Index (CI) Relative Growth Rate (RGR) Approximate Digestibility (AD) and Efficiency of Conversion Ingested (ECI) and digested food (ECD) were calculated. After 24 h treatment 10 larvae from each treatment were removed then the midgut of larvae was dissected out in the normal cold saline. The undigested plant materials present in the gut was removed and the midgut portion was separated, homogenized and pooled in the cold citrate phosphate buffer (pH 7.6). After that ten percentage of the homogenate in 0.25 M sucrose was centrifuged for 20 m at 900 g and the resulting supernatant was employed as enzyme source (Eguchi *et al.*, 1972; Pathak and Sharma, 1996). Amylase and invertase activity was determined (Ishaaya and Swirski, 1970) using 3, 5-dinitro salicylic acid and sucrose as substrates. The lipase activity was evaluated following the method of Sadasivam and Manickam (1997). In all cases the activity of the enzyme was expressed on the protein basis (Finney, 1971).

RESULTS AND DISCUSSION

Crude extracts of the tested plants interfered with the normal development and metamorphosis of *S. litura*, which was manifested at different stages of the life cycle. The majority of the treated nymphal instars were died, either after an extended larval period or after pupation, indicating that the bioactive components of the leaf extracts persisted for a long time in the insect's body and did not get metabolized fully during the larval stage or larval pupal moult (Gebreyesus and Chapya, 1983). The effect of the plant extracts on

Table 1: Biological and morphological features of *S. litura* after IC, PA and AV treatments (No. of larvae 50)

Name of the plant	Treatments	Pupation in (%)	Larval pupal intermediate (in %)**	Pupal deformity (in %)	Adult deformity (%)**
Control	O	100	Nil	Nil	Nil
	D	95.14	Nil	Nil	Nil
	C	100	Nil	Nil	Nil
<i>I. carnea</i>	O	31.25	25.36	23.01	25.33
	D	46.23	29.34	18.36	19.36
	C	51.57	56.32	18.74	14.56
<i>P. murex</i>	O	28.61	25.36	16.38	30.14
	D	35.46	28.69	12.54	27.39
	C	47.6	27.31	14.36	31.25
<i>A. vasica</i>	O	45.35	35.61	14.25	23.28
	D	49.68	31.05	15.35	16.58
	C	56.31	29.65	19.34	18.90

**Indicates oral application

Table 2: Nutritional indices of *S. litura* larvae after AI, CG, PP and VN treatments

Plants	Method of treatment	RCR (g)**	RGR (g)**	AD (%)**	ECI (%)**	ECD (%)**
<i>I. carnea</i>	O	3.18	0.73	67.14	4.08	5.11
	D	3.41	0.87	55.10	4.47	6.35
	C	3.75	0.89	68.41	4.53	5.29
<i>P. murex</i>	O	4.58	0.77	64.29	1.27	1.72
	D	4.73	0.56	67.40	1.74	2.35
	C	4.59	0.61	68.23	2.31	3.82
<i>A. vasica</i>	O	3.69	0.59	64.53	2.47	5.85
	D	3.91	0.71	64.89	2.72	5.24
	C	4.37	0.73	65.44	4.71	5.33
Control	O	4.99	0.88	65.10	5.11	7.91
	D	4.57	0.95	66.31	6.20	8.83
	C	5.12	0.98	67.41	5.37	8.60

**Indicate oral application

S. litura closely resembled the effects of juvenile hormone (JH) on insect larvae, naturally a prolongation of normal instars and adult period's (Srivastava *et al.*, 1965; Sahayaraj and Agnol, 2004; Martin and Gobalakrishnan, 2005). Among the three plant extracts of *I. carnea* and *A. vasicata* had prolonged nymphal and adults followed by *P. murex*. The most of the periods oral application also been appeared to be prolonged duration both the larval and pupal periods. The extract treated larvae showed various types of deformities and high levels of mortality. The dead nymphs and adults were invariably connected with moulted skin. Of these, experimental plant extract, *P. murex* shows two more days additionally taken for fifth nymphal instars to adult stage followed by *A. vasicata* and *I. carnea*. Similarly the nymphal adult intermediaries and adult deformities were also maximum observed under the experiment with *P. murex* (Table 1).

At several stages shown in the extended larval period, growth and development sustained, but in several occurrence the larvae became larger than normal fifth instar nymphs and they were eventually died. From this work clearly articulated the plant extract therefore, seems to have a suppressive action on the moulting process through the metabolic activity, which allowed the treated larvae to live without moulting at few days. In this aspect, the three plant extract, bear a resemblance to the action of excess juvenile hormone, which also hampered with moulting process. Since, this result showed statistically significant for invertase (except the *P. murex*) but not for lipase activity (Table 2). Meanwhile, complete or partial moulting takes place during the time of metamorphosis nymphal instars to adult it might be due to the interference of plant extracts.

The nymphal and adult intermediaries also deformities were higher with when the botanicals were administered orally. Adult longevity was higher in contact toxicity test

(except in the control and AV treatment) followed by the dermal and contact toxicity tests respectively. However, there was no significant differences were observed between the treatments of three plant extracts. Duncan Multiple Range Test (DMRT) demonstrated that all the extracts investigated showed significant difference at 5% level within the treatments, as far as the pupation (except IC and AV) nymphal-adults intermediate (except PM and nymphal deformity (except AV) and adult deformity were concerned among the treatment of plants tested in this experiment. The formation of intermediates may be due to the direct action of the plant extracts through their stimulation of the corpora allata to produce an increased amounts of JH which was sufficient to maintain nymphal characters in the posterior part of the body but insufficient to prevent the pre-adult stage present at the anterior part. Consequently moulting was disrupted and larval, pupal intermediate was produced. Ambika-Devi and Mohandas (1982) and Koul *et al.* (1996) also been reported that plant extracts has antifeedent and growth inhibitory activities on economically affected series pests under various fields.

Enzyme Bio-Assay

Here, this study observed enzyme activities such as amylase and invertase was lower after treatment with following plant extracts *I. carnea*, *P. murex* and *A. vasica*. Among the four experimental plants Protease activity was very low in *P. murex* then *A. vasicata*, finally *C. gigantia* treatments (Table 3). In similarity to this experiment explained amylase and invertase, lipase activity was decreased compared with control categories. Of these three application method tested oral application strongly reduced both amylase and invertase activity, followed by topical and contact applications. Likewise the similar patterns were observed with all four experimental extracts. But in the case of *P. murex* treatment lipase activity was enhanced by the oral applications. In both the control and *A. vasica* treatment lipase activity was higher in the contact application followed by the oral and dermal application respectively. However, the data were statistically significant at 5% level. Ethanol extracts of *Aesculus hippocastanum* L. showed toxic effect against *Thaumetopoea solitaria* (Frey.) larvae (Erturk, 2006). These results suggest that there may be different compounds in extracts possessing different bioactivities, particularly antifeedant and toxicity of insect pests. We can conclude that this study suggest that water extracts of *I. carnea*, *P. murex* and *A. vasica* plants belonging to families taxonomically possesses toxic principles with significant antifeedant effects and could be a potential crop protectants against *S. litura*. These kinds of similar observations have been already found out through the other

Table 3: Digestive enzyme profile of *S. litura* larvae after IC, PM AV treatments. Values expressed in parentheses indicates per cent reduction or (*) increased activity

Plants	Method of treatment	Protease ¹	Lipase ²	Invertase ³
<i>I. carnea</i>	O	4.56 ^{a*}	79.50 [*]	43.56 ^b
	D	5.14 ^{ab}	50.34 ^{bc*}	36.81 ^{bc*}
	C	5.83 ^{a*}	67.31 ^{ab}	32.64 ^{ab}
<i>P. murex</i>	O	2.72 [*]	136.21 ^a	18.37 ^{ab*}
	D	3.37 ^{b*}	123.06 ^c	16.59 ^{bc}
	C	4.20 ^{ab*}	116.87 ^{c*}	17.51 ^{ac}
<i>A. vasica</i>	O	4.10 ^{a*}	79.58 ^{ab}	31.60 ^{bc*}
	D	4.97 [*]	85.42 [*]	24.91 ^{c*}
	C	4.71 ^{a*}	25.39 ^{bc}	20.58 ^{ab}
Control	O	6.60 [*]	122.36 ^{a*}	193.28 [*]
	D	7.11 ^{ab}	108.23 ^c	287.04 ^a
	C	6.07 [*]	114.16 ^c	156.37 ^{ab}

*O: Oral application, D: Dermal application and C: Contact application. Means followed by the same letter are not significantly different at 5% level

medicinal plants having solvent based extracts. It is interesting that certain plant extracts employed in the present study possess antifeedance and toxicity in the lepidopteran pest species studied (Jacobson, 1989; Selvaraj *et al.*, 2005; Ahmad *et al.*, 2009). It appears that these plants contain different chemicals that act upon target cells effectively (Aswad *et al.*, 2003; Sadek and Anderson, 2007). Though the chemical analysis of the active plants is underway and we hope to reveal some interesting similarities between the chemicals isolated as well as their bioactive compounds from the medicinal plants (Murugan *et al.*, 1999; Bell *et al.*, 1990; Sahayaraj, 1998). The activity of these extracts also suggests a future exploitation of the materials in to potential insect management chemicals with a minimum environmental impact. It is advantageous, as the extracts at higher doses act as antifeedant, while the lower dilution of the same plant is oral toxicant (Sedek and Anderson, 2007). The results implying the dual role of a single plant material in castor and groundnut pest management by chosen plant extracts. It also suggests that by a single application of these compounds a complete success of the insect control can be achieved.

Digestive enzymes play a major role in insect physiology by converting complex food materials into micro molecules necessary to provide energy and metabolites for growth, development and other vital functions (Eturk, 2006). The results of this study indicate that there is considerable variation in midgut amylase, invertase and protease activity between the insects treated with the different plant extracts. The present results were agreed with another one pest experiment on digestive enzymes such as protease activity in gut contents and tissue was significantly reduced after precocene treatment to *Acheae janata* (Linn.) (Mathews and Muraleedharan, 1992). However the study demonstrated the increased protease activity after plant extract treatments. The increased activity of midgut lipase all the plant extract treatments might account for a greater utilization of exogenous lipids and resulting in the bio-mass production (Champagne *et al.*, 1992; Babu *et al.*, 1997; Desai and Desai, 2000; Ahmad *et al.*, 2006). They concluded that *I. carnea* treatment decreased the level of lipids probably suggesting the depletion of the reservoir of the fats in *Atractomorpha crenulata* (Fab.). When *S. litura* larvae of that were injected with azadiractin (1 mg g⁻¹) suffered reduced amylase and invertase activity and lower digestibility (Ayyangar and Rao, 1989; Senthil *et al.*, 2006) as well as this study also explained the digestibility differs widely in relation to the food given to an insect. However, they reported that dermal application of precocene II significantly reduced the level of enzyme activity of *A. janata*.

In the present study, we observed that botanical extract treatment decreases the nymphal instars and adult emergence, food consumption and conversion but increases the nymphal instars and adult emergence. Plant compounds interrupted the secretion of digestive enzymes and further led to the disruption of gut physiology. Moreover, feeding is necessary for the stimulation of digestive enzyme activities and might have interfered in enzyme substrate complex and affect the peristaltic movement of the gut, a phenomena which was very clear in the alteration of faecal pellet production by the botanicals treatment. Oral administration of botanical extracts ended to have more impact on growth and development, feeding and digestive processes also relative to dermal and contact treatments. Thus the present study strongly proved to be indicated that the possible use of these plant extracts for the control of series castor and vegetable crop pest of *S. litura*.

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