

## Effect of Some Flavonoids on Survival and Development of *Helicoverpa armigera* (Hübner) and *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae)

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**Abstract:** The aim of the study was to test the effect of three flavonoids on growth and survival of *Helicoverpa armigera* (Hüb) and *Spodoptera litura* (Fab.). A set of experiments were carried out with varying concentrations of three flavonoids namely chlorogenic acid, quercetin and rutin at 23±1°C on growth, development and mortality of larvae of pod borer *Helicoverpa armigera* and tobacco caterpillar *Spodoptera litura* in artificial diets. Rutin caused significant effect on the inhibition of *H. armigera* larvae in higher concentrations, where a proportion of larvae spent between 30-51 days in excess time in larval stage III-V instar which had negative impact on growth because of cessation of feeding by the larvae. *H. armigera* larvae formed cocoons of their diets and lay still inside the cocooned diet. Healthy *H. armigera* moth emergence was common in chlorogenic acid and quercetin, but the moths did not produce any progeny. In case of *S. litura*, rutin alone had a significant effect on arrested larval development, pupal mortality and malformed adults compared to artificial diets with quercetin and chlorogenic acid. The increase in concentrations of rutin did not show proportionate increase in mortality of the larvae. The average excess days at all concentrations of quercetin and chlorogenic acid ranged between 40-55 days. These results indicate a delay in development and suggests that rutin interferes in with physiological processes in both insects at the time of molting.

**Keywords:** Arrested growth, chlorogenic acid, *Helicoverpa armigera*, non-fecund moths, pupal mortality, quercetin, rutin, *Spodoptera litura*

### INTRODUCTION

Durable resistance to insects is mainly due to chemical constituents (allelochemicals) present in the host plants such as alkaloids, flavonoids, terpenoids, sterols, etc., Plant phenolics are a large group of secondary metabolites in plants that affect herbivores larval growth and development either by feeding inhibition, or in post-ingestive phenomena (Treutter, 2006). Among them, flavonoid compounds quercetin, chlorogenic acid and rutin are ubiquitous among diverse crop species and contribute as dietary constituents (Isman and Duffey, 1983) and serve as bases of resistance in several crop plants (Campbell and Duffey, 1979; Elliger *et al.*, 1980, 1981; Kennedy, 2003). A complete photochemistry of two flavonoids is provided by Sisa *et al.* (2010). A recent review on flavonoids in plant resistance has been provided by Treutter (2006). They also showed antibiotic and or antifeedant effect by reducing the growth and extending developmental cycle and decreasing survivorship of many herbivorous insects (Shaver and Lukefahr, 1969;

Harborne, 1979; Duffey *et al.*, 1986; Barbosa, 1988; Stamp, 1990; Stamp and Horwath, 1992) such as *Manduca sexta*; *Heliothis virescens*; *Trichoplusia ni* (Hoffman-Campo *et al.*, 2001); and *S. litura* (Mallikarjuna *et al.*, 2004a). In insects, phytochemicals including the flavonoids interfere with molting, reproduction, feeding behaviour (Reyes-Chilpa *et al.*, 1995; Musayimana *et al.*, 2001; Diaz Napal *et al.*, 2010). In addition, their effects also influence the insects in recognizing their host plants.

Economically important pests include the pod borer, *Helicoverpa armigera* (Hubner) on pigeonpea [*Cajanus cajan* (L.)] chickpea (*Cicer arietinum* L.) and cotton (*Gossypium hirsutum* L.); and the tobacco caterpillar [*Spodoptera litura* (Fab.)] on groundnut (*Arachis hypogaea* L.) and cotton in Asian-African countries (Sharma *et al.*, 2001). Majority of the farmers still heavily rely on synthetic chemicals to control these pests. This has led to increased environmental pollution and alternatively there is an increasing need to introgress resistance present in the wild relatives of pigeonpea as cultivated germplasm lacks the desired

levels of resistance. Wild relatives of pigeonpea, chickpea and groundnut are valuable sources of germplasm with the presence of many desirable traits including resistance to many insect pests and efforts are being made to increase the levels of resistance. In such a scenario it would be desirable to know what causes resistance to insect pests. Studies in groundnut have shown that presence of flavonoids namely chlorogenic acid, quercetin and rutin confer resistance to *Spodoptera litura* (Stevenson *et al.*, 1993) and it was possible to introgress this resistance through interspecific hybridization (Mallikarjuna *et al.*, 2004 a, b).

There is evidence that flavonoids chlorogenic acid, quercetin and rutin are present in wild *Cajanus* species (Mallikarjuna N, unpublished data). The objective of the present investigation was to assess the effect of quercetin, rutin and chlorogenic acid on growth and development in diet-based bioassays of the pod borer, *H. armigera* and tobacco caterpillar, *S. litura* made to increase the levels of resistance.

## MATERIALS AND METHODS

All the experiments were carried out in the Department of Botany, Dr Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra State, India, during 2008-2009, to study the effect of quercetin, chlorogenic acid and rutin on the survival, growth and development of two key pests viz., *H. armigera* and *S. litura*.

**Insect rearing:** *H. armigera*: Larvae of *H. armigera* were collected (approx.200), during the rainy season of 2008-2009, from an unsprayed weed, *Lagascea mollis* usually grown in uncultivated farmers fields. They were reared individually in 7.5 mL cells of 12-well tissue culture plates (Linbro, ICN Flow Ltd.), on chickpea-based artificial diet (Armes *et al.*, 1992). After pupation, they were transferred to small round plastic cups (4.5 cm). Adults were transferred to cylindrical cages, wherein 10% honey-soaked cotton swabs was provided for feeding; and eggs were collected on a nappy liner placed inside the wall of the cylindrical cage.

*S. litura*: Egg-masses of *S. litura* were collected from unsprayed groundnut crop during the post-rainy season. In each egg-mass, there were approximately 200-300 eggs and were pinned on the artificial sorghum-based diet (Armes *et al.*, 1997; Taneja and Leuschner, 1985) to enable the neonates on hatching, to feed. On pupation, they were separated from the diet and kept for adult emergence, which were fed with 10% honey solution in a cylindrical cage. Egg-masses laid on a thick blotting paper were collected from these cages.

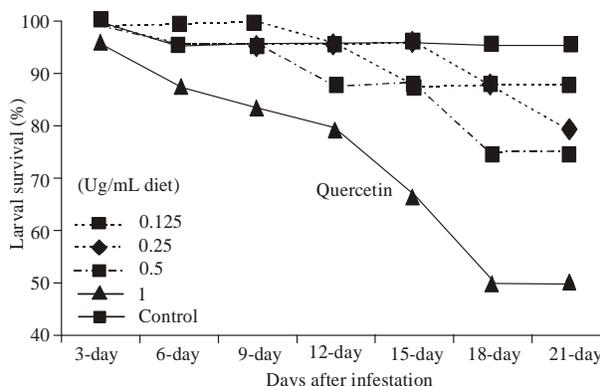


Fig. 1: Larval survival of *H. armigera* on artificial diet based bioassays with quercetin

**Insect bioassays:** Three flavonoids (quercetin, chlorogenic acid and rutin) were obtained from Sigma Chemicals, New York. Bioassays of these compounds were conducted, at four concentrations (0.125, 0.250, 0.500 and 1.0  $\mu\text{g/mL}$ ) by thoroughly mixing them in the artificial diets separately for *H. armigera* and *S. litura* and poured in rearing containers. Controls were solely based on chickpea-based and sorghum-based diets. Soon after hatching, 24 neonate larvae were tested for each concentration in 25 mL plastic cups containing test diets. Data were recorded on the weights, survival and duration of larvae and weight, duration and mortality of pupae for both these insect species. These bioassays were carried out at  $25 \pm 2^\circ\text{C}$ ,  $60 \pm 10\%$  RH and 14:10 (L: D) in a Samsung incubator. The survival of larvae was monitored at 3-day intervals, expressed in percentage, feeding and development time were recorded and expressed in days; and the pupae were weighed two days after pupation and the duration in days and mortality expressed in percentage. In addition, the emergence of healthy and malformed adults was also recorded.

**Statistical analysis:** The data collected for individual larvae were considered as replicates. Data was subjected to Analysis of Variance (ANOVA) with a separation of means following LSD (0.05).

## RESULTS

**Larval survival:** Survival of larvae of *H. armigera* was in general higher on quercetin-based diet as compared to chlorogenic acid and rutin based diets. Among the concentrations of quercetin, the survival was low on 1.0  $\mu\text{g/mL}$  diet. While the survival was relatively high showing gradual decrease proportionate to the increase in concentrations (Fig. 1). In chlorogenic acid-diet based bioassays, a similar trend of larval survival was observed in terms of higher survival at low

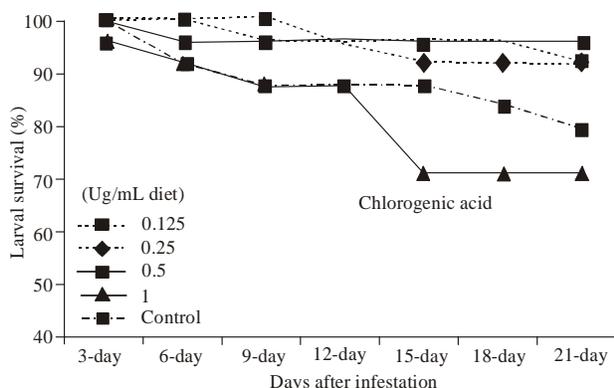


Fig. 2: Larval survival of *H. armigera* on artificial diet based bioassays with chlorogenic acid

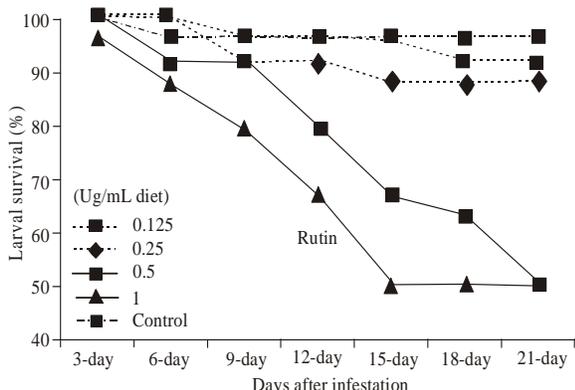


Fig. 3: Larval survival of *H. armigera* on artificial diet based bioassays with rutin

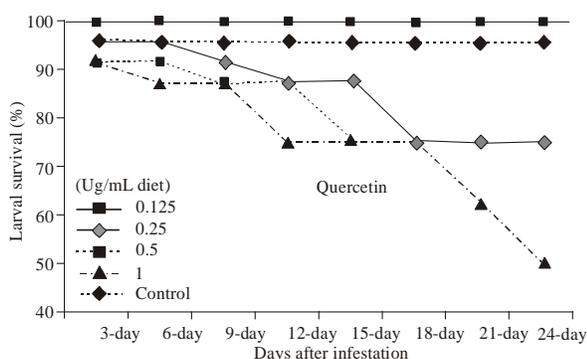


Fig. 4: Larval survival of *S. litura* on artificial diet based bioassays with quercetin

concentrations and with a gradual decrease in survival at higher concentrations. Further, the decrease in survival of larvae was observed up to a period of day 2nd remained status quo at further observation intervals (Fig. 2). Whereas, the larval survival on rutin-based diets displayed greater mortality of larvae at 0.250 ug/mL with a proportionate increase in larval mortality at higher concentrations (0.500 and 1.000 ug/mL). Moreover, the mortality was observed with a

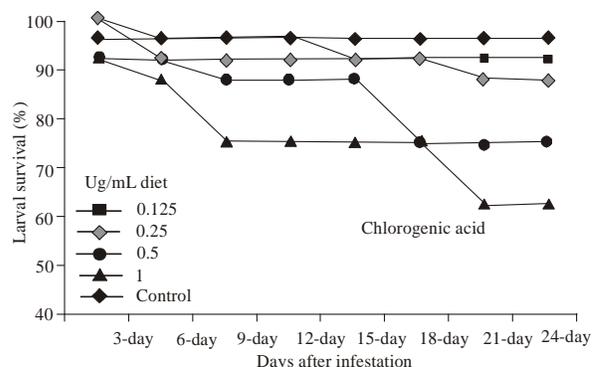


Fig. 5: Larval survival of *S. litura* on artificial diet based bioassays with Chlorogenic acid

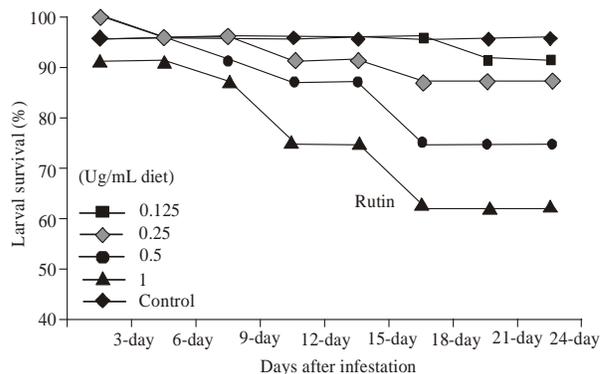


Fig. 6: Larval survival of *S. litura* on artificial diet based bioassays with rutin

gradual increase at all the observations upto a period of day-21 (Fig. 3).

With respect to *S. litura*, the quercetin-based bioassay showed greater mortality of larvae at higher concentration (1.0 ug/mL) as compared to lower concentrations (0.500, 0.250 and 125 ug/mL) (Fig. 4). Whereas the chlorogenic diet-based assay recorded greater survival at lesser concentrations associated with a gradual increase at higher concentrations (Fig. 5). However, the rutin-diet displayed greater percent survival of larvae at all the concentrations tested (Fig. 6). In general, it has been noticed that larval survival was greater on rutin-based diet for *S. litura* contrary to those for *H. armigera*.

**Larval duration:** Larval duration for *H. armigera* was between 18-21 days in all the concentrations except in rutin at 1.0 ug/mL which was of 32 days which is significantly different from the control. The larval duration in *S. litura* to become pupae was exceedingly high in rutin compared to chlorogenic acid and quercetin, at all concentrations in the assayed diet (Fig. 7).

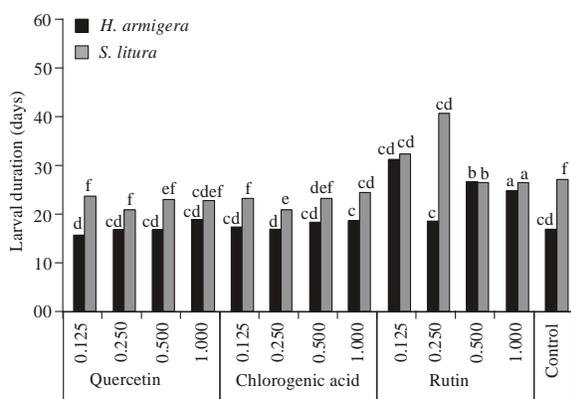


Fig. 7: Larval duration of *H. armigera* and *S. litura* with different concentrations of quercetin, chlorogenic acid and rutin in  $\mu\text{g ml}^{-1}$  diet based bioassays

**Larval weight:** Larval weights for *H. armigera* on flavonoid compound-based diet were terminated at day-

18. Rutin-based diet had a significant effect on the larval weights at all the concentrations tested. Whereas, chlorogenic acid showed an intermediate response in terms of larval weights with a proportionate decline at higher concentrations. On the other hand, quercetin-based diet bioassay displayed greater increase in larval weights at lesser concentrations as compared to higher concentrations. This trend changed completely from day-12 to day-18 with a sudden increase in larval weights on the diet containing chlorogenic acid in relation to quercetin, while more than 80% of the larvae had pupated in the control (Table 1).

Diet-based bioassays of *S. litura* larvae with flavonoid compounds showed a slow increase in larval weights on day-3, 6, 9 and 12 and a significant increase at 3-day intervals, from day 15-24 to day. However, the rutin-based diet had a significant effect in displaying low larval weights as compared to the diets comprising quercetin and chlorogenic acid. In general, there was a significant trend with low larval weights at higher larval

Table 1: Larval weights of *H. armigera* measured at three day intervals when reared on artificial insect diet with different concentrations of chlorogenic acid, rutin and quercetin

Chemical constituents	Larval weights (mg)						
	Days After Infestation (DAI)						
	3	6	9	12	15	18	21
<b>Chlorogenic acid (ug/mL diet)</b>							
0.125	4.09±0.42c	29.28±1.83de	133.07±3.61a	258.70±3.80a	363.10±3.81a	442.21±8.50a	454±3.55a
0.250	3.51±0.19cd	24.87±2.61ef	122.57±2.25abc	225.81±5.61ab	338.82±8.10ab	405.72±8.00ab	412±2.69ab
0.500	3.00±0.25cde	19.63±2.73fg	115.69±6.01abc	212.90±6.45abc	293.32±10.72bc	313.82±11.22de	318±6.51bc
1.000	2.36±0.33ef	11.44±2.35gh	105.04±8.1bc	181.42±4.52bcd	258.50±7.9cd	275.27±15.82ef	283±3.26c
<b>Rutin (ug/mL diet)</b>							
0.125	2.49±0.58def	14.17±3.69gh	30.05±5.98d	77.11±12.61e	163.59±16.34ef	270.31±17.11ef	281±2.75a
0.250	1.85±0.27fg	8.29±1.38gh	23.35±4.71d	46.99±8.78ef	108.19±12.47fg	245.81±18.07f	265±3.13a
0.500	1.48±0.19fg	4.64±3.05h	22.01±3.05d	44.58±4.35ef	113.7±8.81g	193.82±9.31g	213±5.73ab
1.000	1.16±0.31g	4.13±2.21h	20.62±4.05d	33.04±6.16f	75.93±7.96g	145.85±11.12g	151±6.11b
<b>Quercetin (ug/mL diet)</b>							
0.125	6.56±0.38a	55.26±2.78a	111.42±6.58abc	190.11±9.62bcd	261.61±12.31bc	345.71±15.6cd	361±3.15a
0.250	5.31±0.67 b	43.95±4.98bc	108.66±11.75abc	173.12±14.31cd	255.15±12.91cd	318.33±7.51de	332±5.24ab
0.500	4.00±0.46c	35.89±2.75bcd	101.01±7.19bc	164.81±6.32d	248.71±10.73cd	302.41±8.91de	319±3.18b
1.000	3.65±0.35c	34.32±2.37cde	98.61±4.73c	156.32±5.72d	228.31±8.22de	251.91±14.75f	276±1.76b
Control	6.86±0.34a	44.87±6.11b	125.59±8.99 ab	217.39±9.21abc	294.65±9.11bc	369.19±9.42bc	---

> than 50% larvae had pupated on day 18 in the controls; Letters followed by the same letter are not significantly different at <0.05%

Table 2: Larval weights of *S. litura* measured at three day intervals when reared on artificial insect diet with concentrations of chlorogenic acid, rutin and quercetin

Flavonoid compound	Larval weights (mg)							
	Days after infestation (DAI)							
	3	6	9	12	15	18	21	24
<b>Quercetin (ug/mL diet)</b>								
0.125	2.66±0.07bc	7.27±0.15bc	18.24±0.18b	89.63±1.62bc	342.87±4.10b	761.29±8.68ab	902.98±7.98b	1444.27±9.57ab
0.250	2.37±0.08def	6.50±0.12bcde	16.08±0.09bc	61.39±0.92bc	282.93±6.16bc	728.93±3.06bc	882.83±2.16b	1356.29±3.45ab
0.500	1.59±0.07ef	5.63±0.14bcde	11.19±0.15bc	52.52±1.69bc	259.98±5.52bcd	717.20±10.50bcd	716.47±4.58b	1245.35±4.50ab
1.000	1.32±0.07g	4.28±0.16c	9.18±0.16bc	41.53±2.20bc	221.19±3.80ef	617.87±7.41d	888.11±4.86c	1165.62±3.34abc
<b>Chlorogenic acid (ug/mL diet)</b>								
0.125	2.76±0.07bcd	7.15±0.16b	17.76±0.12b	79.54±1.52b	387.21±2.74bcd	919.38±7.61bcd	1343.84±7.58c	1672.54±3.56ab
0.250	2.14±0.04cde	5.44±0.08bcd	12.10±0.13bc	69.77±0.67bc	374.96±5.01bcde	835.85±7.03bcd	1260.96±3.04c	1565.45±4.55abc
0.500	2.09±0.37fg	5.16±0.13bcde	10.65±0.09bc	57.79±0.81bc	342.44±4.57de	810.14±7.69bcd	1187.18±4.74cd	1481.11±7.02abc
1.000	1.28±0.06g	3.61±0.08de	9.15±0.10bc	39.52±1.03bc	226.03±4.84efg	671.55±8.54d	904.95±12.30cde	1299.83±12.01c
<b>Rutin (ug/mL diet)</b>								
0.125	2.92±0.07b	4.43±0.17bcde	11.00±0.23bc	44.51±0.83bc	213.68±4.33efg	404.85±9.16e	675.61±6.39de	882.47±8.27d
0.250	2.43±0.06bcde	4.87±0.17cde	10.24±0.45bc	39.41±0.61bc	117.09±0.16fgh	346.36±0.48e	600.04±2.19e	872.23±4.32d
0.500	2.28±0.05cde	3.46±0.17c	9.65±0.19bc	31.34±0.15bc	110.92±0.11gh	316.70±0.69e	572.97±18.45e	775.05±8.74d
1.000	1.85±0.08efg	2.99±0.19e	8.17±0.18c	29.41±0.88c	92.84±0.62h	258.15±3.55e	332.15±6.94f	449.72±10.19e
Control	5.32±0.31a	20.87±0.29a	65.90±1.22a	396.53±3.62a	801.45±5.52a	1070.16±8.33a	1578.65±19.12a	1688.17±1.81a

Note: > than 75% larvae had pupated after 21 days in the controls

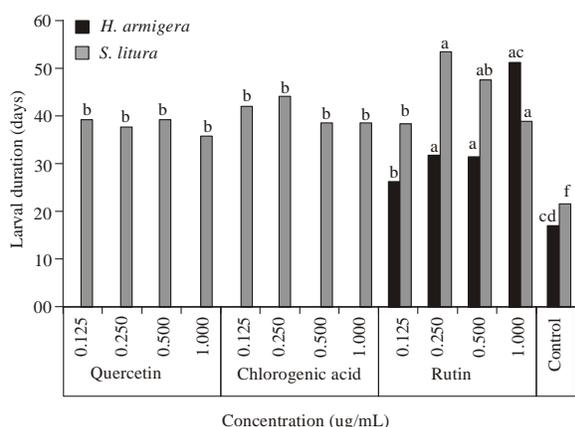


Fig. 8: Extended larval duration and mortality of *H. armigera* and *S. litura* with different concentrations of quercetin, chlorogenic acid and rutin in  $\mu\text{g ml}^{-1}$  diet-based bioassay

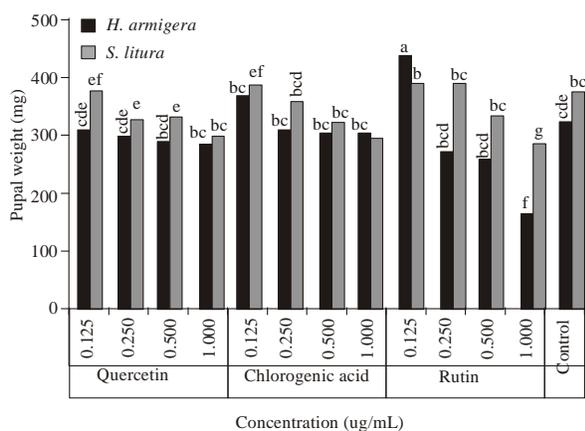


Fig. 9: Effect of flavonoid compounds on pupal weights of *H. armigera* and *S. litura*

weight till day 24 and more than 75% of the larvae had pupated (Table 2).

**Extended larval duration:**

Differences were observed on the extended larval duration of *H. armigera* and *S. litura* bioassayed in flavonoid compound based -diets. In *S. litura*, all the

concentrations over lesser concentrations for all the flavonoid compounds tested in the diet-based bioassays. In comparison to diets with flavonoids, the control without flavonoids showed continuous increase in three acids (quercetin, chlorogenic acid and rutin) caused extended larval duration of more than 40 days causing mortalities in all the concentrations (0.125, 0.250, 0.500 and 1.000 mg/mL). Whereas in *H. armigera* extended larval duration was observed on the diet with rutin alone between 27- 52 days in all the four concentrations causing mortality (Fig. 8).

**Pupal weight:** With respect of pupal weight, significant differences were observed on the weight of pupae of both *H. armigera* and *S. litura*. Among the flavonoid compounds, in *H. armigera*, rutin alone significantly decreased the pupal weight at higher concentrations (0.250, 0.500 and 1.000  $\mu\text{g/mL}$ ) but not at lower concentration (0.125  $\mu\text{g/mL}$ ), while the chlorogenic acid and quercetin-based diets showed moderate increase in pupal weight while in *S. litura*. Rutin concentration of 0.125  $\mu\text{g/mL}$  was comparable to the control although slightly higher in *H. armigera* and *S. litura* (Fig. 9).

**Pupal duration:** Similar trend of significant differences in the duration of pupae of *H. armigera* and *S. litura* was observed. However, quercetin showed significant differences in extending the duration of pupae of *H. armigera* and *S. litura* at high concentration of (1.000  $\mu\text{g/mL}$ ) followed by lower concentrations. While the chlorogenic acid and rutin-based diets showed intermediate duration of pupae of 15-17 days (Table 3 and 4).

**Adult emergence:** Significant differences were observed on healthy and malformed adult emergence of *H. armigera*. Among the flavonoid compounds tested. The emergence of healthy and malformed adults was significantly greater at lower concentrations as compared to higher concentrations of quercetin, chlorogenic acid and rutin-based bioassays. However, rutin had a detrimental effect on emergence of healthy

Table 3: Pupal duration of *Helicoverpa armigera* at different concentrations of chlorogenic acid, quercetin and rutin in the artificial diet

Chemical constituents (ug/mL diet)		Pupal duration (days)			
		Concentrations (ug/mL diet)			
		0.125	0.125	0.500	1.000
Quercetin	Mean±SE	17.38±0.16 a	17.38±0.16a	14.76±0.40b	18.47±0.46a
	Range	(16-18)	(16-18)	(13-17)	(14-21)
Chlorogenic acid	Mean±SE	15.79±0.42a	15.79±0.42a	15.79±0.58a	14.75±0.45b
	Range	(13-20)	(13-20)	(13-22)	(12-17)
Rutin	Mean±SE	16.00±2.00a	16.00±2.00a	21.50±1.45a	18.0±00a
	Range	(14-18)	(14-18)	(17-29)	(18.00±00)
Controls	Mean±SE	12.90±0.34b	12.90±0.34b		
	Range	(10-16)	(10-16)		

Letters followed by the same letter are not significantly different at <0.05%

Table 4: Pupal duration of *Spodoptera litura* at different concentrations of chlorogenic acid, quercetin and rutin in the artificial diet

Chemical constituent (ug/mL diet)		Pupal duration (days) Concentrations (ug/mL diet)			
		0.125	0.250	0.500	1.000
Chlorogenic acid	Mean±SE	13.44±0.51a	15.75±0.16a	16.33±0.19a	16.09±0.21a
	Range	(11-18)	(15-17)	(15-17)	(15-17)
Quercetin	Mean±SE	15.13±0.29a	16.83±0.32a	17.18±0.18a	16.92±0.36a
	Range	(14-18)	(10-13)	(16-18)	(14-18)
Rutin	Mean±SE	16.50±0.50a	15.26±0.5a	16.71±0.61a	18.0±00a
	Range	(16-17)	(11-14)	(9-14)	(17-19)
Controls	Mean±SE	11.41±0.33b			
	Range	(8-13)			

Letters followed by the same letter are not significantly different at <0.05%

adults compared to chlorogenic acid and quercetin-based diets.

Adult emergence of *S. litura* also showed differences for healthy and malformed adults. Among the flavonoid compounds tested, rutin had a significant effect for emergence of healthy adults over malformed adults. Whereas quercetin-based bioassay showed moderate effect and chlorogenic acid had a significant effect on healthy adult emergence. In general, the rutin had a significant effect. While the malformed adult emergence was significantly higher on chlorogenic acid diet followed by quercetin and rutin-based bioassays.

## DISCUSSION

One of the interesting research studies of this experiment is that rutin has significant effect on the inhibition of larval growth by *H. armigera*. A considerable proportion of the larvae in the increasing concentrations of 0.500 and 1.000 ug/mL of diet spent between 30-51 days of excess time in larval stage in the higher/late instar period which had a negative impact on the growth because of cessation of feeding by the larvae. The individual larvae had formed a cocoon of the diet and were passive for longer period without feeding, which ultimately resulted in their mortality. Though there were larval mortalities in the 0.125 and 0.250 ug/mL diets, the mean time spent in cessation period was 27-32 days. Rutin tended to prolong the non-feeding period and the stadium duration and onset of molting. These results indicate that rutin did not affect the larval weight at 0.125 and 0.500 ug/mL, but had considerably lesser weight gain at 0.250 and 1.000 ug/mL. Earlier studies have indicated poor growth of larvae of *H. zea* and *S. exigua* when rutin was incorporated into the artificial diet (Isman and Duffey, 1981, 1982; Duffey and Bloem, 1986; Kennedy, 2003). In contrast, rutin has a greater impact in the process of moulting by interfering with the prothoracicotropic hormone and ecdysteroid action causing mortality (Nijhout and Williams, 1974; Curtis *et al.*, 1984; Riddiford, 1985; Sehna, 1985).

In the present study, the finding that rutin induced an increase in the proportion of time spent by *H. armigera* in the non-feeding period is consistent with

other studies (Stamp and Horwath, 1992, 1993; Hoffman-Campo *et al.* 2001) found that rutin negatively affected the survival of cabbage looper *Trichoplusia ni* larvae at higher and lower concentrations of 0.25 and 0.5% and decreased their pupal weights considerably. It may be the negative effect of rutin on growth in these studies and reflects prolongation of growth period including the extension of molt period or vice versa. Presently, we are unable to explain the mechanism by which rutin exerts these oxidizing effects on molting delay.

However, the other two flavonoids used in this experiment chlorogenic acid and quercetin did not cause growth inhibition in the larvae of *H. armigera*. However, Ali *et al.* (1999) did not find any correlation of larval weights of *H. virescens* raised on artificial diet with various concentrations of chlorogenic acid derived from tobacco foliage. Isman and Duffey (1983) working with chlorogenic acid and rutin demonstrated the uptake of these plant phenolics into the haemolymph of 5<sup>th</sup> instar *Heliothis zea* (Boddie) larvae by oral administration within 1 hr amounting to 5% or less of the ingested dose. They also fed these phenolics through artificial diet to *H. zea* larvae and results indicated through thin layer chromatography that either through chronic or by acute feeding 90% of the ingested phenolic was excreted by the larvae. Though healthy looking adult emergence was quite common amongst all the concentrations of chlorogenic acid and quercetin, the moths could not produce any progeny. There was some percentage of moths with malformed emergence. Beninger *et al.* (2004) found significantly reduced growth of cabbage looper *Trichoplusia ni* and gypsy moth *Lymantria dispar* larvae when chlorogenic acid was added to the artificial diet at both 100 and 1000 ppm and reduced pupal weights of *Trichoplusia ni*. Chlorogenic acid levels correlate with resistance to carrot fly *Psila rosae* F. (Ellis, 1999) and are a major factor in the resistance of corn to both fall armyworm, *Spodoptera frugiperda* and corn earworm, *Helicoverpa zea* (Guedner *et al.*, 1992). Kranthi *et al.* (2003) reported that semilooper *Anomis flava* Fab., feeding on in vivo plants induced an increased concentration of quercetin, which caused growth inhibition of the larvae

of *H. armigera*. While laboratory bioassays with tobacco budworm, *H. virescens* indicated that 0.2 and 0.063% concentration of quercetin showed greater mortality (Shaver and Lukefahr, 1969) and 75% weight inhibition of the larvae, respectively (Jenkins *et al.*, 1983). On the other hand, higher concentration of rutin and chlorogenic acid in the trichomes of tomato leaves are reported to be toxic to corn earworm, *H. zea* (Bi *et al.*, 1997). Simmonds and Stevenson (2001) and Simmonds (2001) reported that chlorogenic acid tested in combination with other isoflavonoids at 50 ppm induced an antifeedant response by larvae of *H. armigera*. Similarly, rutin also deters feeding by *H. zea* and *H. armigera* at concentrations in excess of  $10^{-3}$  (Blaney and Simmonds, 1983). Kimmins *et al.* (1995) observed significant retardation of *H. armigera* larvae and increased days to pupation at 9 mM with chlorogenic acid in the artificial diet. Onyilagha *et al.* (2004) observed that dihydroquercetin in *Brassica napus* reduced both the larval weight as well as larval and pupal development time in *Mamestra configurata* Walker. Similarly (Guerra *et al.* (1990) observed high larval mortality and increased the time required for the larvae to reach pupation and reduced rate of larval development with catechol phenolics supplemented in the artificial diet against *H. zea*. Larval weights were not correlated with the level of chlorogenic acid in tobacco foliage (Ali *et al.*, 1999). Bernays *et al.* (2000) studied behavioral aspects of *H. virescens* and *H. subflexa* and demonstrated that higher concentrations of chlorogenic acid caused post-ingestive effects in *H. virescens*, but deterred feeding by *H. subflexa* on first contact. This was attributed to the differences in tradeoffs involved in different diet breadths by *H. virescens* and *H. subflexa*.

In case of *S. litura*, rutin alone had significant effect on arrested larval development, pupal mortality and malformed adults compared to quercetin and chlorogenic acid contents in artificial diets. Although increasing concentrations of rutin did not show proportionate increase in mortality of the larvae, the average excess days at all the concentrations of quercetin and chlorogenic acid ranged between 40-55 days. Unlike *H. armigera* larvae, the larvae of *S. litura* did not form any cocoons during their cessation of feeding. The high degree of repellence was observed from wild tomato suggesting that both the larvae of *H. zea* and *S. exigua* cease feeding altogether and starved to death at high sesquiterpene carboxylic acids (Frelichowski and Juvik, 2001) and acylglucoses (Juvik *et al.*, 1994). While Stevenson *et al.* (1993) terminated the experiments with *S. litura* with pupation in control diets. Mallikarjuna *et al.* (2004a) demonstrated a combined effect of all the three flavonoids (quercetin, chlorogenic acid and rutin) in lines derived from wild *Arachis* spp. various developmental stages of larval,

pupal and moth deformities, thus leading to significant mortalities. Upasani *et al.* (2003) also indicated the role of flavonoids in *Ricinus communis* L. as insecticidal and antimicrobial agents against the bruchid, *Callosobruchus chinensis* L. Traugott and Stamp (1997) found no effect of chlorogenic acid mixed in the artificial diet on *Manduca sexta*, including its predator, *Podisus maculiventris*.

From these experiments with bioassay tests on artificial diets containing rutin and chlorogenic acid indicated the presence of antibiosis factors affecting the larval development. Increased duration or retardation of larvae suggests either inability to feed or utilize the food, or molt and predispose the larvae to parasites and predators or influence the hormonal imbalance leading to delay/extended larval period. Thus, these chemicals may play a significant role in tritrophic interactions extending the larval duration and fall prey to parasites and predators (Van Emden, 1987). The ecological consequences could be many if the larvae feeding for extended duration on plants could face, are, changes in the quality of the food material i.e., extended duration means more time will be spent on poor quality of food, which will necessitate either pupating at lower weight, which will be correlated with poor fecundity. Also the larvae if they are molting are defenseless against parasitoids/predators (Kennedy, 2003). Duffey *et al.* (1986) suggested breeding of plants with relative levels of phenolics to maintain compatibility with the parasitoids without having any adverse effect on them. The results from this study indicate that the presence of three flavonoids may play an important role for resistance to *H. armigera* and *S. litura* not only in groundnut as indicated by the studies of Mallikarjuna *et al.* (2004 a, b) but also in pigeonpea. Many of the wild relatives of pigeonpea have been successfully crossed with cultivated pigeonpea and stable pre-breeding lines have been generated. Some of the lines have multiple disease and pest resistance including resistance to *H. armigera* (Mallikarjuna *et al.*, 2004a, b). The results of the present study throw light on the biochemical basis of resistance.

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