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Incorporation of lyophilized leaves and pods into artificial diet to assess antibiosis component of resistance to pod borer in pigeonpea

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ABSTRACT

***Helicoverpa armigera*, is the most damaging insect pest of grain legumes including pigeonpea in the semi-arid tropics, and host plant resistance is an important component for the management of this pest. Because of the variation in insect density over space and time, it is difficult to assess the contribution of different components of resistance to this insect under field conditions. Therefore, we standardized a bioassay involving incorporation of lyophilized leaves or pods into the artificial diet to assess antibiosis component of resistance to *H. armigera*. Antibiosis was assessed in terms of larval mortality, larval and pupal weights, adult emergence, and duration of development on fresh leaves, flowers and pods, and through incorporation of lyophilized leaves and pods of different pigeonpea genotypes into the artificial diet. Incorporation of 10 g of lyophilized leaf or pod powder into the artificial diet (300 ml) of diet resulted in maximum differences in survival and development of *H. armigera* larvae on the resistant (ICPL 332) and susceptible (ICPL 87) genotypes. Reduced larval and pupal weights, and prolongation of larval and pupal development periods were observed in insects reared on intact leaves or pods of ICPL 332, ICPL 84060, ICP 7035, ICPL 88039 and T 21. Similar effects were also observed in larvae reared on artificial diet impregnated with lyophilized leaves or pods of ICPL 332, ICPL 84060, ICP 7035, ICPL 187-1, ICPL 88039, and ICP 7203-1. Larval and pupal periods, pupal weight, and pupation and adult emergence were positively correlated between the insects reared on fresh leaves or pods, and on artificial diets impregnated with lyophilized leaves or pods. However, there was no correspondence in terms of larval weight and mortality between the fresh plant parts and diet impregnation assay. Incorporation of lyophilized leaves or pods of pigeonpea into artificial diet for assessing the antibiosis component of resistance to *H. armigera* has been discussed.**

Key words: Antibiosis, Diet impregnation assay, *Helicoverpa armigera,* Host plant resistance, Pigeonpea

The cotton bollworm or legume pod borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae), is the most important pest of grain legumes including pigeonpea in the semi-arid tropics (Sharma 2005). Its control on pigeonpea and other high value crops depends heavily on synthetic insecticides. It has long been recognized that host plant resistance can play an important role in minimizing the extent of losses due to *H. armigera* (Sharma *et al*. 2005a). Pigeonpea genotypes with resistance to pod borer, *H. armigera* have been reported by several workers (Nawale and Jadhav 1983, Patnaik *et al*. 1989, Lateef and Pimbert 1990, Sachan 1990, Borad *et al*. 1990, Kalariya *et al*. 1998, Venkateswarlu and Singh 1999). However, the levels of resistance in the cultivated germplasm have been found to be low to moderate, and the expression of resistance is not stable across seasons and locations (Lateef and Pimbert 1990, Sharma *et al*. 2005a). Some of the variation in genotypic reactions to damage by *H. armigera* is due to changes in onset of insect infestation, severity of infestation, and genotype x environment interactions. Therefore, it is important to increase the levels of resistance to *H. armigera* in pigeonpea through gene pyramiding (combining 2 to 3 genes from diverse sources for different mechanisms of resistance), which requires an in-depth understanding of different mechanisms of resistance to the target insect, and precise evaluation of the available sources of resistance for different components of resistance. It is difficult to evaluate pigeonpea genotypes for different components of resistance to *H. armigera* under field conditions because of staggered flowering of different pigeonpea genotypes, and variation in insect density over space and time. As a result, the success in screening and breeding for resistance to *H. armigera* has not been as efficient as for plant pathogens. In addition, assessment of antibiosis component of resistance on fresh plant parts under laboratory conditions is influenced by possible changes in the relative amounts of primary and secondary plant metabolites. Therefore, we evaluated a bioassay technique involving incorporation of lyophilized leaves or pods of different pigeonpea genotypes into the artificial diet to assess antibiotic component of resistance to *H. armigera*.

MATERIALS AND METHODS

Insect culture

H. armigera larvae were obtained from the laboratory culture maintained in the insect rearing laboratory at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. The laboratory culture was regularly supplemented with field-collected insects to maintain heterogeneity in the insect

culture. The larvae were reared on the chickpea based artificial diet (Armes *et al*. 1992) at 27$\pm$1^{0}C, 65 $\pm$ 5% RH, and 12 h photoperiod. The adults were released in a 30 x 30 x 30 cm cage having nappy liners on the sides for oviposition. The adults were fed on 10% sucrose solution in absorbent cotton. Eggs laid on the nappy liners were sterilized with 1% sodium hypochlorite, and transferred into 200 ml plastic cups smeared with 2 mm thick layer of artificial diet for rearing groups of 250 larvae. After 5 days, the larvae were transferred to six cell-well plates (having 5 to 7 ml artificial diet in each cell well), and reared individually till pupation. Neonate larvae from this culture were used for assessing antibiosis component of resistance in fresh leaves and pods of different pigeonpea genotypes, and through incorporation of lyophilized plant parts into the artificial diet.

Plant material

The antibiosis component of resistance was studied in 12 pigeonpea genotypes in terms of survival and development of neonate larvae of *H. armigera* on as leaves, flowers and pods, and by incorporating lyophilized leaf and pod powder into the artificial. The test material included a diverse array of *H. armigera* – resistant (ICP 7035, ICP 7203-1 and T 21 – germplasm sources of resistance to pod borer; ICPL 98001, ICPL 98008 and ICPL 88039 – early-maturity improved indeterminate type genotypes with less susceptibility to pod borer; and ICPL 187-1, ICPL 332 and ICPL 84060 – medium maturity improved pod-borer resistant genotypes) and susceptible genotypes (ICPL 87, ICPL 87119 and ICPL 87091). The plants were grown at the farm of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, in the field (black Vertisol soils) on ridges 75 cm apart. Each genotype was planted in 4 row plots, 4 m long, and there were three replications in a randomized complete block design. The plants were thinned to a spacing of 15 cm between the plants 15 days after seedling emergence. The crop was raised under rainfed conditions between June to December. There was no insecticide application in this crop. The survival and development of neonate larvae of *H. armigera* was studied on fresh plant parts brought to the laboratory and on artificial diet containing the lyophilized leaves or pods of different genotypes.

Survival and development of neonate larvae of Helicoverpa armigera on leaves, flowers, and pods of different pigeonpea genotypes

Under natural conditions, *H. armigera* larvae feed on tender leaves of pigeonpea when flowers and pods are not available before flowering or during heavy infestations. During the reproductive stage of the crops, the neonate larvae feed on flowers. The larger sized larvae (3rd to 5th instar) feed on seeds in the green pods. Therefore, we studied the antibiosis component of resistance to *H. armigera* on leaves, and on flowers and pods to follow the natural feeding behavior of this insect on pigeonpea.

Leaves

Survival and development of neonate *H. armigera* larvae was studied on first fully expanded leaves of pigeonpea genotypes under laboratory conditions. The leaf material obtained from the field was placed in 250 ml plastic cups using detaches leaf assay (Sharma *et al*. 2005b), and 10 neonate larvae were released on the leaves with the help of a fine camel hair brush. A moistened filter paper (7.5 cm diameter) was attached to the inner side of the lid, and the plastic cups were covered immediately. The plastic cups were kept in the laboratory at 27 ± 2^{0}C and 45 to 65% RH. The leaves were changed every alternate day. From fifth day onwards, the larvae were reared individually to avoid cannibalism. Larval weights and mortality were recorded at 10 days after initiating the experiment. Data were also recorded on larval and pupal periods, pupal weights, and adult emergence. Pupal weights were recorded one day after pupation. There were 5 replications in a completely randomized design.

Flowers and pods

Under natural conditions, the larvae first feed on flowers, and then on pods. Therefore, neonate *H. armigera* larvae were first fed on the flowers for 5 days, and then transferred to pods of respective pigeonpea genotypes. The flowers and pods were kept in a Petri dish (7.5 cm diameter) having a moistened filter paper attached to the lid. Ten larvae were released in each Petri-dish. After five days, the larvae were reared individually. The Petri dishes were kept under laboratory conditions as described above. Larval weights were recorded at 10 days after release. Data were also recorded on larval and pupal periods, pupal weights, and adult emergence. The experiment was conducted in a completely randomized design, and there were five replications.

Impregnation of lyophilized leaves and pods of pigeonpea into artificial diet to assess antibiosis component of resistance to Helicoverpa armigera

Leaves and pods of different pigeonpea genotypes were collected from plants grown under field conditions. Three leaves from the terminal end were collected at two months after seedling emergence, and freeze-dried in lyophilizer (-50^{o}C, and 250 mbar pressure) for 36 h to avoid changes in chemical composition of the leaves. The material was powdered in a Willey mill to <80 mesh size, and stored in a desiccator for use in diet impregnation assay. The pods were harvested at 10 to 15 days after flowering, lyophilized, and powdered as described above.

To determine the amount of leaf or pod powder needed in the artificial diet to quantify the antibiosis component of

resistance to *H. armigera*; 5, 10, 15, and 20 g of pigeonpea leaf powder of ICPL 87 (susceptible check) and ICPL 332 (resistant check) was added in diet components enough for 300 ml of artificial diet. Pigeonpea leaf powder was soaked in 100 ml of warm water (70°C) and blended with fraction-A of the artificial diet (Table 1) for two minutes. Agar-agar was boiled in 80 ml of water (fraction-B), cooled to 40°C, and then poured into the blender containing the fraction A. After adding formaldehyde, all the constituents were blended for three minutes. The diet thus prepared was poured into 50 ml plastic cups. Each cup had 20 ml diet, and one neonate larva was released in each cup. The cups were kept under laboratory conditions as described before. Observations were recorded on larval mortality and larval weights on 10th day after initiating the experiment. Observations were also recorded on pupal weight, pupation, adult emergence, and larval and pupal development periods.

To assess antibiosis component of resistance to *H. armigera* in different pigeonpea genotypes, leaves or pods (10 g per 300 ml diet) was incorporated into 300 ml of artificial diet. The artificial diet was prepared as described above. One neonate larva was released in each cup, and the cups were placed in the laboratory as described above. There were three replications in a completely randomized design. Each replication had ten larvae. Data were recorded on larval mortality and larval weight on 10th day, pupal weight, pupation and adult emergence, and larval and pupal development periods.

Statistical analysis

Data were subjected to analysis of variance. The significance of differences between the treatments was tested by F-test, while the significance of differences between the treatments was judged by least significance difference (LSD) at P 0.05.

RESULTS AND DISCUSSION

Survival and development of Helicoverpa armigera on leaves and pods of different pigeonpea genotypes

Leaves

Weights of the larvae at 10 days after initiating the experiment ranged from 55.2 mg on ICP 7203-1 to 87.6 mg on T 21 (Table 2). The larval mortality ranged from 36 to 52%, although the differences among the genotypes tested were not significant. Duration of larval development ranged from 22 to 32 days. The larval development was prolonged significantly when the insects were reared on the leaves of ICP 7203-1, ICPL 88039, ICPL 98008, ICPL 332, and ICPL 84060 (>29 days) as compared to that on the susceptible check, ICPL 87 (22 days). Pupal weights ranged from 181.7 to 237.3 mg, but the differences among the genotypes tested were not significant. Pupal period lasted for 13 to 17 days, and was significantly prolonged in insects reared on T 21 and ICPL 332 (16 to 17 days) as compared to the larvae reared on ICPL 87 and ICPL 87091, and ICPL 87119 (13 to 14 days). Percentage pupation was significantly lower (<20%) when the larvae were reared on the leaves of ICPL 98008, T 21, and ICPL 332 as compared to those reared on the susceptible check, ICPL 87 (40%). Adult emergence (computed in relation to the number of larvae released in each replicate) was also significantly lower (<20%) in larvae reared on the leaves of ICPL 98008, ICPL 84060, ICPL 87119, and ICPL 332 as compared to the larvae reared on the susceptible check, ICPL 87 (36%). The results indicated that there were significant differences in survival and development of *H. armigera* on the leaves of different pigeonpea genotypes. However, leaves were not suitable as a source of food for *H. armigera*, as the *H. armigera* larvae rarely feed on the leaves during heavy infestation.

Table 1. Composition of artificial diet impregnated with lyophilized leaf and pod powder

Fraction-A	Quantity
Chickpea flour	75 .00g
Ascorbic acid	1.175 g
Methyl-p-hydroxybenzoate	1.25 g
Sorbic acid	0.75 g
Aureomycin	2.875 g
Vitamin stock solution	2.5 ml
Water	112.5 ml
Yeast	12.0 g
Fraction B	
Agar	4.375 g
Water (for yeast and agar)	200 ml
Leaf or pod powder	5 - 20 g*

* Variable amounts in diets with different amounts of leaf and pod powder.

Flowers and pods

Weights of the 10-day-old larvae were significantly lower when reared on flowers and pods of ICPL 187-1, ICP 7203-1, ICPL 98008, ICPL 84060, and ICPL 332 (148.3 to 184.4 mg per larva) as compared to the larvae reared on the susceptible check, ICPL 87 (238.7 mg per larva) (Table 3). Larval mortality ranged from 14 to 40%, and significantly more number of larvae died (>34%) when reared on ICPL 187-1, ICPL 98008, T 21, ICPL 84060, ICPL 87119, and ICPL 322 as compared to 14% mortality on ICPL 88039. Larval period was prolonged (>24 days) in larvae reared on ICPL 98008, T 21, ICPL 84060, and ICPL 332 as compared to those reared on susceptible check, ICPL 87 (19 days). Pupal period lasted foru 14 days on ICPL 84060 and ICPL 332 as compared to 10 days on ICPL 87. Percentage pupation and adult emergence were significantly lower in larvae reared on T 21, ICPL 84060, and ICPL 87119 (38 to 48% pupation and 24 to 34% adult emergence) as compared to 60% pupation and 56% adult emergence on ICPL 87.

Table 2. Survival and development of *Helicoverpa armigera* on leaves of 12 pigeonpea genotypes (ICRISAT, Patancheru, 2002)

Genotype	Larval weight (mg) 10th day	Larval mortality (%) 10th day	Larval period (days)	Pupal period (days)	Pupal weight (mg)	Pupation (%)	Adult emergence (%)
ICPL 187-1	86.0	46	27	15	223.2	28	28
ICP 7203-1	55.3	48	29	15	216.3	28	26
ICPL 88039	70.8	52	29	15	235.6	34	32
ICPL 98001	85.8	42	26	14	227.6	32	28
ICPL 98008	73.1	50	30	14	230.2	16	16
ICPL 87091	80.3	36	23	13	228.3	50	44
T 21	87.6	50	25	16	221.4	20	20
ICPL 84060	55.4	46	32	15	209.5	22	14
ICPL 87119	80.8	46	27	13	181.7	28	16
ICP 7035	79.4	50	27	14	227.2	24	20
Control							
ICPL 332 (R)	55.2	48	30	17	215.0	20	18
ICPL 87 (S)	79.2	40	22	14	237.3	40	36
Fp	<0.001	0.442	<0.001	<0.001	0.32	0.015	<0.001
LSD at *P* 0.05	14.59	NS	2.72	1.64	NS	14.72	12.03

R = Resistant check. S = Susceptible check. NS = Non-significant.

Table 3. Survival and development of *Helicoverpa armigera* on flowers and pods of 12 pigeonpea genotypes (ICRISAT, Patancheru, 2002)

Genotype	Larval weight (mg) 10th day	Larval mortality (%) 10th day	Larval period (days)	Pupal period (days)	Pupal weight (mg)	Pupation (%)	Adult emergence (%)
ICPL 187-1	183.1	34	23	12	212.0	52	40
ICP 7203-1	162.0	32	22	13	207.2	54	42
ICPL 88039	259.9	14	18	10	246.5	66	58
ICPL 98001	193.8	26	21	11	234.3	56	44
ICPL 98008	178.8	34	24	13	203.1	52	38
ICPL 87091	191.5	30	23	12	200.8	52	34
T 21	189.1	40	24	13	220.0	38	24
ICPL 84060	148.3	38	24	14	203.9	48	34
ICPL 87119	204.4	36	23	12	224.7	44	32
ICP 7035	223.1	28	21	11	272.5	58	52
Control							
ICPL 332 (R)	184.4	34	24	14	222.0	50	44
ICPL 87 (S)	238.7	30	19	10	278.1	60	56
Fp	<0.001	<0.001	<0.001	<0.011	0.001	0.001	<0.001
LSD at *P* 0.05	37.83	7.76	2.52	1.35	33.99	11.11	12.4

R = Resistant check. S = Susceptible check.

Standardization of artificial diet impregnation assay to assess antibiosis mechanism of resistance to Helicoverpa armigera

Leaf powder

There was considerable reduction in larval weights when reared on artificial diets having 10 to 20 g leaf powder of ICPL 332 (18.1 to 50.1 mg per larva) as compared to that on the standard artificial diet (388.3 mg per larva) (Table 4). However, the reduction in larval weight was not as much in insects reared on artificial diet impregnated with leaf powder of the susceptible check, ICPL 87 (102.6 to 211.9 mg in diets with 10 to 20 g of leaf powder). Larval mortality at 10 days after initiating the experiment was 50 to 60% in diets with 10 to 20 g of lyophilized leaf powder of ICPL 332 as compared to 16.7 to 28.3% in diets with leaf powder of ICPL 87. The larval period was prolonged by 11 to 14 and 3 to 6 days in diets containing lyophilized leaf powder of ICPL 332 and ICPL 87, respectively. Percentage pupation ranged from 40 to 63.3% and 56.7 to 66.7% in diets with lyophilized leaf powder of ICPL 332 and ICPL 87, respectively, compared to 66.7% pupation on the standard artificial diet. Adult emergence was 20 to 36.7% and 43.3 to 53.3% in diets with leaf powder of ICPL 332 and ICPL 87, respectively, compared to 60% adult emergence on the standard artificial diet. The results indicated that 10 g of lyophilized leaf powder per 300 ml of diet was optimum to assess the antibiosis component of resistance to *H. armigera*.

Pods powder

Larval mortality increased with an increase in the amounts of pod powder, and ranged between 15.0 to 26.7% in ICPL 332, and 8.8 to 13.3 % in case of ICPL 87 (Table 5). Pupal weights ranged from 159.0 to 284.2 mg in larvae reared on artificial diet having lyophilized pod powder of ICPL 332 compared to 191.9 to 293.2 mg in case of ICPL 87, and 276.4 mg on the standard artificial diet. Larval period was prolonged by 10 to 13 days on artificial diets having pod powder of ICPL 332, and to 4 to 9 days on diets having pod powder of ICPL 87 compared to that on the standard artificial diet. Pupal period varied from 9 to 12 days, but the differences were not substantial. Pupation was 40.0 to 43.3% on diets with ICPL 332 pod powder, 40.0 to 66.7% on diets having pod powder of ICPL 87, and 73.3% on standard artificial diet. Adult emergence was 20.0 to 53.3% and 30.0 to 53.3% in diets having 5 to 20 g pod powder of ICPL 332 and ICPL 87, respectively, and 60% on the standard artificial diet. Incorporation of 10 g of pod powder resulted in significant differences in larval survival and development on the resistant (ICPL 332) and susceptible (ICPL 87) genotypes of pigeonpea, this can be used to assess the antibiosis component of resistance in pigeonpea to *H. armigera*.

Survival and development of Helicoverpa armigera on artificial diets with lyophilized leaf and pod powder of different pigeonpea genotypes

Leaf powder

There was a drastic reduction in larval weights at 10 days after initiating the experiment in diets with leaf powder of different pigeonpea genotypes (11.5 to 51.9 mg per larva) as compared to that on the standard artificial diet (210.2 mg per larva) (Table 6). The larval weights were significantly lower (<22 mg per larva) in diets having leaf powder of ICPL 187-1,

Table 4. Incorporation of lyophilized leaf powder into artificial diet for assessing antibiosis mechanism of resistance to *Helicoverpa armigera* (ICRISAT, Patancheru, 2002)

Amount of leaf powder per 300 ml diet	Larval weight (10^{th} day) (mg)	Larval mortality (10^{th} day) (%)	Larval period (days)	Pupal period (days)	Pupal weight (mg)	Pupation (%)	Adult emergence (%)
ICPL 332 (R)							
5 *	257.8	27	22	9	333.1	63.3	53.3
10	50.1	50	28	11	298.9	40.0	20.0
15	30.7	60	25	11	296.7	40.0	36.7
20	18.1	60	27	12	300.0	40.0	33.3
ICPL 87 (S)							
5	295.7	28	16	10	319.3	66.7	56.7
10	211.9	28	17	12	299.7	56.7	43.3
15	102.6	18	20	12	339.8	66.7	50.0
20	147.4	17	18	11	297.6	63.3	53.3
Standard artificial diet	388.3	7	14	11	313.7	66.7	60.0
Fp	<0.001	<0.001	<0.001	0.003	0.005	0.002	0.001
LSD at *P* 0.05	17.29	14.64	4.290	1.32	23.32	14.64	14.94

R = Resistant. S = Susceptible. Number of larvae = 50. DAI = Days after initiation of experiment. * Amount of lyophilized pod powder added to artificial diet.

Table 5. Impregnation of lyophilized pod powder into artificial diet for assessing antibiosis mechanism of resistance to *Helicoverpa armigera* in pigeonpea (ICRISAT, Patancheru, 2002)

Amount of leaf powder per 300 ml diet	Larval weight (mg) (10^{th} day)	Larval mortality % (10^{th} day)	Larval period (days)	Pupal period (days)	Pupal weight (mg)	Pupation (%)	Adult emergence (%)
ICPL 332 (R)							
5 *	77.7	15	16	9	284.2	63.3	53.3
10	50.9	20	24	11	247.6	40.0	20.0
15	7.8	27	22	10	161.8	43.3	33.3
20	5.2	33	25	10	159.0	43.3	33.3
ICPL 87 (S)							
5	68.5	9	15	10	293.2	73.3	53.3
10	48.0	2	16	12	249.6	60.0	43.3
15	18.8	11	19	11	226.4	66.7	50.0
20	9.8	13	21	11	191.9	40.0	30.0
Artificial diet	301.2	10	12	10	276.4	73.3	60.0
Fp	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	0.01
LSD at *P* 0.05	18.0	5.72	2.52	1.204	52.0	15.40	15.4

R = Resistant. S = Susceptible.

ICPL 87119, ICP 7035, and ICPL 88039 compared to the larvae reared on diets with leaf powder of the susceptible check, ICPL 87 (51.9 mg per larva). Pupal weights ranged from 245.9 to 332.9 mg per pupa in diets with leaf powder of different pigeonpea genotypes as compared to 371.4 mg on the standard artificial diet. Low (<280 mg) pupal weights were recorded in diets having leaf powder of ICP 7035, ICPL 84060, ICPL 87119, and ICPL 87, but the differences were not significant statistically. There were no significant differences in larval mortality at 10 days after infestation, which ranged from zero per cent in standard artificial diet to 13.3% in diet with ICPL 332 leaf powder. Larval period was prolonged by >5 days in larvae reared on artificial diet with leaf powder of ICPL 187-1, ICP 7203-1, ICPL 84060, ICPL 87091, ICPL 87119, and ICPL 332 as compared to the larvae reared on standard artificial diet. The differences in duration of larval development on standard artificial diet and the diet with leaf powder of ICPL 87 were non-significant. Pupal period was prolonged on diets with leaf powder of different pigeonpea genotypes. Pupation was <50% in larvae reared on artificial diets with leaf powder of ICPL 187-1, ICPL 84060, and ICPL 332 as compared to 63.3% pupation in diets with ICPL 87 leaf powder, and 80% pupation on standard artificial diet. Adult emergence ranged from 20.0 to 63.3% on diets with leaf powder of different pigeonpea genotypes compared to 76.7% on standard artificial diet. Low adult emergence (<50%) was recorded in larvae reared on diets with lyophilized leaf powder of ICPL 187-1, ICP 7035, ICP 7203-1, ICPL 84060, ICPL 87119, ICPL 98008, T 21, and ICPL 332 compared to 63.3% adult emergence on diets with leaf powder of ICPL 87.

Pod powder

Weights of the larvae reared on artificial diets, impregnated with 10 g of lyophilized pod powder, ranged from 47.9 to 319.8 mg per larva compared to 387.5 mg in larvae reared on standard artificial diet (Table 7). Weights of the larvae were significantly lower (47.9 to 138.3 mg per larva) in diets having pod powder of ICP 7035, ICPL 84060, ICPL 87119, ICPL 332, ICPL 98008, and T 21 as compared to that on susceptible check, ICPL 87 (319.8 mg). Pupal weights were lower on artificial diets with pod powder of ICP 7203-1, ICPL 84060, ICPL 87091, and T 21 (211.7 to 217.1 mg) as compared to 267.6 mg on ICPL 332 and 275.5 mg on ICPL 87. Larval mortality ranged from 0 to 16.7% in diets with pod powder of different pigeonpea genotypes, but the differences were nonsignificant. Larval period was prolonged by 5 days when the insects were reared on artificial diets with pod powder of ICPL 84060, ICPL 87119, T 21 and ICPL 332 as compared to those reared on diets with pod powder of ICPL 87. Pupal period was prolonged by 2 to 3 days in diets with pod powder of different pigeonpea genotypes, but the differences among the genotypes tested were not very large. Pupation was <60% in larvae reared on diets with pod powder of ICPL 187-1, ICPL 332 and ICPL 87 compared to 90% pupation on standard artificial diet. Adult emergence was <50% in larvae reared on diets having pod powder of ICP 7035, ICP 7203-1, ICPL 87119, T 21, and ICPL 332 as compared to 53.3% in diets with ICPL 87 pod powder, and 86.6% on standard artificial diet.

Table 6. Survival and development of *Helicoverpa armigera* in artificial diet impregnated with lyophilized leaf powder of different pigeonpea genotypes (ICRISAT, Patancheru, 2002)

Genotype	Larval weight (10^{th} day) (mg)	Larval mortality (10^{th} day)	Larval period (days)	Pupal period (days)	Pupal weight (mg)	Pupation (%)	Adult emergence (%)
Artificial diet	210.2	0	20	10	371.4	80.0	76.7
ICPL 187-1	15.6	7	28	13	329.3	43.3	36.7
ICP 7203-1	36.2	7	25	12	328.4	60.0	50.0
ICPL 88039	21.6	0	21	12	332.9	63.3	56.7
ICPL 98001	28.4	7	18	11	331.7	63.3	53.3
ICPL 98008	44.3	0	18	11	327.3	53.3	46.7
ICPL 87091	28.8	0	25	10	332.6	60 .0	60.0
T21	32.4	3	24	12	321.8	60 .0	50.0
ICPL 84060	32.4	10	30	14	245.9	33.3	20.0
ICPL 87119	14.8	3	27	12	249.5	56.7	50.0
ICP 7035	11.5	3	23	11	279.8	56.7	50.0
Control							
ICPL 332 (R)	41.5	13	31	12.	311.0	40.0	33.3
ICPL 87 (S)	51.9	3	22	11	261.6	63.3	63.3
Fp	<0.001	0.21	0.013	0.003	0.064	0.147	<0.001
LSD *P* 0.05	24.63	NS	7.04	1.81	NS	NS	19.60

R = Resistant check. S = Susceptible check. NS = Non-significant.

Relationship between survival and development of Helicoverpa armigera on fresh plant parts and diet impregnation assay

Adult emergence (r = 0.63*, significant at P <0.05, df = 10), larval period (r = 0.32), pupal period (r = 55*), pupal weight (r = 0.66*), and percentage pupation (r = 0.48*) were positively associated between the insects reared on fresh leaves and those reared on artificial diets with lyophilized leaves of different genotypes. However, there was no association for larval weight and larval morality between diet impregnation assay and the *in vivo* studies. In case of larvae reared on pods and in artificial diets with lyophilized pod powder, there was a positive correlation for adult emergence (r = 0.29), larval period (0.91*), larval mortality (r = 0.29), pupal period (0.74*), and pupal weight (r = 0.79*). However, there was no association for larval weight and parentage pupation.

Antibiosis is an important component of resistance to insects (Painter 1951). It leads to reduction in size and weight, low fecundity, prolongation of development period, and increased mortality. The present study has shown that there were significant differences in survival and development of *H. armigera* larvae reared on leaves and pods of different pigeonpea genotypes. Differences in survival and development of *H. armigera* on different pigeonpea genotypes have earlier been reported by Sison and Shanower (1994). Larval and pupal weights, and larval survival were greater in insects reared on diets containing lyophilized leaf and pod powders compared to the larvae reared on leaves, flowers, and pods collected from field. This may be due to availability of more nutrients in the artificial diet as compared to that in the intact plant parts, in addition to the possible changes in chemical composition of different plant parts during sample collection and drying, although efforts made to reduce such effects by freeze-drying the material immediately. Similar observations have earlier been made by Yoshida and Shanower (2000). Reduced larval and pupal weights and prolonged larval and pupal periods were observed in insects reared on ICPL 332, ICPL 84060, ICP 7035, ICPL 88039 and T 21 as compared to those reared on ICPL 87 and ICPL 87091, suggesting that antibiosis is one of the components of resistance to *H. armigera* in these genotypes. Reduction in larval and pupal weights, and prolongation of larval and pupal periods has been observed in insects fed on developing pods of resistant genotypes (Lateef *et al*. 1981, Dodia and Patel, 1994, Dodia *et al*. 1996). In the present studies, adverse effects of resistant varieties were observed on the survival and development of *H. armigera,* both on the fresh plant parts, and in artificial diet impregnation assay.

Larval survival, larval and pupal weights, and adult emergence were lower on diets having leaf or pod powder of the resistant genotypes than the diets with leaf or pod powder

Table 7. Survival and development of *Helicoverpa armigera* on artificial diet impregnated with 10 g of lyophilized pod powder of different pigeonpea genotypes (ICRISAT, Patancheru, 2002)

Genotype	Larval weight 10th day (mg)	Larval mortality 10th day (%)	Larval period (days)	Pupal period (days)	Pupal weight (mg)	Pupation (%)	Adult emergence (%)
Artificial diet	387.5	0	16	9	219.0	90.0	86.6
ICPL 187-1	146.8	17	21	12	232.3	60.0	53.3
ICP 7203-1	156.0	17	20	12	211.7	70.0	46.7
ICPL 88039	58.1	3	18.5	10	223.6	73.0	70.0
ICPL 98001	184.4	7	19	12	256.9	80.0	66.7
ICPL 98008	84.0	7	21	11	245.9	70.0	63.3
ICPL 87091	278.9	7	18	11	213.9	73.0	63.3
T 21	119.8	7	22	13	217.1	66.7	50.0
ICPL 84060	104.2	17	22	14	215.4	70.0	56.7
ICPL 87119	47.9	0	22	12	234.7	70.0	40.0
ICP 7035	138.3	13	19	11	292.8	66.7	50.0
Control							
ICPL 332 (R)	131.8	13	25	13	267.6	56.7	43.3
ICPL 87(S)	319.8	7	17	11	275.5	56.7	53.3
P	<0,001	0.297	<0,001	0.02	0.06	0.033	0.005
LSD at *P* 0.05	110.3	15.58	2.9	2.18	69.68	17.21	19.95

R = Resistant check. S = Susceptible check.

of susceptible genotypes. Expression of resistance to *H. armigera* in artificial diet impregnated with lyophilized leaf or pod powder of different pigeonpea genotypes was quite consistent, and comparable to that observed on fresh plant parts. However, the results of such assays were slightly different than those observed with the fresh plant parts due to availability of more nutrients in the artificial diet. Some of these differences may also be due to biochemical changes in the nutritional quality of the pigeonpea plant parts impregnated in artificial diets. Another factor resulting in such differences may be greater incidence of pathogenic viruses, bacteria, and fungi in insects reared on artificial diet than in the insects reared on fresh plant parts. Therefore, efforts should be made to establish a clear relationship in terms of survival and development of *H. armigera* on artificial diets impregnated with lyophilized leaf and pod powder with that on the fresh plant parts, and overall expression of resistance to *H. armigera* under field conditions.

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REFERENCES

Armes NJ, Bond GS and Cooters RJ. 1992. The Laboratory Culture and Development of *Helicoverpa armigera*. Natural Resources Institute Bulletin No.57. Natural Resources Institute, Chatham, UK.

Borad PK, Patel JR and Patel MG. 1991. Evaluation of vegetable pigeonpea (*Cajanus cajan*) genotypes resistant to gram-pod borer (*Helicoverpa armigera*), plume moth (*Marasmarcha liophanes*) and podfly (*Melanagromyza obtusa*). Indian Journal of Agricultural Sciences **61**: 682-684.

Dodia DA and Patel JR. 1994. Antibiosis in pigeonpea to *Helicoverpa armigera* Hubner. International Chickpea and Pigeonpea Newsletter **1**: 39-40.

Dodia DA, Patel AJ, Patel IS, Dhulia FK and Tikka SBS. 1996. Antibiotic effect of pigeonpea wild relatives on *Helicoverpa armigera*. International Chickpea and Pigeonpea Newsletter **3**: 100-101.

Kalariya GB, Judal GS and Patel GM. 1998. Reaction of pigeonpea genotypes against important insect pests. Gujarat Agricultural University Research Journal **23**: 33–38.

Lateef SS, Reddy LJ, Reed W, Faris DG. 1981. *Atylosia scarabaeoides*: a source of resistance to *Heliothis armigera*. International Pigeonpea Newsletter **1**: 32-34.

Lateef SS and Pimbert MP. 1990. The search for host plant resistance of *Helicoverpa armigera* in chickpea and pigeonpea at ICRISAT. In: Proceedings of the Consultative Group Meeting on the Host Selection Behavior of *Helicoverpa armigera,* 5-7 March 1990, pp 14-18. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India.

Nawale RN and Jadhav LD. 1983. Relative susceptibility of some varieties of pigeonpea to pod borer damage. Journal of Research, Maharashtra Agriculture Universities **8**(1): 94-95.

Painter RH. 1951. Insect Resistance in Crop Plants, Macmillan, New York, USA. 520 pp

Patnaik HP, Senapati B, Behera PK, Mohapatra HK. 1989. Relative susceptibility of some pigeonpea cultivars to *Heliothis armigera* (Hubner). Indian Journal of Plant Protection **17**: 279–282.

Sachan GC. 1990. Progress of host plant resistance work in chickpea and pigeonpea against *Helicoverpa armigera* in India. In: Proceedings, Host Selection Behavior of *Helicoverpa armigera*, 5-7 March 1990, pp 19-22. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India.

Sharma HC. (ed.). 2005. *Heliothis/Helicoverpa* Management: Emerging Trends and Strategies for Future Research. Oxford and IBH, New Delhi, India. 469 pp.

Sharma HC, Ahmad R, Ujagir R, Yadav RP, Singh R and Ridsdill-Smith TJ. 2005a. Host plant resistance to cotton bollworm/legume pod borer, *Heliothis*/*Helicoverpa*. In: Sharma HC (ed), *Heliothis*/*Helicoverpa* Management: Emerging trends and Strategies for Future Research, pp. 167-208. Oxford and IBH Publishers, New Delhi, India.

Sharma HC, Pampapathy G, Dhillon, MK and Ridsdill-Smith TJ. 2005b. Detached leaf assay to screen for host plant resistance to *Helicoverpa armigera*. Journal of Economic Entomology **98**: 568-576.

Sison MJ and Shanower TG. 1994. Development and survival of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on short-duration pigeonpea. Journal of Economic Entomology **87**: 1749–1753.

Venkateswarlu O and Singh TVK. 1999. Response of pigeonpea genotypes against important insect pests. Insect Environment **5**: 123–124.

Yoshida M and Shanower TG. 2000. *Helicoverpa armigera* larval growth inhibition in artificial diet containing freeze dried pigeonpea pod powder. Journal of Agricultural and Urban Entomology **17**: 37-41.