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Screening of Pigeonpea Varieties through Nylon Bag No-choice Bioassay for Host Plant Resistance to *Helicoverpa armigera*

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ABSTRACT

Background: The legume pod borer, *Helicoverpa armigera* (Hübner), is one of the most damaging crop pests, including pigeonpea. Host plant resistance is a component of pest management and therefore, we standardize a nylon bag No-Choice Bioassay technique to screen for resistance to *H. armigera* under field conditions.

Methods: Pigeonpea plants were infested with 24 h old 1, 2, 3, 4 and 5 larvae per plant inside the nylon bag. Observations were recorded on pod damage, larval survival, larval weight, pupation, adult emergence and fecundity after 10 days.

Result: Pigeonpea varieties AL-201, H03-41 and PAU-881 exhibited lower pod damage (15.89 to 19.77%) and larval weight (12.02 to 13.82 mg). The expression of resistance to *H. armigera* was associated with trichome density, pod wall thickness and higher amount of phenolic compounds and condensed tannins. Lower trichome density and thin pod walls and higher amounts of sugars rendered the varieties Paras, Manak and Pussa-992 more susceptible to *H. armigera*. Nylon bag assay can be used to screen and select pigeonpea cultivars for resistance to *H. armigera*.

Key words: *Helicoverpa armigera*, Host-plant resistance, Nylon bag assay, Pigeonpea.

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a multipurpose, hardy pulse legume grown in the tropics and sub-tropics. Insect pests cause an average of 30% loss in pulses, valued at US$ 815 million throughout the world. However, the legume pod borer, *Helicoverpa armigera* (Hübner) (Sharma, 2016) is the most important yield reducing factor, resulting in a loss of over US$2 billion million annually in the semi-arid tropics, despite the use of insecticides costing more than $500 million annually. Since *H. armigera* has developed high levels of resistance to insecticides, it has become difficult to manage this pest with conventional insecticides. In this context, host plant resistance is an important component for managing *H. armigera* in different crops and cropping systems. Screening of more than 14,000 accessions of pigeonpea for resistance to *H. armigera* showed low to moderate levelsof resistance in the cultivated genotypes (Jat *et al*., 2021; Reed and Lateef, 1990), but a few accessions of the wild relatives found resistant to *H. armigera* (Green *et al*., 2006).

Various morphological traits have been reported to be associated with resistance to *H. armigera* (Jat *et al*., 2021). Besides these traits, chemical compounds in the trichome exudates also influence host plant selection and colonization by *H. armigera* (Green *et al*., 2003; Hartlieb and Rembold, 1996).

Due to variations in the flowering times, the infestation of *H. armigera* varies over space and time, results in variations in the infestation levels across seasons and locations. According to Smith *et al*. (1994), it is important to screen the test genotypes for resistance to the target insects under the optimum and uniform level of insect infestation at the most susceptible stage of the crop. A technique that results in more than 80% infestation/ pod or leaf damage in the susceptible check or maximum differences in leaf/pod damage between resistant and susceptible checks can be used to screen for resistance to insect-pests. Sharma *et al*. (2005) standardized a detached leaf assay in chickpea for rapid screening of germplasm for resistance to *H. armigera* in a short span of time, with minimal cost and under uniform insect infestation, which also provides useful information on antibiosis component of resistance to the target insect pest. Therefore, we used a nylon bag no-choice bioassay technique to screen for host plant resistance to *H. armigera* in pigeonpea under field conditions.

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MATERIALS AND METHODS

The experiments were conducted during the 2013 and 2014 rainy season at Pulses Farm, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar.

Number of treatments : 24 (6 varieties and 4 dates of sowing).
a) Varieties : Manak, Paras, Pusa-992, AL-201, PAU-881 and H03-41.
b) Date of sowing : D1 (3rd week of June), D2 (1st week of July).
D3 (2nd week of July) and D4 (3rd week of July).

Plants

The experiment was replicated thrice in a factorial random complete block design. Plot size was 1.8 × 4 m (4 rows × 4 m long) with 45 cm × 15 cm spacing. Varieties were sown on four different planting dates by following recommended agronomic practices. At flowering, 10 plants were randomly covered with a nylon bag (60 × 35 cm).

Insects

The culture was maintained on artificial diet in the laboratory for infestation of the pigeonpea cultivars tested.

Infestation of varieties with the larvae

Plants were infested with 24 h old 1, 2, 3, 4 and 5 larvae per plant inside the nylon bag by removing naturally presence of eggs and larvae. Uninfested plants served as a control. Larva were allowed to feed for 10 days and data were recorded on pod damge, and larval weight and the larvae were shifted to artificial diet until pupation. The plant were again covered with the nylon bag for further studies. Adult emergence and fecundity data were also recorded. At maturity, the data were recorded on percentage pod damage.

Morpho-physiological interactions between pod borer and varieties

25 days old fresh pods of pigeonpea were picked randomly from each genotype. Trichome density on the pods (top, middle and bottom canopy) were recorded (Sass, 1964). Pod wall thickness, pod length, and seed length and width (in mm) were measured by using Vernier Calipers.

To study the biochemical constituents of the seeds as well as the pod wall, the sufficient number of 15 day old pods were plucked from each replication. The pods were kept in paper bags in an airtight plastic container and stored at 4°C until chemical analysis. One set of pods kept in a paper bag, oven dried at 60°C for 3 days, powdered them, and again oven dried at 50°C for 1 day to ensure complete drying of pods. Biochemical constituents were estimated by adopting following methods *viz* crude protein AOAC (1985), moisture (Mehta and Lodha, 1979), total soluble sugars (Dubios *et al*., 1956), fat (AOAC 1975), total phenols (Bray and Thorpe, 1954), tannins (AOAC 1965) and chlorophyll content (Hiscox and Israelstam, 1979).

Statistical analysis

Data were subjected to analysis of variance Steel and Torrie (1980) by factorial analysis. Significance of differences between the genotypes was judged by F-test and the genotypic means were compared by the least significant difference (LSD) at *P 0.05*.

RESULTS AND DISCUSSION

Insect density × pod damage relationships

There were significant differences between varieties and larvae released per plant ($F_{8,32}$ = 0.516; $p \leq 0.05$) and sowing dates×varieties×larvae released per plant ($F_{8,32}$ = 1.032; $p \leq 0.05$) (Table 1). There were significant differences in larval weight, pupation and adult emergence across sowing dates and varieties ($F_{8,32}$ = 0.256; $p \leq 0.05$), ($F_{8,32}$ = 0.313; $p \leq 0.05$), ($F_{8,32}$ = 0.626; $p \leq 0.05$) (Table 2), varieties ($F_{8,32}$ = 6.42; $p \leq 0.05$) and sowings×varieties ($F_{8,32}$ = 12.85; $p \leq 0.05$) and sowing dates ($F_{8,32}$ = 5.63; $p \leq 0.05$), varieties ($F_{8,32}$ = 6.90; $p \leq 0.05$) and sowings×varieties ($F_{8,32}$ = 19.50; $p \leq 0.05$).

Pod damage is the most common parameter for assessing genotypic resistance or susceptibility to *H. armigera*. Maximum chickpea pod damage was observed when six third-instar larvae per three plants released in the greenhouse and eight larvae per plant under field conditions (Sharma *et al*., 2005). Under detached leaf assay, significantly lower larval weight gain and lowest pod damage was in chickpea cultivars ICCV 097105 and ICCV 92944.

Susceptibility of a test genotype in the field conditions and under detached leaf assay is also influenced by non-preference for oviposition and feeding, tolerance and antibiosis. As these factors are important component of resistance, nylon bag no-choice bioassay technique can be

Table 1: Pod damage by *H. armigera* larvae in different pigeonpea varieties (Pooled).

Variety	No. of larvae released/plant						Mean
	0	1	2	3	4	5	
Paras	0.00 (2.87)	14.63 (22.43)	19.69 (26.07)	23.26 (28.65)	28.19 (31.91)	38.50 (38.09)	20.71 (25.00)
Manak	0.00 (2.87)	14.65 (22.25)	17.93 (24.85)	22.90 (28.56)	31.85 (34.24)	41.04 (39.73)	21.40 (25.42)
AL-201	0.00 (2.87)	8.67 (16.99)	13.59 (21.34)	17.06 (24.05)	23.66 (28.92)	32.36 (34.64)	15.89 (21.47)
Pusa-992	0.00 (2.87)	13.90 (21.85)	19.92 (26.37)	22.65 (28.32)	29.41 (32.80)	35.86 (36.75)	20.29 (24.83)
PAU-881	0.00 (2.87)	11.88 (20.11)	15.87 (23.41)	21.20 (27.22)	29.65 (32.85)	40.00 (39.14)	19.77 (24.26)
H03-41	0.00 (2.87)	11.86 (20.02)	15.36 (22.94)	19.35 (25.97)	25.75 (30.44)	33.41 (35.23)	17.62 (22.91)
Mean	0.00 (2.87)	12.60 (20.61)	17.06 (24.16)	21.07 (27.13)	28.08 (31.86)	36.86 (37.26)	-
LSD		B = 0.211		C = 0.211		B × C = 0.516	
	($p \leq 0.05$)			A × B × C = 1.032			

The figures in parentheses are √+1 transformed values.

used to evaluate germplasm and breeding lines under uniform insect pressure and environmental conditions.

Association of morphological traits with expression of resistance to *H. armigera*

Trichome density of pods of top canopy

In pooled over years results, type A and B trichomes were significantly and negatively correlated with pod damage (r = -0.730*, r = -0.768*, r- = -0.531*, r = -0.729*) and (r = -0.864*, r = -0.734*, r = -0.662*, r = -0.776*, respectively) (Table 3) in D_1, D_2, D_3 and D_4 sown crop.

Trichome density of pods of middle canopy

In pooled results, type A and B trichomes were significantly and negatively correlated with pod damage (r = -0.751*, r = -0.766*) in D_1 and D_2 and (r = -0.729*, r = -0.730*, r = -0.742*, respectively) in D_1, D_3 and D_4 sown crop.

Trichome density of pods of lower canopy

In pooled over years results, type A and B trichomes were significantly and negatively correlated with pod damage (r = -0.725*) in D_2 sown crop.

But, C type of trichomes were positively correlated (r = 0.794*, r = 0.760*), (r = 0.646*, r = 0.803*) and (r = 0.964**, r = 0.639*, r = 0.510*, r = 0.832*) with pod damage in D_1, D_2, D_3 and D_4 sowings (Table 3).

Trichomes type A and B of top and middle pod canopy (slope = -0.50; -1.11, -0.25; -0.32) and (slope = -0.37; -0.50; -0.46; -0.62) were negatively correlated with pod damage, with a negative slope in D_1, D_2, D_3 and D_4 sowings (Fig 1 and 2). Trichomes type B of top and middle pod canopy (slope = -2.23; -3.55; -1.89; -0.72) and (slope = -3.18; -2.20; -3.03; -2.52) were negatively correlated with pupation, with a negative slope.

Trichomes type A of middle canopy in D_1 and D_2 sowings (slope = -0.24; -0.30) were negatively correlated with fecundity, with a negative slope (Fig 5).

However, trichomes of type C of top and middle canopy in D_1, D_2, D_3 and D_4 sowings (slope = 0.45; 0.50; 0.57, 0.50)

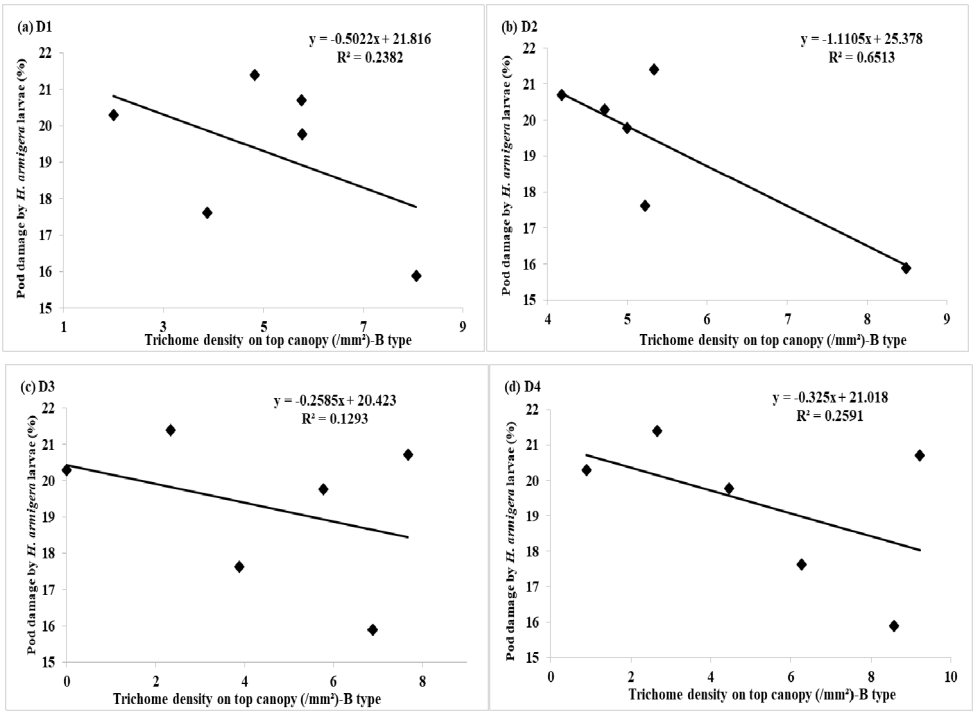


Fig 1: Association of a, b, c, d trichome density on top canopy-B type with resistance to *H. armigera.*

Table 2: Effect of sowing dates and varieties on larval weight (Pooled).

Sowing	Variety						Mean
	Paras	Manak	AL-201	Pusa-992	PAU-881	H03-41	
D_1	15.18 (3.96)	15.46 (4.10)	12.44 (3.60)	14.35 (3.88)	12.38 (3.62)	13.51 (3.79)	13.89 (3.81)
D_2	18.55 (4.40)	17.83 (4.29)	14.57 (3.92)	17.20 (4.24)	15.35 (3.93)	17.38 (4.26)	16.81 (4.17)
D_3	14.29 (3.88)	15.52 (3.92)	12.51 (3.52)	15.63 (4.02)	12.50 (3.53)	13.78 (3.77)	14.04 (3.77)
D_4	11.91 (3.50)	12.81 (3.66)	8.57 (2.88)	12.22 (3.50)	8.48 (2.71)	10.61 (3.17)	10.77 (3.24)
Mean	14.98 (3.94)	15.40 (4.10)	12.02 (3.48)	14.85 (3.91)	12.18 (3.45)	13.82 (3.75)	-
LSD ($p \leq 0.05$)		A = 0.256			B = 0.313		A × B = 0.626

The figures in parentheses are square root transformed values.

and (slope = 0.1.53; 0.93; 1.74; 1.31) (Fig 3 and 4), (slope = 2.04; 1.53; 1.82; 1.69), (slope = 1.48; 1.83; 1.63; 2.21) were positively associated with pod damage, pupation and fecundity with a positive slope.

Pod wall thickness

Pod wall thickness was significantly and negatively correlated with pod damage (*r* = -0.909**, -0.739*, -0.612*, -0.801*) (Table 3) with a negative slope (slope = -2.43; -3.17; -4.54; -4.11) (Fig 6) in D_1, D_2, D_3 and D_4 sowings.

Association of biochemical traits with expression of resistance to *H. armigera*

Chlorophyll content (mg g^{-1})

Chlorophyll content of seeds as well as pod wall was significantly and positively correlated with pod borer damage

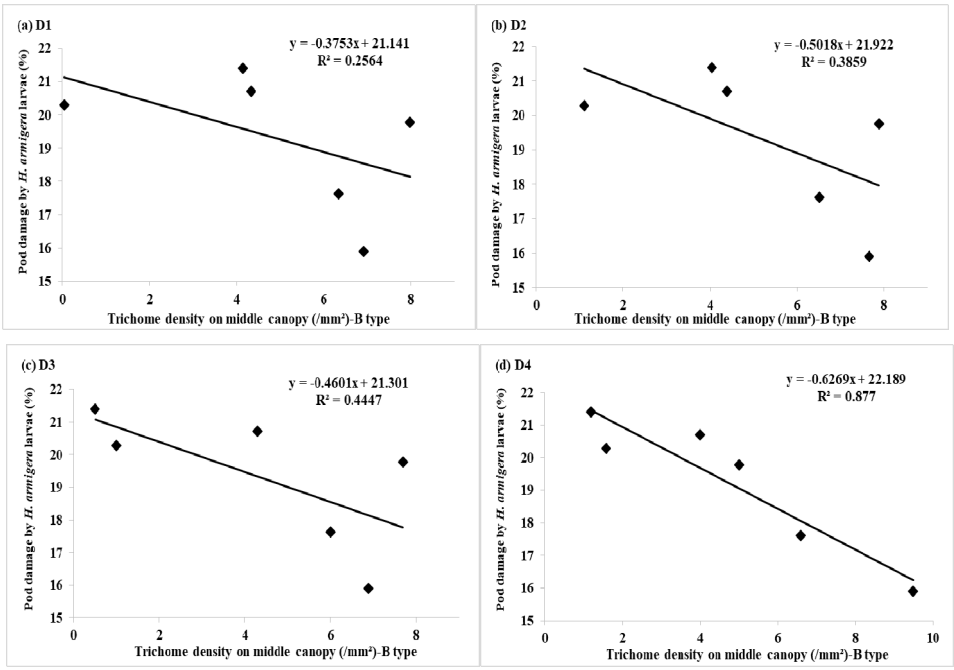


Fig 2: Association of a, b, c, d trichomes density on middle canopy-B type with resistance to *H. armigera*.

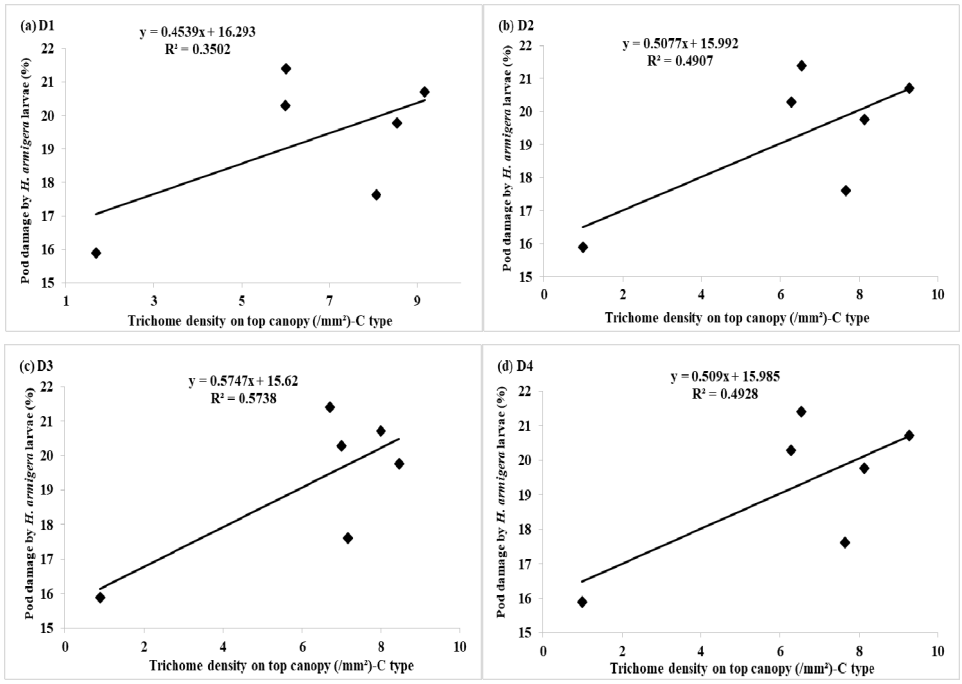


Fig 3: Association of a, b, c, d trichomes density on top canopy-C type with susceptibility to *H. armigera*.

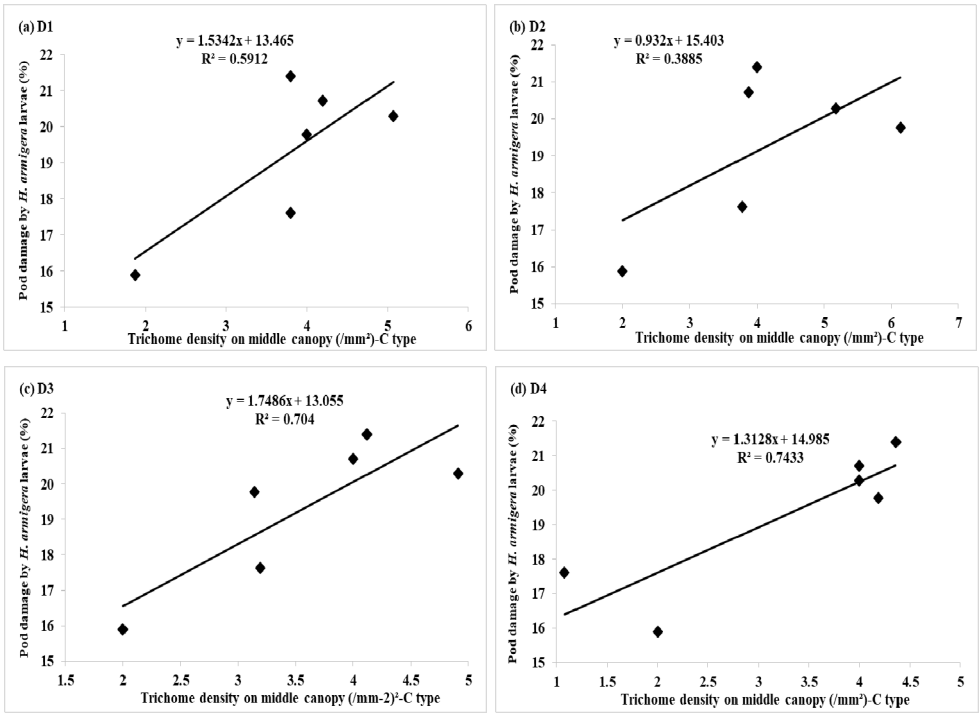


Fig 4: Association of a, b, c, d trichomes density on middle canopy-C type with susceptibility to *H. armigera.*

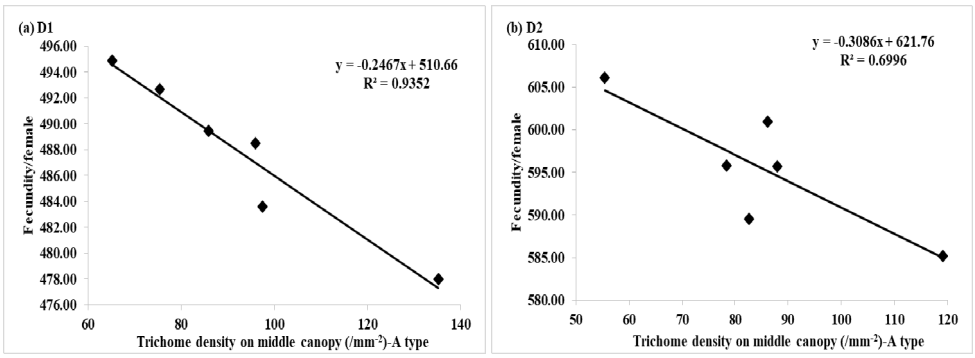


Fig 5: Association of a, b trichomes density on middle canopy-A type with resistance to *H. armigera.*

Table 3: Correlation coefficient (r) between morphological traits and *H. armigera* pod damage.

Sowing	Morphological traits									
	Trichomes (/mm²)									Pod wall thickness (mm)
	Top canopy			Middle canopy			Lower canopy			
	A	B	C	A	B	C	A	B	C	
	Pooled (2013-2014)									
D_1	-0.730*	-0.864*	0.646*	-0.751*	-0.729*	0.964**	0.129	0.059	0.019	-0.909**
D_2	-0.768*	-0.734*	0.803*	-0.766*	0.048	0.639*	-0.725*	-0.099	0.129	-0.739*
D_3	-0.531*	-0.662*	0.139	0.414	-0.730*	0.510*	-0.088	-0.384	0.794*	-0.612*
D_4	-0.729*	-0.776*	0.133	0.416	-0.742*	0.832*	-0.337	-0.492	0.760*	-0.801*

A = Non-glandular pod trichomes.

B = Glandular pod trichomes.

C = Non-glandular lengthy pod trichomes.

*Significant at $p = 0.05$; **Significant at $p = 0.01$.

in D_3 and D_4 sowings (r = 0.655*, r = 0.753*) and in D_1 sowing (r = 0.626*) (Table 4).

Crude protein (%)

Crude protein content of seeds as well as pod wall was significantly and positively correlated with pod borer damage in D_2, D_3 and D_4 sowings (r = 0.639*, r = 0.810*, r = 0.711*) and in D_1 sowing (r = 0.740*) (Table 4). Path coefficients shows positive slope with larval weight (slope = 1.13; 0.93; 1.24; 0.21) (Fig 7), pupation (slope = 10.30; 5.13; 8.67; 9.72) (Fig 8), adult emergence (slope = 5.56; 2.08; 6.44; 12.95) (Fig 9) and fecundity (slope = 4.17; 3.79; 7.03; 5.52) (Fig 10), respectively in D_1, D_2, D_3 and D_4 sowings.

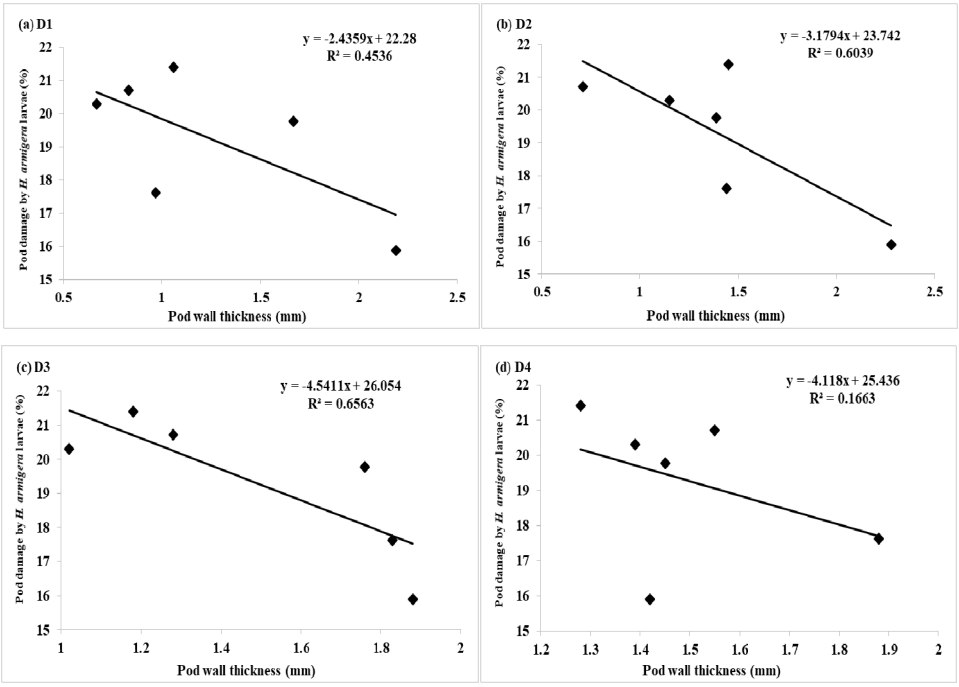


Fig 6: Association of a, b, c, d pod wall thickness with resistance to *H. armigera* pod damage.

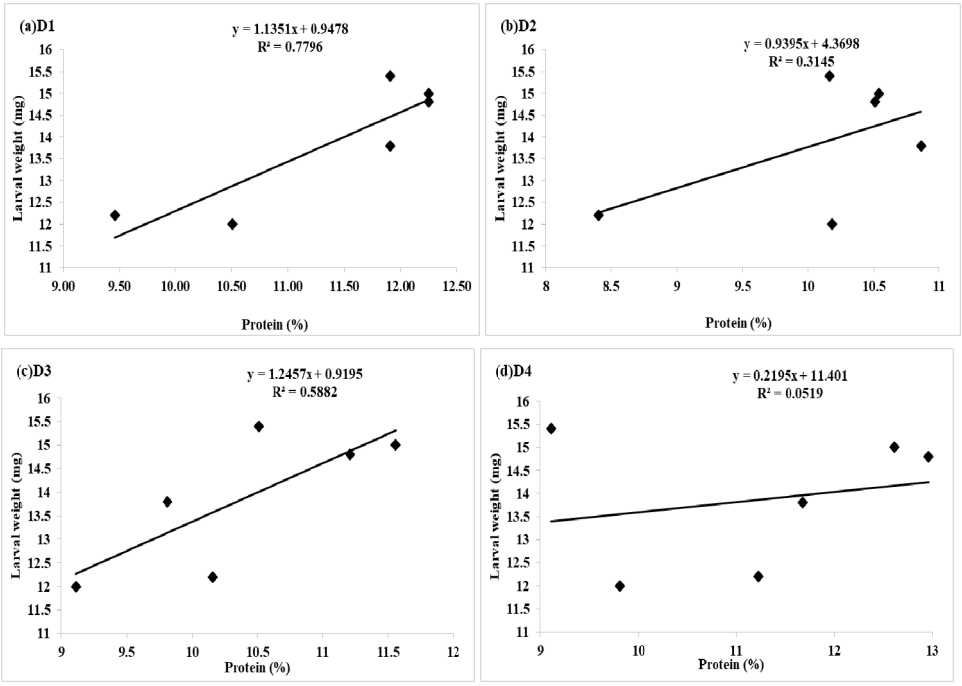


Fig 7: Association of a, b, c, d protein content of pod wall with susceptibility to *H. armigera* larval weight.

Total soluble sugar (%)

Total soluble sugars of seeds as well as pod wall were significantly and positively correlated with pod borer damage in D_1, D_2 and D_4 sowings (r = 0.738*, r = 0.793*) and (r = 0.698*, r = 0.898**, r = 0.819*), respectively (Table 4).

Total soluble sugar content (slope = 6.42; 2.88; 1.06; 1.0) was positively correlated with pod borer damage (Fig 11), larval weight (slope = 4.50; 1.43; 0.99; 0.62), pupation (slope = 40.16; 20.31; 30.97; 30.94) (Fig 12) and fecundity (slope = 20.47; 3.95; 2.90; 3.03) with a positive slope in D_1, D_2, D_3 and D_4 sowings, respectively.

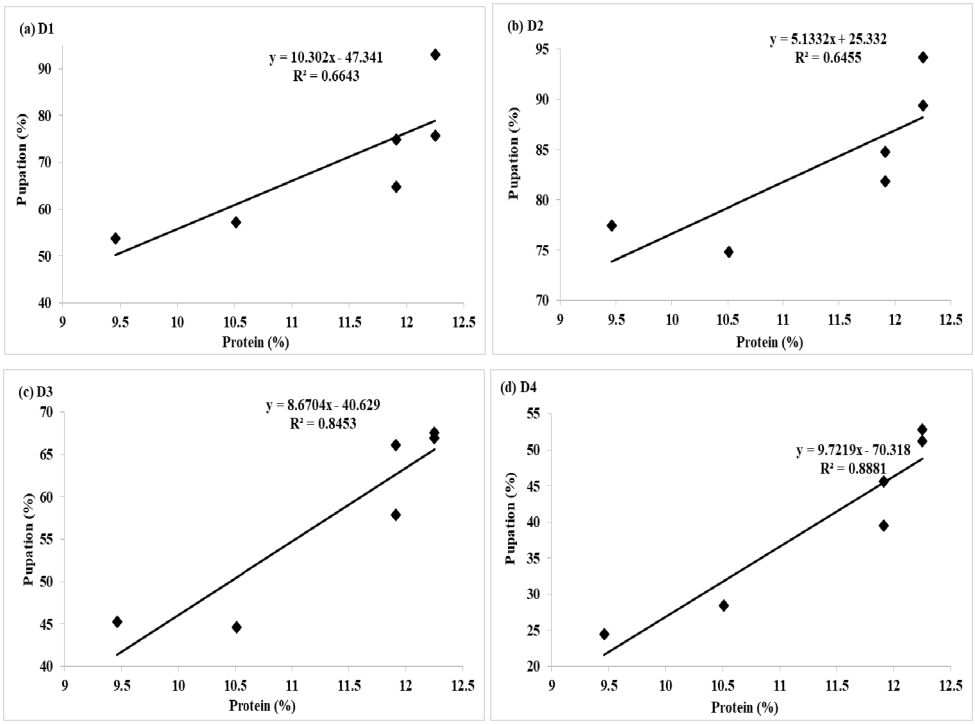


Fig 8: Association of a, b, c, d protein content of pod wall with susceptibility to *H. armigera* pupation.

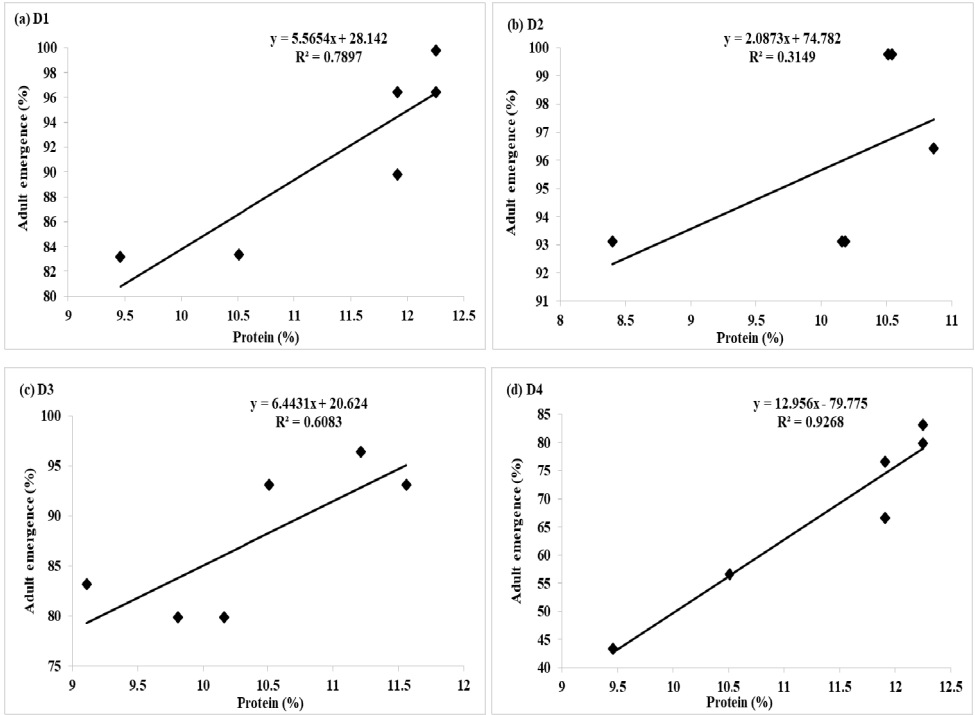


Fig 9: Association of a, b, c, d protein content of pod wall with susceptibility to *H. armigera* adult emergence.

Fat content (%)

Fat content of seeds as well as pod wall was significantly and negatively correlated with pod borer damage (r = -0.884**, r = -0.675*) and (r = -0.743*) in D_1 and D_2 sowings (Table 4).

Phenol content (mg g^{-1})

Phenol content of seeds as well as pod wall was significantly and negatively correlated with pod borer damage (r = -0.900**, r = -0.625*) and (r = -0.656*, r = -0.697*) in D_1 and D_2 sowings (Table 4).

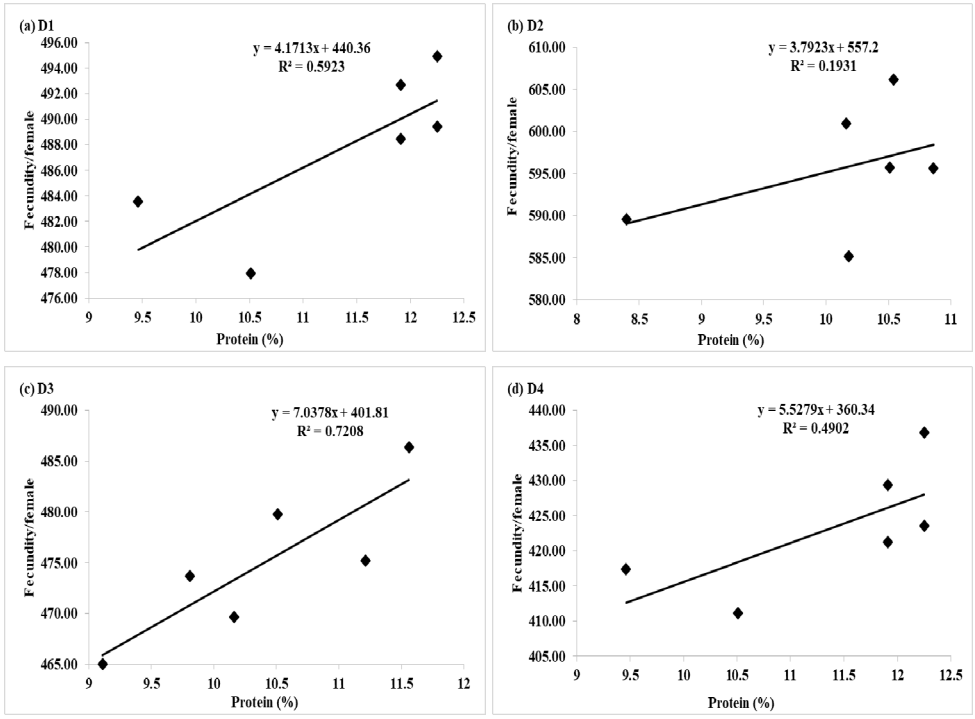


Fig 10: Association of a, b, c, d protein content of pod wall with susceptibility to *H. armigera* female fecundity.

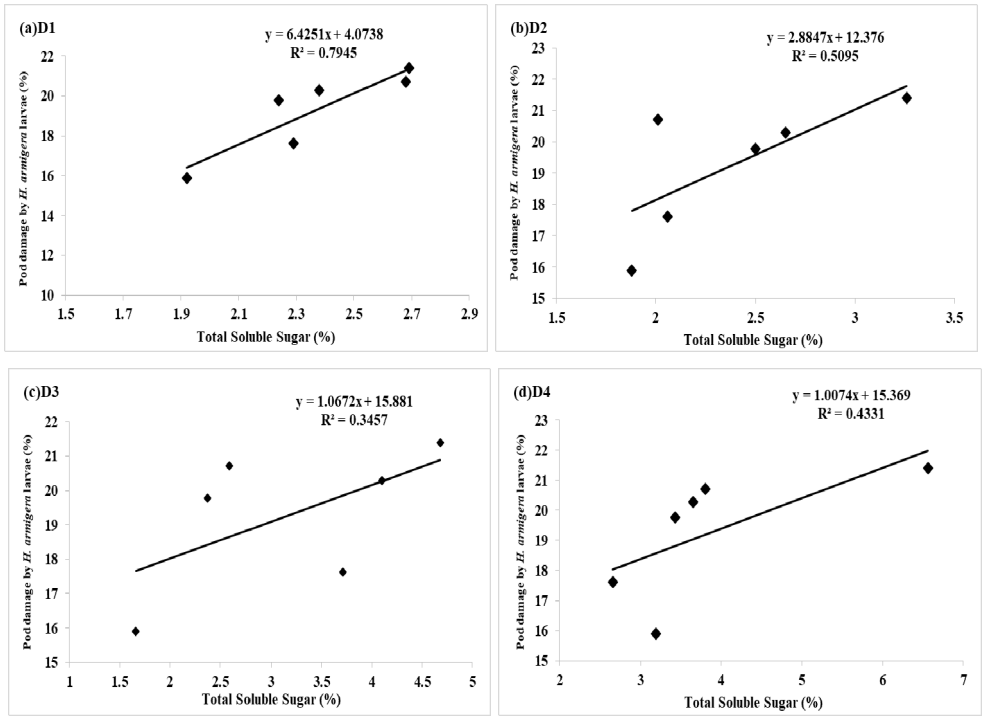


Fig 11: Association of a, b, c, d total soluble sugar of pod wall with *H. armigera* pod damage.

Phenol content was negatively correlated with pod borer damage (slope = -2.40; -2.50; -2.30; -3.75) (Fig 13), larval weight (slop = -1.73; -1.92; -0.72; -1.89) (Fig 14), pupation (slope = -16.70; -8.87; -13.08; -14.23) (Fig 15), adult emergence (slope = -8.96; -4.38; -6.67; -13.84), and fecundity (slope = -6.51; -9.20; -1.73; -11.50) (Fig 16) respectively, with a negative slope in D_1, D_2, D_3 and D_4 sowings.

Tannin content (µg g^{-1})

The tannin content of the seeds as well as pod wall was also significantly and negatively correlated with borer damage

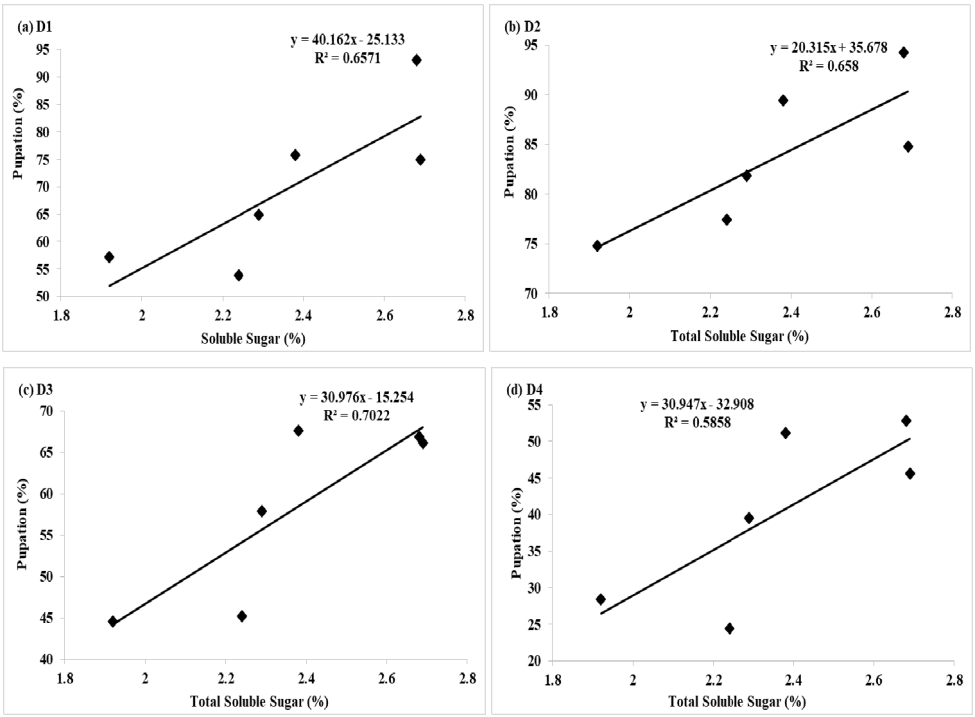


Fig 12: Association of a, b, c, d total soluble sugar of pod wall with susceptibility to *H. armigera* pupation.

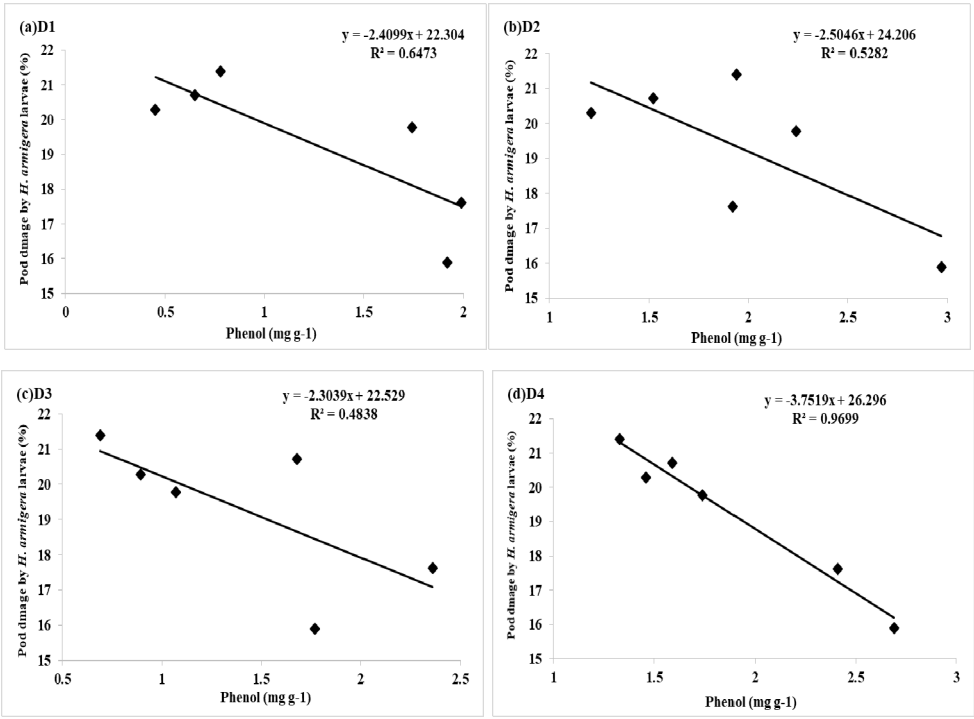


Fig 13: Association of a, b, c, d phenol content of pod wall with resistance to *H. armigera* pod damage.

(r = -0.792*, r = -0.812*, r = -0.676*) and (r = -0.630*), respectively in D_1, D_2 and D_4 sowings.

Path coefficients of trichome density, pod wall thickness, phenol and tannins content exhibited direct effects and correlation in the same direction (-ve) suggesting the importance of these traits against *H. armigera* resistance and these traits can be used as a resistance source criteria. To understand the mechanisms of expression of resistance to *H. armigera* under field conditions is a long-term process. And hence, it is difficult to identify stable source of resistance under natural infestation in the field.

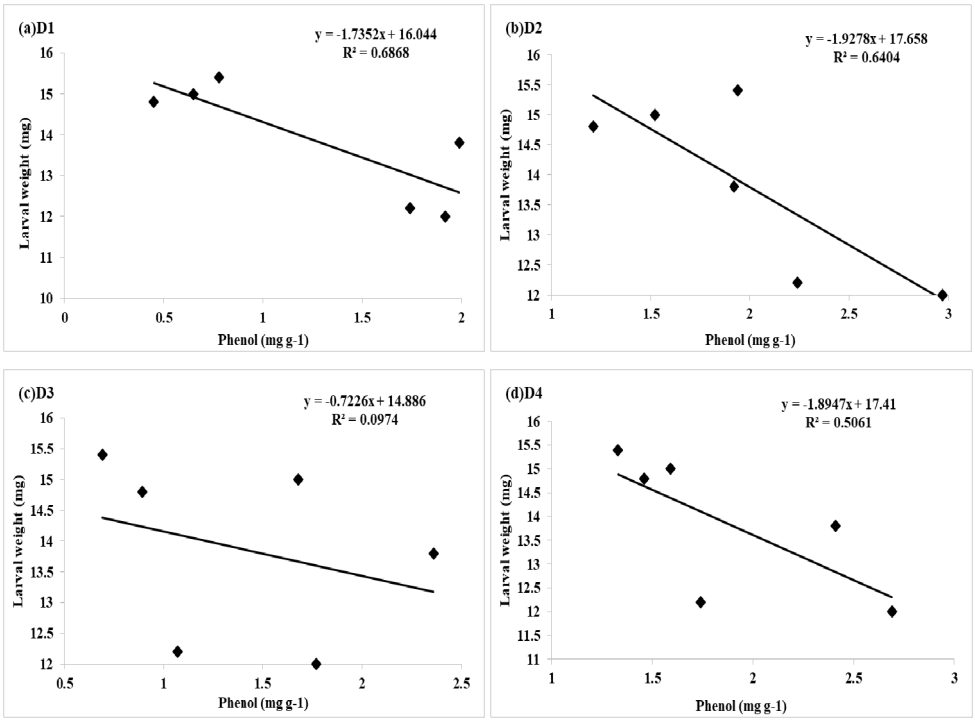


Fig 14: Association of a, b, c, d phenol content of pod wall with resistance to *H. armigera* larval weight.

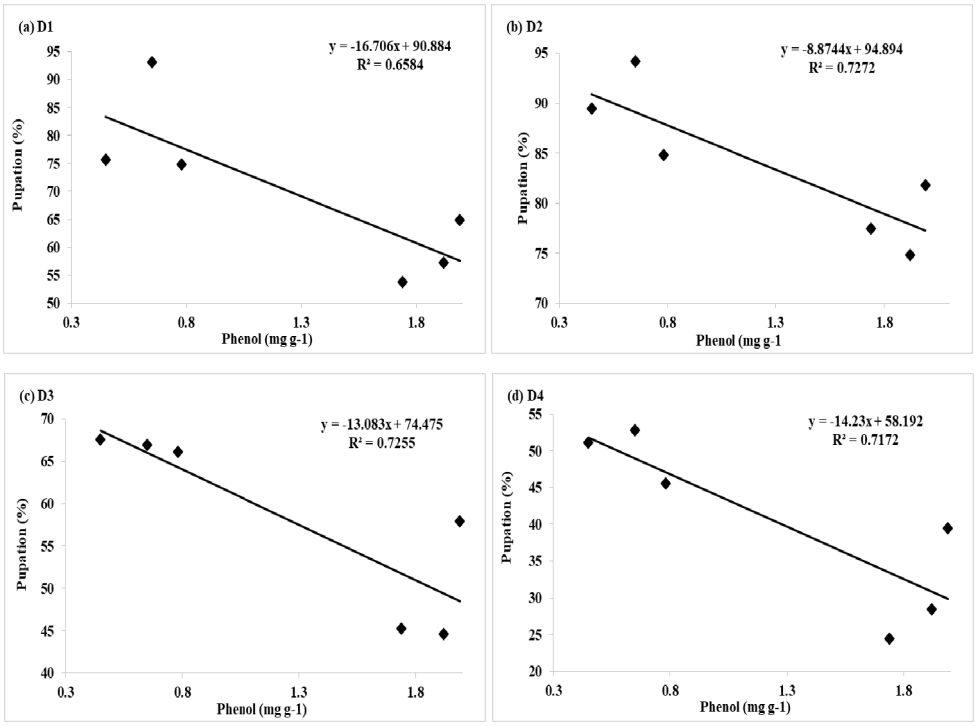


Fig 15: Association of a, b, c, d phenol content of pod wall with resistance to *H. armigera* pupation.

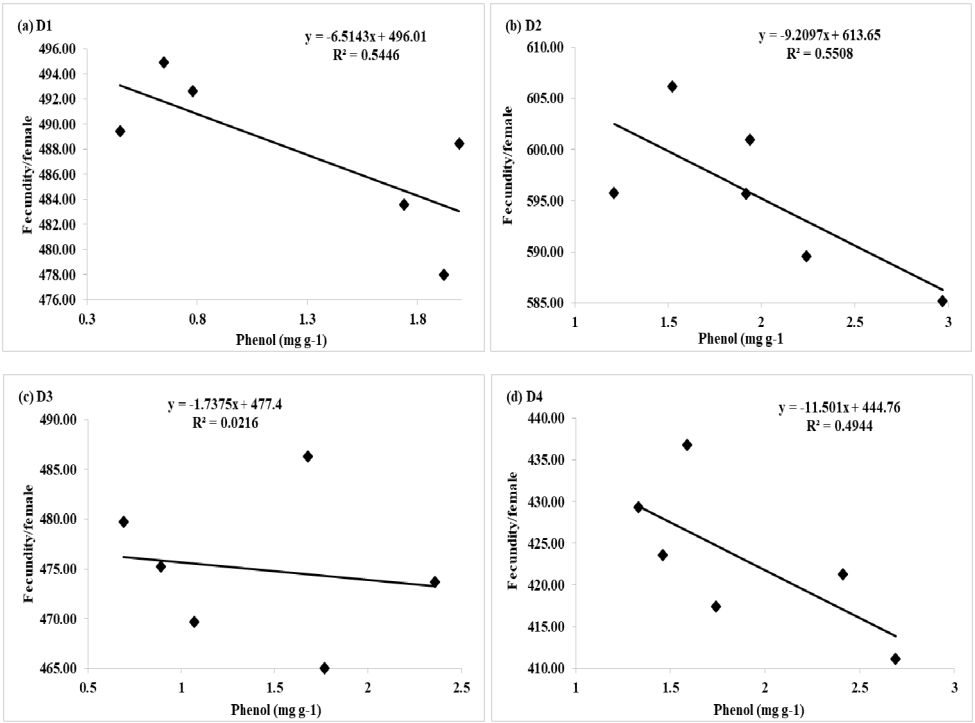


Fig 16: Association of a, b, c, d phenol content of pod wall with resistance to *H. armigera* female fecundity.

Table 4: Correlation coefficient (r) between biochemical constituents and *H. armigera* pod damage.

Sowing	Biochemical constituents											
	Chlorophyll (mg g^{-1})		Crude protein (%)		Fat (%)		Phenol (mg g^{-1})		Total soluble sugar (%)		Tannin (µg g^{-1})	
	Seed	Pod wall	Seed	Pod wall	Seed	Pod wall	Seed	Pod wall	Seed	Pod wall	Seed	Pod wall
	Pooled (2013-2014)											
D_1	0.485	0.626*	0.304	0.740*	-0.884**	-0.060	-0.900**	-0.656*	0.738*	0.698*	-0.792*	-0.086
D_2	0.384	0.465	0.639*	0.104	-0.675*	-0.743*	-0.625*	-0.697*	0.793*	0.898**	-0.812*	-0.630*
D_3	0.655*	0.219	0.810*	0.497	0.580	-0.547	-0.547	0.361	0.250	0.543	0.149	-0.585
D_4	0.753*	0.206	0.711*	-0.253	-0.080	-0.113	0.028	0.181	0.356	0.819*	0.676*	0.262

*Significant at *p = 0.05*; **Significant at *p = 0.01*.

Trichome density, orientation and their types also influences the expression to insect pests in pigeonpea (Aruna *et al*., 2005; Jat *et al*., 2021; Sharma *et al*., 2009). Total phenolic content, phenols and flavonoids were negatively correlated, while sugar content and green pods were positively associated with susceptibility to insect pests in pigeonpea and cowpea (Jakhar *et al*., 2017; Tripathi and Purohit, 1983).

CONCLUSION

One of the main reasons for low yield in pigeonpea crop is the susceptibility to pod borers. The legume pod borer is one of the most important pest of this crop. Relationship between insect, excised leaves and performance of a genotype in the field, depends on insect-host plant relationship, preference by insect and induced resistance. Susceptibility of a test genotype in the field conditions and detached leaf assay to insect-pest is also influenced by non-preference for oviposition and feeding, tolerance and antibiosis. As these factors are important component of resistant breeding programme, nylon bag assay technique can be used as a rapid screening of germplasm under a uniform insect population and suitable environmental conditions. As the results showed, this technique also provides information on anti-feedant and antibiosis components of resistance.

Conflict of interest

Authors do not have conflict of interest to disclose. All authors declare that they have no conflict of interest at all.

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