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Escape resistance in fieldpea against *Phytomyza horticola* Goureau, as influenced by sowing time and its correlation with plant metabolites

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ABSTRACT: Fieldpea or dry pea (*Pisum sativum* L.) is one of the important, highly productive cool season food legume crops and pea leaf miner, *Phytomyza horticola* Goureauis is a pest of high economic importance, associated with the crop**.** Twenty eight diverse field pea genotypes including checks were screened for their susceptibility to the pest and to study the influence of sowing time on the extent of infestation of pea leaf miner. The correlation of infestation with the host primary and secondary metabolites was also examined. In late sown condition, only eight genotypes were categorized as 'Susceptible', 2 genotypes under 'Moderately Resistant', 3 genotypes under 'Intermediate', whereas 15 genotypes were categorized as 'Moderately Susceptible'. None of the genotypes could be categorized as 'Very Highly Resistant', 'Highly Resistant', 'Resistant', 'Highly Susceptible' and 'Very Highly Susceptible' in case of late planting. In case of timely planting, only 9 genotypes were categorized under 'Moderately Susceptible', 7 genotypes each under 'Moderately Resistant' and 'Intermediate' and 5 genotypes grouped under 'Highly Resistant' category. None of the genotypes categorized as 'Susceptible' or beyond that in case of timely planting. Most of the genotypes which showed susceptibility under late sown condition had expressed more level of resistance in the timely sown crop. Although, different fieldpea genotypes differed significantly in terms of quantity of primary and secondary metabolites estimated, only the total phenol content was found significantly and positively correlated with the per cent infestation in the late sown genotypes.

Keywords: Genotypes, leaf miner, *Pisum sativum* L. resistance, screening, susceptibility

INTRODUCTION

Pea (*Pisum sativum* L.) is one of the oldest domesticated crops on the planet (Ambrose, 1995; Zohary and Hopf, 2000) as well as one of the important, highly productive cool season food legume crops grown around the world to consume as food, feed and fodder (Rubiales *et al*., 2019). Since it is an excellent source of protein, starch, fibre and micro-nutrients, hence, widely used as an ingredient in many food industries around the world (Burstin*et al*., 2011; Dixit *et a*l., 2014; Gupta and Parihar, 2015; Parihar *et al*., 2016). Seeds also contain vitamins, minerals, polyphenolics, galactosides, saponins, and phytic acid which are being studied for their health-promoting properties (Arnoldi *et al*., 2015; Dahl *et al*., 2012; Marles *et al*., 2013; Mitchell *et al*., 2009). It is a prominent pulse crop and an important part of sustainable cropping system (Duc *et al*., 2010; Nemecek *et al*., 2008).

Among the various biotic stresses associated with the Fieldpea crop, pea leaf miner, *Phytomyza horticola* Goureau, taxonomically described under the family Agromyzidae of the Order Diptera, is a pest of high economic importance (Spencer, 1973). This insect is highly polyphagous, with over 127 recognized host plants (Singh and Havi, 1982) recorded from 268 genera from 36 families but most commonly on Brassicaceae, Fabaceae and Asteraceae (Spencer, 1990). It is one amongst the major insect pests of pea crop (Singh *et al*., 1992) widely distributed over Africa, Asia and Europe (Crop Protection Compendium, 2007). The pea leaf miner is a serious and persistent pest of peas and Brassicas in northern India, wreaking havoc on these crops (Atwal *et al*., 1969; Bhalla and Pawar, 1977; Prasad *et al*., 1984). It can cause up to 90% damage to the crop by mining young leaves which results in stunting and low flower production (Tariq *et al*., 1991). The tiny adults are two winged flies having greyish black mesonotum. The frons is yellow in colour. Eggs are laid by making punctures through inserting ovipositor into the leaf tissues. The adult females' activity, which punctures fragile leaves in several places with their sharp and pointed ovipositor for the purpose of oviposition or eating, is the first sign of damage to the leaves (Ahmad and Gupta, 1941). The damaging stages are larvae which are minute and slender and form a narrow, linear mine on the upper or lower leaf surface (Spencer, 1973) and feed on mesophyll between the upper and lower epidermis. Maggots that mine into the leaves, eating through the mesophyll while leaving the two epidermal layers intact, causes the most catastrophic harm to the crop (Ahmad and Gupta, 1941; Ancev and

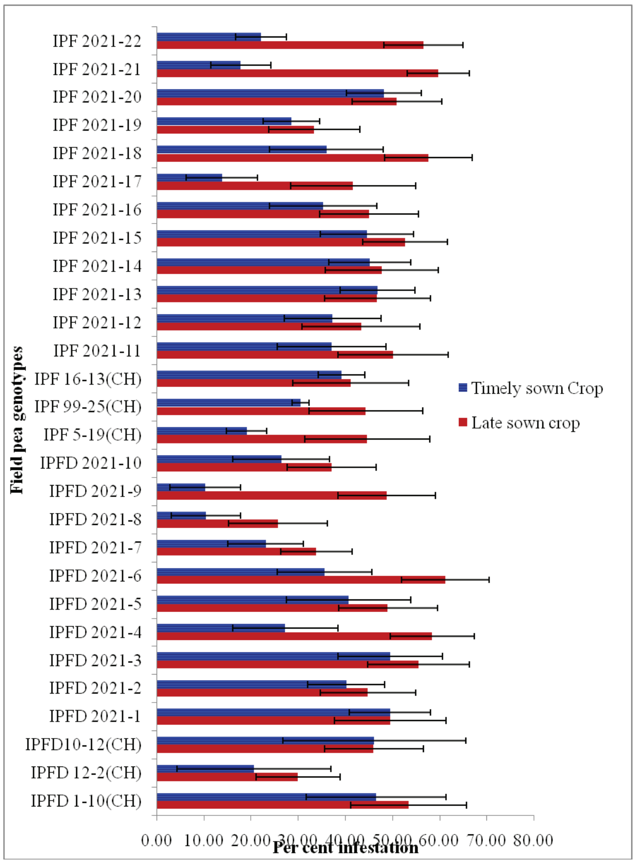

Fig 1. Per cent leaf infestation of different fieldpea genotypes caused by pea leaf miner in timely and late sown condition

Postolovski, 1978). Mining by larvae results in loss of chlorophyll which, in turn, affects the proper growth of plant and crop yield. Photosynthetic activity is severely hampered, and in severe infestations, leaves wither completely. The ability of afflicted plants to bloom and produce fruit is harmed. Under severe infestations, leaf miners cause withering of leaves and reduced flowering and fruiting (Molitas and Gabriel, 1978).

The majority of pest management methods are insecticidal in nature (Chopade, 1975; Dash, 1990; Khajuria and Sharma, 1995; Mehta *et al*., 1995). However, due to their limits, insecticide spraying on vegetable crops has been severely restricted in recent years. As a result, there is a pressing need to investigate and implement new environmentally benign pest management strategies, such as the use of resistant/tolerant types to reduce the use of harmful chemicals. In the present scenario of preferring ecofriendly management avenues over chemical insecticides, development of varieties resistant to insect pest always gets the first thought. Deployment of host plant resistance in insect pest management strategies will render reduced losses, less insecticide use, better crop yields and safer environment (Howe and Jander, 2008). Selections of varieties less prone to insect attack and studies related to adjustment in the date of sowing to gain escape resistance is the present day way forward (Chandra *et al*., 2021).The mechanism of plant resistance rotates around biochemical host attributes too which affects the oviposition and feeding preferences of an insect pest. In view of this, the study was taken up to investigate how the fieldpea crop escapes leaf miner infestation by changing the time of its sowing and to see the correlation of infestation with some primary and secondary plant metabolites.

MATERIALS AND METHODS

Screening of fieldpea genotypes

A field experiment was conducted during rabi 2020-21 at the research farm of ICAR-Indian Institute of Pulses Research, Kanpur, Uttar Pradesh, India, to study the influence of sowing time on the level of infestation of pea leaf miner, *Phytomyza horticola* Goureau. The site of experiment was geographically situated between 26.49188^0 N and 80.27748^0 E with an altitude of 128 m above mean sea level. A panel of twenty eight diverse field pea genotypes including checks was selected for the trial. Timely sowing was done on November 1, whereas another set for late sowing was done on December 9. The experiment was executed in randomized complete block design with three replications. In each replication, genotypes were planted in three rows of 4.0 m length with inter and intra row spacing of 60 cm and 10 cm, respectively. The crop was raised following recommended package of practices to maintain a normal healthy crop. No control measures adopted for insect pest control.

The incidence of leaf miner in field pea crop noticed from first week of February, at the time crop reached the flowering stage. For estimation, done at weekly interval, ten plants were tagged randomly in each genotype. The per cent leaf infestation was calculated by counting the infested leaves out of the total number of leaves present in the plant. On the basis of per cent leaf infestation, the genotypes were categorized using a scale of 1-9 ('1' being 'Very highly resistant' and '9' being 'Very highly susceptible'), following the method given by Singh and Weigand (1994).

Estimation of plant metabolites

Protein content, total sugar, total phenol and tannin content were quantified in randomly selected 10 different field pea genotypes. Protein present in sample was estimated using Lowry's method (Lowry *et al*., 1951). The principle behind the Lowry method of determining protein concentrations lies in the reactivity of the peptide nitrogen[s] with the copper [II] ions under alkaline

Table 1. Categorization of fieldpea genotypes on the basis of degree of susceptibility against pea leaf miner during different planting time

S.N.	Field Pea Genotypes	susceptibility category	
		Timely sown	Late sown
1	IPFD 1-10(CH)	MS	S
2	IPFD 12-2(CH)	MR	MR
3	IPFD 10-12(CH)	MS	MS
4	IPFD 2021-1	MS	MS
5	IPFD 2021-2	I	MS
6	IPFD 2021-3	MS	S
7	IPFD 2021-4	MR	S
8	IPFD 2021-5	MS	MS
9	IPFD 2021-6	I	S
10	IPFD 2021-7	MR	I
11	IPFD 2021-8	HR	MR
12	IPFD 2021-9	HR	MS
13	IPFD 2021-10	MR	I
14	IPF 5-19(CH)	HR	MS
15	IPF 99-25(CH)	MR	MS
16	IPF 16-13(CH)	I	MS
17	IPF 2021-11	I	MS
18	IPF 2021-12	I	MS
19	IPF 2021-13	MS	MS
20	IPF 2021-14	MS	MS
21	IPF 2021-15	MS	S
22	IPF 2021-16	I	MS
23	IPF 2021-17	HR	MS
24	IPF 2021-18	I	S
25	IPF 2021-19	MR	I
26	IPF 2021-20	MS	MS
27	IPF 2021-21	HR	S
28	IPF 2021-22	MR	S

VHR = Very highly resistant; 2. HR = Highly resistant; 3.R = Resistant; 4.MR = Moderately resistant; 5.I = Intermediate; 6. MS = Moderately susceptible; 7. S = Susceptible; 8. HS = Highly susceptible; 9. VHS = Very highly susceptible

Table 2. Quantity of protein, total sugar, total phenol and tannin in different field pea genotypes

Field pea genotypes	Protein (%)	Total sugar(mg/g of Leaf sample)	Total phenol (mgGAE/g of leaf sample)	Tannin (mgTAE/g of leaf sample)
IPED 1-10(CH)	5.63 (13.71)	13.16 (3.76)	2.81 (1.95)	3.31 (2.07)
IPFD 2021-1	5.36 (13.38)	14.88 (3.98)	2.31 (1.82)	2.72 (1.92)
IPFD 2021-3	3.65 (10.99)	6.65 (2.76)	2.25 (1.80)	2.80 (1.94)
IPFD 2021-5	4.20 (11.81)	13.16 (3.76)	2.31 (1.81)	3.09 (2.02)
IPFD 2021-8	5.50 (13.54)	5.77 (2.60)	2.15 (1.77)	3.17 (2.04)
IPF 2021-11	5.28 (13.27)	19.31 (4.50)	3.23 (2.05)	3.61 (2.14)
IPF 2021-15	6.22 (14.42)	29.53 (5.52)	3.16 (2.04)	3.97 (2.22)
IPF 2021-16	5.38 (13.39)	31.59 (5.70)	2.89 (1.97)	3.65 (2.15)
IPF 2021-21	5.40 (13.43)	18.21 (4.38)	3.29 (2.07)	3.64 (2.15)
IPF 2021-22	4.92 (12.80)	17.71 (4.32)	2.80 (1.94)	3.45 (2.11)
C.D	0.563	0.118	0.030	0.029
SE(m)	0.188	0.039	0.010	0.010
SE(d)	0.266	0.056	0.014	0.014
CV	2.490	1.651	0.911	0.807

Figures under parenthesis, corresponding to protein values,are arcsine transformation of the original percent values; Figures under parenthesis, other than protein values, are square root transformation of the original values.

conditions and the subsequent reduction of the Folin-Ciocalteu phosphomolybdic phosphotungustic acid to heteropoly molybdenum blue by the copper-catalyzed oxidation of aromatic acids. The absorbance was taken at 660 nm in spectrophotometer. A calibration curve was plotted using BSA (Bovine serum albumin) as standard and the protein content in the sample was calculated in percentage using plotted standard curve of BSA. Total phenolic content of the sample extracts was determined using Folin–Ciocalteu reagent following the method of Singleton and Rossi (1965).The absorbance of the samples was measured at 650 nm against reagent blank in spectrophotometer. The Calibration curve was plotted using gallic acid as standard and phenols in the sample was calculated as gallic acid equivalents (mgGAE/g sample) using standard curve of gallic acid.Tannins in the sample was estimated using Folin–Denis method based on the principle that tannin like compounds reduce Phosphomolybdic acid in alkaline solution to produce highly coloured blue solution, the intensity of which is proportional to the amount of tannins (Schanderl, 1970). The absorbance of the samples was taken at 700 nm in spectrophotometer. The calibration curve was plotted using tannic acid as standard and the content in the sample was calculated as tannic acid equivalents (mgTAE/g sample) using standard curve of tannic acid. The total sugar in the sample was estimated by the method of Dey (1990). The absorbance was recorded at 490 nm in spectrophotometer. The calibration curve was plotted by using glucose as standard. The total soluble sugar content in the sample was calculated in mg/g of leaf sample using standard. The chlorophyll content in all the 28 field pea genotypes was estimated using SPAD-502 and expressed in relative SPAD meter values which are proportional to the chlorophyll content present in the leaf (Ling *et al*., 2011).

Table 3. Correlation matrix showing Pearson's correlation coefficient amongst metabolites and per cent leaf infestation in field pea genotypes

	Chlorophyll	Per cent Infestation (late sown genotypes)	Per cent Infestation (timely sown genotypes)	Protein	Total sugar	Total Phenol
% Infestation (late sown genotypes)	0.285 NS					
% Infestation (timely sown genotypes)	0.232 NS	0.127 NS				
Protein		-0.017 NS	-0.165 NS			
Total sugar		0.259 NS	0.097 NS	0.538 NS		
Phenol		<u>0.636*</u>	-0.121 NS	0.538 NS	0.703*	
Tannin		0.324 NS	-0.312 NS	0.632*	0.768**	0.864**

Correlation analysis

Correlation analysis was done by calculation of Pearson's correlation coefficient through the OPSTAT analytical software to see relation, if any, between per cent leaf infestation done by pea leaf miner with quantity of protein, total phenol, tannin, total sugar and chlorophyll.

RESULTS AND DISCUSSION

Screening of field pea genotypes

The per cent leaf infestation of 28 field pea genotypes and their comparison under timely and late sown conditions has been depicted in the Figure 1. In timely sown condition, the per cent leaf infestation ranged from 3.44 (IPFD 2021-9) to 49.44 (IPFD 2021-1 and IPFD 2021-3). Other genotypes observed with comparatively higher per cent leaf infestation in timely sown condition were IPF 2021-20 (48.19%), IPFD 1-10 (Check) (46.53%), IPFD 10-12 (Check) (46.11%), IPF 2021-13 (46.81%), IPF 2021-14 (45.14%) and IPF 2021-15 (44.58%). Range of leaf infestation was found increased (25.67 for IPFD 2021-8 to 61.17 for IPFD 2021-6) in case of late sown crops.

Kooner and Singh (1980) observed the per cent infestation of pea leaves by leaf miner which was ranged from 2.0 to 31.7 in different varieties whereas Kashyap *et al*. (1982) found 33.6, 20.3, and 22.9 per cent pea leaf miner infestation on Bonneville, Arkel and Harabona-B cultivars of field pea, respectively. In the present study, IPFD 12-2 (Check), IPFD 2021-7, IPFD 2021-10 and IPFD 2021-19 were some of the genotypes observed with low per cent leaf infestation of 29.92, 33.83, 37.08 and 33.33, respectively. On the other hand, genotypes like IPFD 1-10 (Check), IPFD 2021-3, IPFD 2021-4, IPF 2021-11, IPF 2021-15, IPF 2021-18, IPF 2021-20, IPF 2021-21 and IPF 2021-22 were recorded with high per cent leaf infestation of 53.42, 55.50, 58.42, 50.08, 52.67, 57.58, 50.92, 59.75 and 56.58, respectively. Genotypes when sown under late condition, had recorded higher per cent leaf infestation as compared to timely sown genotypes. In both timely and late sown genotypes, it was observed that the per cent leaf infestation increased with progress of time.

In timely sown crops, mean per cent leaf infestation was recorded minimum on 8 February with only 5.96, followed by 14.98 on 15 February, 23.99 on 24 February and maximum of 41.48 on 1 March 2021. Similarly, in late sown condition, minimum per cent leaf infestation of 7.13 was observed on 3 February, which increased to 12.25 on 10 February, to 21.05 on 17 February, to 36.29 on 24 February and a maximum of 57.16 was recorded on 3 March 2021.

All the 28 field pea genotypes were rated using 1-9 scale and were categorized from Very highly resistant (1) to Very highly susceptible (9). In case of timely planting, only 9 genotypes were categorized under 'Moderately Susceptible', 7 genotypes each under 'Moderately Resistant' and 'Intermediate' and 5 genotypes grouped under 'Highly Resistant' category (Table 1). None of the genotypes categorized as 'Susceptible' or beyond in case of timely planting. In late sown condition, among 28 genotypes, 8 genotypes grouped under 'Susceptible' category, 2 genotypes under 'Moderately Resistant', three genotypes under 'Intermediate', whereas 15 genotypes were categorized as 'Moderately Susceptible'. None of the genotypes could be categorized as 'Very Highly Resistant', 'Highly Resistant', 'Resistant', 'Highly Susceptible' and 'Very Highly Susceptible' in case of late planting.

Kashyap *et al*. (1982) reported no substantial differences in resistance to this pest among the various potential pea cultivars. Mahobe and Narsinghani (1986) found none of the pea lines with desired level of resistance to this miner; however, they reported some degree of resistance in the cultivars JP-179, JP-854, JP-747, JP-169-1 and JP-Batri Brown. Also, Dash *et al*. (1988) found no cultivars resistant to pea leaf miner attack; however, "early wonder" showed considerable resistance, followed by Arkel. Bhat (1988) examined 20 lines of pea for resistance to *C. horticola*, *Lampides boeticus* L., and *Bruchus pisorum* L. in Kashmir and found that different cultivars had various levels of infestation, but no variety was totally resistant to this pest. Sharma and Sharma (1991) classified 8 pea cultivars (IP-3, JP-169, PG-2, S-143, Sel-35, Sel-93, Sel— 3487, VG-1) as resistant to pea leaf miner, 22 as moderately susceptible and 11 as highly susceptible in field trials conducted in Himachal Pradesh. Thakur and Patial (2019) screened ninety-two pea germplasm against pea leaf miner where six pea genotypes (DPP 25G, DPPLMR 41, JI 1766 (2), JP 179, LMR 100, S143) exhibited high resistance, nineteen as resistant and seventeen as moderately resistant to the pest. In the present study, only two genotypes i.e. IPFD 2021-8 and IPFD 12-2 could demonstrate good level of resistance in both late and timely planting. These identified genotypes after further validation can be used as donor in resistance breeding program.

Quantification of plant metabolites in field pea germplasm

The estimated values of protein, total sugar, total phenol and tannin in selected field pea genotypes are shown in Table 2.

The difference in the contents of these bio-chemicals among the genotypes studied was found statistically significant.The protein content was estimated highest in IPF 2021-15 with 6.22 per cent and lowest in IPFD 2021-3 with only 3.65 per cent. The total sugar was observed maximum in IPF 2021-15 with 29.53 mg/g leaf sample while minimum quantity was observed in IPFD 2021-8 and IPFD 2021-3 with 5.77 and 6.65 mg/g of leaf sample, respectively. Sepehya *et al*. (2015) reported maximum sugar content of 4.21 mg/g in field pea. Minimum content of total phenol, 2.15 mg GAE/g leaf sample was estimated in IPFD 2021-8 to the maximum of 3.29 mg GAE/g leaf sample in IPF 2021-21. Wang *et al*. (1998) estimated total phenolics in field pea which differed significantly among cultivars, ranging from 162 mg/kg DM (dry matter) (CE, catechin equivalents) to 325 mg/kg DM (CE). Zia-ul-Haq *et al*. (2013) showed that the contents of total phenols, flavonoids, and condensed tannins in seeds of four Pakistani pea cultivars ranged between 0.84–0.99, 0.09–0.17, and 0.57–0.68 mg/g, respectively. Hegedusova *et al*. (2015) reported the total polyphenol contents of six garden pea varieties as 1179.995±28.081 mg GAE/kg as the highest value and the lowest value as 674.505± 26.541 mgGAE/kg. The tannin content was found minimum in IPFD 2021-1 with only 2.72 mgTAE/g of leaf sample while maximum tannin content was recorded in IPF 2021-15 with 3.97 mgTAE/g leaf sample. Overall, it was observed that IPFD 2021-15 was the genotype observed with maximum content of protein, total sugar, total phenol and tannin content, whereas, IPFD 2021-3 and IPFD 2021-8 was found low in all of these parameters.

The chlorophyll contents in all the 28 field pea genotypes were estimated and expressed in relative SPAD meter values which remain proportional to the chlorophyll content present in the leaf (Ling *et al*., 2011). The SPAD meter values ranged from 26.63, 27.41 and 28.99 in IPF 16-13 (Check), IPF 99-25 (Check) and IPF 2021-11 to maximum of 51.02, 48.71 and 47.68 in IPFD 2021-3, IPFD 10-12 (Check) and IPFD 2021-4, respectively. Golawska *et al*. (2010) reported chlorophyll ranging from 36.57 to 39.82 (SPAD meter values) in *P. sativum*. The difference among genotypes in terms of chlorophyll content was found statistically significant.

Correlation between per cent infestation and physiological attributes

Correlation analysis were done among per cent infestation observed in timely sown crops, late sown crops, their protein content, total sugar, total phenol and tannin content in randomly selected 10 genotypes. Correlations of chlorophyll content of all 28 genotypes were analysed with the corresponding per cent leaf infestation in both timely and late sown conditions. Of all the correlations, only per cent infestation observed in late sown condition was found significantly and positively correlated with the total phenol content with a correlation coefficient of 0.64 (Table 3).

Also, Sharma and Aggarwal (1983) observed positive correlation between population of jassid, *Amrasca biguttula biguttula* Ishida, with free phenol and the tannin content of cotton. On the other hand, Woodhead *et al*., (1980) found high phenolic acid concentrations linked to reduce eating by several grasshoppers and the plant hoppers. Dass and Odak (1987) reported total phenols to be adversely linked with pod fly (*Melanagromyza obtusa* Malloch) infestation in *Cajanus cajan*. Nomura and Itioka (2002) discovered that tannin inhibits cutworm larvae from growing and that the inhibitory impact was proportional to the amount of tannin consumed. According to Chandra (2012), total chlorophyll, carotenoid and sugar in maize germplasm were significantly and positively correlated with the Leaf Injury Rating (LIR) recorded with respect to *Chilo partellus*. Contrastingly, Singh (1984) reported that chlorophyll content of a genotype was not related to resistance or susceptibility in *Brassica* strains to pea leaf miner. In another study, Padmavathi *et al*. (2013) found leaf folder damage leading to 57% loss in chlorophyll content, a 23% drop in PS II activity and a 23% reduction in relative water content. Saheb *et al.* (2018) reported proteins with a significant negative correlation with incubation period and fifth instar larval duration, phenols with a significant positive correlation with fifth instar duration, reducing sugars showed positive correlation with incubation period, fifth instar duration and a negative significant correlation with post-oviposition period of leaf bud borer in groundnut. Saleem *et al*. (2019) observed significant negative correlation between *S. litura* damage and protein and phenol content while significant positive correlation was found with reducing and total sugar.

CONCLUSION

Although, there exist a large variation in results pertaining to the nature of correlation between plant biochemical and their impact on biology of insect pests; in the present study, physiological attributes like protein, total sugar, tannin, chlorophyll content found no significant correlation with per cent infestation in the field pea crop. The significant positive correlation observed between per cent leaf infestation in late sown condition and phenol content of the genotypes may also be seen as an induction or plant response to higher infestation of leaf miner in late sown crop. Secondary metabolites like phenol may prolong the life stages of the insect, facilitating prolonged feeding and thereby increase the level of infestation. Most of the genotypes which showed susceptibility under late sown condition had expressed more level of resistance when timely sowing was done.

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