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Antibiotic effects of resorcinol, gallic acid and phloroglucinol on *Heliothis armigera* Hubner (Insecta: Noctuidae)

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Abstract. The antibiotic effects of 3 phenolic substances viz. resorcinol, gallic acid and phloroglucinol on the cotton bollworm *Heliothis armigera* Hubner were evaluated with particular reference to the feeding regimes, survival and pupation. Some of the characteristic antibiotic effects observed for resorcinol and phloroglucinol include reduction in consumption index and approximate digestability, increased efficiency of conversion of ingested food and efficiency of conversion of digested food, extended larval duration and high mortality. However, there was no alteration in the various food utilization efficiencies in the case of gallic acid treated individuals. The defensive response of the larvae to these toxic substances seems to be excessive defaecation. Frass analysis through thin-layer chromatography and ultraviolet characterisation indicated the presence of the ingested antibiotics, opening further avenues of investigation on the possible role of these antibiotics present in the frass in biological control.

Keywords. Antibiotic effects; *Heliothis armigera*; resorcinol; gallic acid; phloroglucinol; frass analysis.

1. Introduction

In the recent past much attention has been focussed on the chemical basis of insect-host specificity and host plant resistance (Beck 1965; Dethier 1970; Chapman 1974; Schoonhoven 1972; Gallun *et al* 1975; Hanover 1975; Kogan 1975). Most such attempts have been concentrated on chemical substances which create immediate response involving such behavioural parameters as feeding stimulants and feeding incidents (Beck and Stauffer 1957; Beck 1956, 1957, 1960). Several chemicals have been found to be responsible for the non-preference mechanism in resistant plant varieties through non-preference for oviposition and allied activities for successful establishment of an insect species on a crop (Pathak and Dale 1983). Besides this, the presence of extraneous chemicals of the host plants also prevent higher damage to the crop as has been reported in the past (Maxwell *et al* 1972; Lukefahr *et al* 1965, 1971). However, all these effects tend to be of short term duration being unable to withstand the changing pest status, which is basically different from the chronic effects of feeding, growth and survival offered by a number of chemical substances (Matsumato 1962).

Very little work has been attempted on the chronic effects of allelochemics on different insect groups. Most investigators (Lukefahr and Martin 1966; Williams *et al* 1980; Wiseman *et al* 1976; Waiss *et al* 1979) have reported the antibiosis process influencing insect-plant interactions particularly to *Heliothis* spp. With special reference to such chemicals as gossypol, 2-tridecanone, d-tomatine etc. several investigators (Beck 1957, 1960; Beck and Stauffer 1957; Harley and Thorsteinson 1967; Todd *et al* 1971; Feeny 1968; Reese and Beck 1976a, b, c) studied the effects of chronic ingestion of certain allelochemics on growth, survival and pupation. Fraenkel (1969) explained the possible role of allelochemicals on the feeding activity of herbivorous insects. Several other workers (Shaver *et al* 1970; Soo Hoo and Fraenkel 1966; Erickson and Feeny 1974) demonstrated the role of several plant allelochemicals on the feeding activity of herbivorous insects.

The present paper attempts to highlight the antibiotic effect of allelochemicals with special reference to resorcinol (1,3-dihydroxybenzene), gallic acid (3,4,5-trihydroxybenzoic acid) and phloroglucinol (1,3,5-trihydroxybenzene) on the feeding regimes, survival and pupation of *H. armigera.* Most of the resistant crop varieties screened for the preference of *Heliothis* exhibit the presence of such phenolic substances (Annadurai *et al* 1989) which provide indepth information in understanding the relationship between chemical defenses of host plants and their biological activity against insect pests.

2. Materials and methods

2.1 *Stock culture*

H. armigera culture was maintained in the laboratory using artificial diet following the methods slightly modified from Singh (1971) and Callahan (1962). Adults were kept in cages (30 × 30 cm) and the females oviposited on *Cicer arietinum* potted plants as well as on *Gossypium hirsutum* (MCU 5) twigs bearing buds and flowers. Eggs were treated with 0·1% formaldehyde by dipping the plants and air dried. The larvae were reared with *C. arietinum* plants for the first 4 days and subsequently transferred to artificial diet in individual culture vials (5 × 3 cm). Pieces of the diet were placed inside the culture vials for stock culture. Distilled water was used in diets and wherever water came into contact with the insects. Contamination was kept to a minimum by autoclaving the glassware at 100°C for a few hours. Containers were also washed in 0·5% formaldehyde. The larvae were reared at 29 ± 2°C under 12D:12L photoperiodic regime. The pupae were placed in a netlon mesh cage (30 × 30 cm).

2.2 *Experimental cultures*

Authentic phenolic substances (Sigma chemical Co., USA) were incorporated with the standard diet at concentrations of $2{\cdot}48 \times 10^{-4}$ M through 10^{-1} M. Similarly, for gallic acid and phloroglucinol the concentrations used were $4{\cdot}23 \times 10^{-1}$ through 10^{-4} and $3{\cdot}65 \times 10^{-4}$ through 10^{-1} M respectively. All the chemicals to be tested were dissolved in water and appropriate amounts of the solution were incorporated with the ingredients of the standard diet and homogenized. The diet was kept open for 24 h and the larvae were introduced. The appropriate controls were used for these experiments with distilled water. Each treatment consisted of 10 replicates and all the larvae were screened for survival at 10 days feeding period, 10 days pupation and survival.

2.3 *Nutritional parameters*

In order to have an understanding of the role of allelochemicals on weight gain, the various nutritional indices (Waldbauer 1964, 1968) were calculated. Fifth or sixth day old larvae of 3rd instar from the stock culture were weighed and placed individually on weighed containers containing standard as well as experimental diets. The size of the diet was such that the control larvae would eat a large part of the diet given to them, but none of the larvae were in danger of running out of food before the end of the experiment. Faecal pellets were carefully separated from the left over diet piece weighed and dried to constant weight. Feeding experiments were conducted for 10 days larval period starting from sixth day. Final feeding data on 15th or 16th day of larval life, were avoided (the period during which the larvae masticate the diet for preparing pupal cells). All weights were taken on a Yamato analytical balance to the nearest accuracy of 1 mg.

2.4 *Frass analysis*

Thin layer chromatography (TLC) was performed to separate the phenolic compounds both from the host tissues and faecal material in the respective experiments. The R_f values and spectral ranges (UV Spectrophotometer, Hitachi 150–20) of standard phenols and phenolic acids were compared with the eluted fractions (Harborne and Williams 1969).

3. Results

Resorcinol is a substance most abundant as a compound as umbelliferone (Robinson 1963) and is a frequent constituent of more complex plant compounds (Onslow 1923). Phloroglucinol is structurally related to resorcinol and found free or in a combined state in plants (Doby 1965; Whittaker 1970), while gallic acid is a hydrolytic product of tannins and is one of the most common phenolic derivatives in tannins (Robinson 1963; Wong 1973) (figure 1).

3.1 *Resorcinol*

An analysis of the food consumption and the relative influence on nutritional indices indicated clear variation when fed on diets mixed with resorcinol, gallic acid

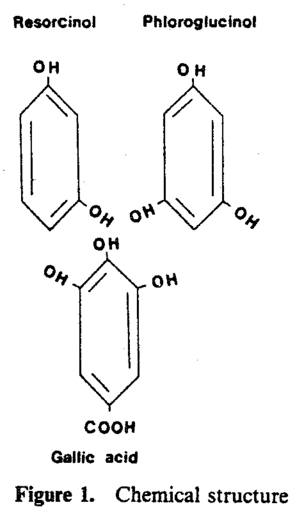

Figure 1. Chemical structure of the antibiotic substances.

and phloroglucinol. Nutritional indices were estimated for diet mixed with resorcinol and the results showed that larvae of *H. armigera* ingested only 25% of food ingested by control larvae at higher concentrations and the rate of ingestion increased at the lower concentrations. A closer look on the weight gain of the larvae also showed that larvae fed on the incorporated diet had a poor weight gain as compared to control (table 1). Larvae fed on the phenol incorporated diets excreted excess faecal pellets when compared to normal individuals presumably in an act to egest the toxic substances in the diet. The consumption index (CI) as well as approximate digestability (AD) showed similar values being higher in control and considerably reduced in treated individuals. The values of efficiency of conversion of digested food (ECD) doubled in higher concentrations of resorcinol treated diets when compared to control and the values showed considerable reductions at moderate and lower concentrations. The efficiency of conversion of ingested food (ECI) showed higher values in the case of controls and relatively lesser ECI values were recorded in treatments. The higher ECD and lower ECI values in treatments compared to controls could presumably be attributed to the fact that the larvae defaecate excessively the diet containing toxic substance in a physiological event to remove the toxic substance and hence relatively less quantity of food material is allocated to body tissue.

A comparison of the larval duration and larval weight clearly indicated that the larvae fed on resorcinol significantly extended their larval duration with a significant difference in their body size (table 2). Among the various treatments, $2{\cdot}75 \times 10^{-1}$ concentration increased the larval duration several folds compared to control. The mean pupal weight also varied among treatments and the size being very small in higher concentrations. An overall analysis of larval survival showed that 38% of larval mortality occurred in higher concentrations of resorcinol and larval mortality decreased considerably at lower concentrations. Considerable pupal mortality was also observed among resorcinol-fed individuals especially at higher concentrations (table 2). Behavioural as well as feeding activities of the treated larvae confirmed the hypothesis that most of the larval mortality and prolonged larval duration are largely attributed to the inhibition of ingestion of food as well as disproportionate defaecation during larval stages. Several larvae were also noticed in a struggling condition to remove their arval exuviae during the moulting process.

3.2 *Gallic acid*

A totally different picture was obtained when individuals of *H. armigera* were subjected to diet with gallic acid. The nutritional indices and related parameters showed that almost similar quantity of diet was ingested as that of control at higher concentrations of gallic acid, the quantities being 93·6, 66·3, 63·7 and 76·3% of that of control when fed on concentrations $4{\cdot}73 \times 10^{-1}$, 10^{-2}, 10^{-3} and 10^{-4} respectively. On the other hand the amount of faeces ejected was greater in treatments compared to control and the values being 344·4, 255·8, 244·3 and 185·3% at various concentrations indicated in table 3. The experimental larvae assimilated a much smaller proportion of their food (AD). The efficiency of conversion of assimilated food (ECD) was much lower at all concentrations compared to control. The inhibition of both these processes caused a marked reduction in the overall efficiency of ingested food (EC1) (table 3).

Table 1. Effect of resorcinol on nutritional indices and related parameters of *H. armigera*.

		Parameters (% of control)				
Parameters	Control	$2{\cdot}75 \times 10^{-1}$ M	$2{\cdot}75 \times 10^{-2}$ M	$2{\cdot}75 \times 10^{-3}$ M	$2{\cdot}75 \times 10^{-4}$ M	CD value at 5%
Average food consumed/day (mg)	330·3	025·0	042·9	059·3	064·5	31·96
Final wt. gain (mg)	118·3	028·4	042·0	034·8	050·9	16·46
Average wet wt. of faeces/day (mg)	065·5	089·0	203·2	213·1	148·9	20·57
CI	001·6	043·8	068·8	075·0	112·5	00·261
AD	076·2	048·6	045·0	053·1	061·3	08·59
ECD	039·4	171·3	133·5	112·7	110·4	08·18
ECI	025·4	088·9	074·4	047·2	060·2	05·04

Values are mean of 10 replicates.

Table 2. Effect of resorcinol on larval duration, pupation and survival of *H. armigera*.

		Treatments				
Parameters	Control	$2{\cdot}75 \times 10^{-1}$ M	$2{\cdot}75 \times 10^{-2}$ M	$2{\cdot}75 \times 10^{-3}$ M	$2{\cdot}75 \times 10^{-4}$ M	CD value at 5%
Larval duration (days)	014·8	022·4	021·4	021·4	018·3	2·04
Mean larval growth prior to pupation (mg)	362·5	169·2	224·6	236·8	243·6	—
Mean pupal wt. (3 days after pupation) (mg)	302·4	132·6	172·3	204·2	231·8	—
Percentage larval survival (at the end of 5th larval instar)	100·0	62·0	71·0	70·0	80·0	—
Percentage pupal mortality	—	23·0	26·0	7·0	3·0	—

Values are mean of 10 replicates.

Table 3. Effect of gallic acid on nutritional indices and related parameters of *H. armigera*.

		Parameters (% of control)				
Parameters	Control	$4·23 \times 10^{-1}$ M	$4·23 \times 10^{-2}$ M	$4·23 \times 10^{-3}$ M	$4·23 \times 10^{-4}$ M	CD value at 5%
Average food consumed/day (mg)	330·3	93·6	66·3	63·7	76·3	37·48
Final wt. gain (mg)	118·3	08·5	07·5	19·2	34·3	09·35
Average wet wt. of faeces/day (mg)	065·5	344·4	265·8	244·3	085·2	32·36
CI	001·6	181·3	131·3	156·3	150·0	00·39
AD	076·2	029·9	031·5	036·4	059·7	07·76
ECD	039·2	059·0	039·9	083·2	082·7	06·21
ECI	025·4	014·9	017·3	046·9	074·4	02·78

Values are mean of 10 replicates.

Table 4. Effect of gallic acid on larval duration, pupation and survival of *H. armigera*.

		Treatments				
Parameters	Control	$4·23 \times 10^{-1}$ M	$4·23 \times 10^{-2}$ M	$4·23 \times 10^{-3}$ M	$4·23 \times 10^{-4}$ M	CD value at 5%
Larval duration (in days)	014·8	020·5	020·2	019·4	018·9	12·18
Mean larval growth prior to pupation (mg)	362·5	103·2	122·4	116·8	143·8	—
Mean pupal wt. (3 days after pupation) (mg)	302·4	092·2	098·2	108·2	114·6	—
Percentage larval survival (at the end of 5th larval instar)	100·0	40·0	46·0	48·0	65·0	—
Percentage pupal mortality	—	66·0	60·0	50·0	50·0	—

Values are mean of 10 replicates.

Extended larval duration as well as reduction in larval and pupal size was well evident at all concentrations as compared to control. Higher larval and pupal mortality were recorded at higher concentrations of gallic acid compared to control (table 4). Behavioural observations clearly indicated that the larvae tend to feed continuously as well as defaecate excessively especially at higher concentrations of gallic acid. Further, it was also observed that larvae developed muscular lesions and convulsions in the posterior half of the body which make them totally immobile and the individuals tend to display a crawling movement.

3.3 *Phloroglucinol*

A new type of antibiotic effect was observed in the larvae fed on diet with phloroglucinol, where the larvae hardly ingest any diet. Food utilization experiments indicated that larvae fed only 27% of control diet at $3{\cdot}65 \times 10^{-1}$ concentration compared to control. A moderate faecal output was also recorded in all concentrations. A significant difference in the final weight gain was observed between control and various experimental individuals at different concentrations (table 5). Phloroglucinol did inhibit AD at all concentrations. ECD was reduced significantly at higher concentrations of phloroglucinol and gradual increase was noticed at lower concentrations. As a result of it, the ECI was reduced to a highly significant level by phloroglucinol at higher concentrations and a gradual increase is recorded in corresponding concentrations (table 5). Similar to other compounds, larvae fed on phloroglucinol extended their larval duration with a poor larval and pupal weight gain. High larval and pupal mortality was also evident compared to control individuals (table 6). The higher larval mortality can greatly be attributed to starvation due to inhibition of ingestion in experimental individuals compared to control. Secondly, the pupal mortality may presumably be due to low hormonal titers ultimately preventing adult emergence.

3.4 *Frass analysis*

In order to assess the defensive response of the larvae to plant allelochemicals as well as to substantiate the excretion of considerable amount of allelochemicals along with faecal pellets which tend to either attract/repel the natural enemies, the faeces of *H. armigera* when fed on different chemical regimes were subjected to TLC and the presence of allelochemicals were characterised based on UV spectral analysis (figure 2). A comparison of UV spectra of the phenolic substances (resorcinol, gallic acid and phloroglucinol) both from host plant samples as well as from faecal pellets of *H. armigera* indicated the presence of all the compounds in the faeces especially at higher concentrations confirming the hypothesis that insect herbivores can avoid toxic effects of plant allelochemicals through excessive defaecation as one possible defensive act against plant growth reducing substances. Table 7 provides the spectral ranges and R_f values of all the 3 compounds from various host tissues were analysed.

4. Discussion

The present investigation considerably substantiates the hypothesis that plant allelochemicals may have chronic effects on rate of growth, ingestion and utilization

Table 5. Effect of phloroglucinol on nutritional indices and related parameters of *H. armigera*.

Parameters	Control	Treatments (% of control)				CD value at 5%
		$3{\cdot}65 \times 10^{-1}$ M	$3{\cdot}65 \times 10^{-2}$ M	$3{\cdot}65 \times 10^{-3}$ M	$3{\cdot}65 \times 10^{-4}$ M	
Average food consumed/day (mg)	330·8	027·1	023·5	029·9	037·4	26·4
Final wt. gain (mg)	118·3	005·5	008·5	009·0	017·3	06·95
Average wt. of faeces/day (mg)	065·5	074·0	074·6	078·8	119·8	58·05
CI	001·6	076·3	075·0	091·3	112·5	00·33
AD	076·2	060·8	071·2	053·7	048·7	07·85
ECD	039·2	078·1	070·2	059·0	114·5	07·01
ECI	025·4	046·1	062·1	038·6	053·4	03·77

Values are mean of 10 replicates.

Table 6. Effect of phloroglucinol on larval duration, pupation and survival of *H. armigera*.

Parameter	Control	Treatments				CD value at 5%
		$3{\cdot}65 \times 10^{-1}$ M	$3{\cdot}65 \times 10^{-2}$ M	$3{\cdot}65 \times 10^{-3}$ M	$3{\cdot}65 \times 10^{-4}$ M	
Larval duration (in days)	014·3	021·9	020·4	019·8	019·4	12·13
Mean larval growth prior to pupation (mg)	362·5	096·4	103·2	102·2	139·8	—
Mean pupal wt. (3 days after pupation) (mg)	302·4	083·8	092·6	088·6	106·6	—
Percentage larval survival (at the end of 5th larval instar)	100·0	034·0	040·0	040·0	060·0	—
Percentage pupal mortality	—	060·0	060·0	050·0	050·0	—

Values are mean of 10 replicates.

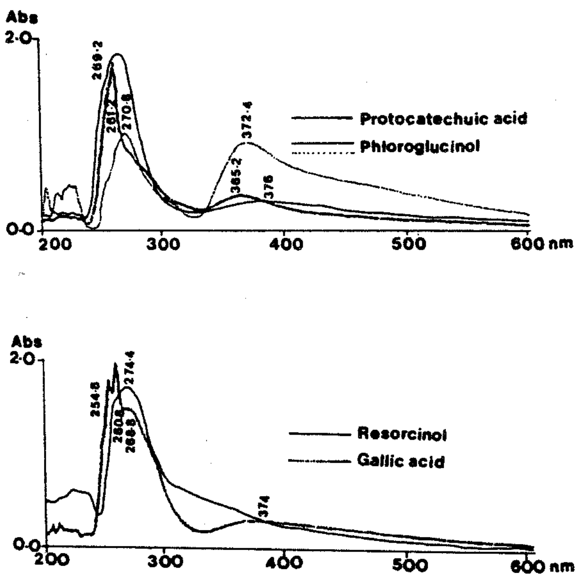

Figure 2. UV spectra of the antibiotic phenolic substances separated from the frass of *H. armigera* fed on different host tissues as well as individuals fed on phenol incorporated semi-synthetic diet.

of food by herbivores (Whittaker 1970). Evidences in support of this phenomenon have been indicated by Beck and Reese (1976) and Reese and Beck (1976a, b, c) on black cutworm, *Agrotis ipsilon.* Nutritional index experiments showed that resorcinol did not exert much adverse effect on any of the nutritional indices, but still tend to affect growth especially at higher concentrations. Added to this, resorcinol inhibited normal growth, but did not inhibit the larvae from pupation and thus it appears that resorcinol might exhibit growth inhibition mainly through inhibition of ingestion (Reese and Beck 1976a, b, c). The increased survival percentage of larvae as well as pupae when fed on resorcinol among all compounds may also be attributed to large scale excretion of resorcinol along with faeces. Unlike resorcinol, gallic acid inhibited growth by primarily reducing the efficiency of conversion of assimilated food and inhibition of the ECI. Gallic acid also reduced survival and pupation presumably through its action against its digestive enzymes and gut cells in the hind gut. This was well evident in *H. armigera* by exhibiting continous defaecation and muscular lesions of hind gut and inactivity of the larvae. Dissected individuals also revealed the fact that a large amount of faecal material accumulate in the hindgut making the larvae immobile. Similarly, phloroglucinol inhibited larval weight gain mainly through inhibition of food ingestion and affecting ECD and ECI values. The survival and pupation of *H. armigera* is severely affected when fed on phloroglucinol compared to normal individuals and resorcinol treated individuals. However, resorcinol as well as other compounds at lower concentrations did not show much impact on larval survival and pupation compared to higher concentrations. The apparent stimulation of growth, survival and pupation at low concentrations of certain plant compounds may have been due to hormoligosis, wherein the harmful substances of stress agents may stimulate growth etc. (Luckey 1968). Similar reports of enhancing the growth and survival

Table 7. List of phenolic substances characterised from the host tissues and frass of *H. armigera*.

Host plants	Phenols identified from host plant tissues	Phenols recovered from frass	Solvent system (mobile phase)	R_f value (× 100)	Spectral maxima (nm)
G. hirsutum		*Benzene derivatives*			
Bolls	Phloroglucinol	Resorcinol	HOAc–$CHCl_3$	19	274·4
Young leaves	Protocatechuic acid				
Mature leaves	Phloroglucinol	Phloroglucinol	Benzene–MeOH–HOAc	12	269·2 270·8
Senescent leaves	Resorcinol				
C. cajan		*Benzoic acid derivatives*			
Pod	Phloroglucinol	Gallic acid	HOAc–$CHCl_3$	07	260·8
Young leaves	Protocatechuic acid				
Senescent leaves	Resorcinol	Protocatechuic acid (3,4-dihydroxybenzoic acid)	Benzene–MeOH–HOAc	18	261·2
A. esculentus					
Pod					
Young, mature and senescent leaves	Protocatechuic acid				

of *A. ipsilon* at lower concentrations of phloroglucinol was reported by Reese and Beck (1976a, b, c). The poor rate of ingestion of diet with phloroglucinol may be attributed to the direct action on the chemoreceptory system of the larvae as described earlier in *A. ipsilon* by Reese and Carlson (1974). The reduction in growth, survival and pupation of *H. armigera* may also be the result of chronic effects of lower assimilation and efficiency of conversion of food. In the present investigation, although the larvae consume thrice the quantity of diet mixed with gallic acid compared to control, growth and survival are still affected in *H. armigera* in a greater way. Erickson and Feeny (1974) similarly found that sinigrin did not inhibit ingestion, but reduced assimilation of food by larvae of *Paplio polyxenes asterius* Stoll. It was observed that unlike gallic acid and sinigrin, phloroglucinol and resorcinol affected ingestion thereby affecting larval growth and survival.

The other possible factor related to the lower survival and reduced growth of *H. armigera* in various treatments of resorcinol, gallic acid and phloroglucinol is apparently due to the change in the digestive enzymes or membrane proteins of the microvilli in the gut, as a result altering the digestion and assimilation efficiencies of the larvae. Reese and Beck (1976a, b, c) hypothesized that *p*-benzoquinone may change the sulphydryl-disulphide equilibrium of important digestive enzymes and membrane proteins of the microvilli in the gut. The possible other mode of action of this compound on both enzyme and hormonal regulation of the larvae which may also play a major role in the larval survival and growth requires greater attention. In the present study, gallic acid and phloroglucinol is found to be more effective compared to resorcinol which can largely be attributed to the differential nature of gut pH facilitating the action of the concerned compound. Goldstein and Swain (1965) reported that a high gut pH is suggested as a mechanism of defence against tannins, since it would inhibit complexing of tannins with proteins. Higher faecal output in the case of *H. armigera* individuals fed on gallic acid may also act as a defensive strategy of the larvae to avoid toxicity. Studies of Self *et al* (1964) supported the present view, wherein they have indicated that excretion and egestion of intact nicotine along with frass is an adaptive mechanism in the tobacco hornworm *Protoparce sexta* (John) when fed on tobacco. Convulsion and immobility are the injurious effects of gallic acid on the gut as well as posterior muscles of the larvae. Bernays (1978) observed that *Locusta migratoria* fed on diet with tannic acid showed midgut and caecum lesions associated with gut staining and broken gut epithelial cells.

Several other reports of antibiotic activity of several allelochemicals exist in plants on the survival and growth of Lepidopterans. Sesamin was found to be a growth inhibitor for silk worm, *Bombyx mori* L. (Kamikado *et al* 1975). Chan *et al* (1978) reported that the condensed tannins have higher antibiotic activity for *H. virescens*. Similarly gossypol has been implicated as the primary substance responsible for the cotton plant resistance to several pests (Bottger and Patana 1966; Shaver *et al* 1970; Bell 1974). Meanwhile, several phenolic compounds are important in several respects during the development of *B. mori* (Kato 1978).

It is also a well known fact that several allelochemicals of plant origin not only affect the survival and growth of the herbivores, but also make them available to natural enemies for a longer period and raises the probability of mortality. Therefore, the extended larval period and poor growth rate of *H. armigera* make themselves available to an array of natural enemies in a natural ecosystem. Feeny (1976) indicated that plants with digestibility reducing substances (DRS), support herbivores that are more heavily attacked by natural enemies. The foregoing results therefore, clearly indicate the antibiotic effects of 3 allelochemicals viz. resorcinol, gallic acid and phloroglucinol which largely affect the nutritional indices, survival and growth in addition to morphological malformations. Though an evidence of clear role of antibiotics in growth inhibition and survival of *H. armigera* is highlighted, the host plant tissues rich in these compounds at appropriate concentrations may impose precise mechanisms of non-preference to herbivores, which warrants a deeper analysis of the problem.

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