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Physio-morpho and biochemical basis of resistance in soybean germplasms against *Spodoptera litura* (Fabricius)

Kota Sahithi Chowdary and BP Katlam

Abstract
Correlation studies were conducted between different physio-morphological and biochemical parameters with per cent leaf damage due to *Spodoptera litura* on soyabean under field conditions. The correlation studies revealed that the per cent leaf damage had significant and positive correlation with different parameters like leaf succulency (r = 0.841**), chlorophyll content (r = 0.580**) and reducing sugars (r = 0.850**). Whereas significant and negative correlation was found between per cent leaf damage and trichome density (r = - 0.905**), phenol content (r = -0.866**). For plant height (r = -0.168), leaf shape there was non-significant correlation with per cent leaf damage.

Keywords: Correlation, *Spodoptera litura*, Soybean, physio-morphological and biochemical parameters

Introduction

Soybean, *Glycine max* L. Merrill, is known as the "wonder crop", "Golden Bean" of 20th century, "miracle crop" of 21st century, and "gold from soil "and" cow of the field in light of its different uses. Insect pests are major drawbacks in realising the yield potential and responsible to cause more than 27% yield loss (Sharma and Shukla 1997) [16]. During early seventies, soybean was considered to be the safest crop with regard to insect pest attack. But with rapid increase in area under soybean, its extension to newer areas added the new insect pests which are causing great concern to its productivity. For the management of insect-pests, host-plant resistance is highly useful strategy as it does not require any special action from growers. It is advised to employ resistant plants, which have several advantages over insecticides and to stabilise yield. It has also been shown to be environmentally friendly, has lower production costs, doesn't need to transfer new technology, and is thought to work well with other insect management control methods (Suharsono and Sulistyowati, 2012) [17]. It is also a cheaper and practical input in the integrated pest management system. Further, it does not require any monetary investment and is an added benefit to protect the environment from the toxic chemical residues, etc.

Each plant species has a unique defence strategy that uses a variety of physical characteristics and has an effect on the reproduction and survival of pests. In order to counteract the impacts of insect damage, plants adapt to it through a variety of morphological (Antixenosis), molecular (Tolerance) and bio-chemical (Antibiosis) methods. It is recognised that morphological and biochemical characteristics help plants to withstand insect infestations (Norris *et al*., 1980) [12]. Plant morpho-physiological characters like trichome density, leaf succulency, chlorophyll content play an important role in the insect oviposition and feeding activities. Biochemical characters like phenols, sugars in the leaf tissues also play an important role in feeding activities of insect

Materials and Methods

A total fifty-four soybean germplasms were studied including three checks among them against tobacco caterpillar *Spodoptera litura* (Fabricius). In which each germplasm was sown in 3 rows each of 3 m length with a row to row spacing 45 cm and plant to plant spacing 10 cm. These germplasms were replicated twice with a plot size of 3m x 1.35m.

Method of observation

Calculation of per cent leaf damage

The reaction of germplasms for *Spodoptera litura* infestation was recorded based on the visual observation of leaf damage at peak period of pest infestation and the per cent leaf damage is calculated on five randomly selected plants by the formula given by Abdul Fattah *et al*. (2018) [1].

$$I = \frac{\sum_{i=0}^{N}(n_i v_i)}{ZN} x\, 100$$

I = Intensity of damage;
ni = the number of leaves with vi scale;
N = the number of leaves observed;
Z = the higher vi.
Scale value vi:
1 = leaf damage > 0%-20%;
3 = leaf damage > 20%-40%;
5 = leaf damage > 40%-60%;
7 = leaf damage > 60%-80%;
9 = leaf damage > 80%-100%.

Collection of experimental data
Observation on different physio-morpho and bio-chemical parameters of soybean germplasms were recorded on randomly selected plants from each plot at 45 days after sowing (DAS).

Physio-morphological characters Leaf succulency
Leaf succulency was expressed as relative water content (RWC). Leaf sample after 45 DAS was taken and fresh weight was recorded, followed by turgid weight after flotation on water for 4 hours and the leaf tissue was subsequently oven-dried to a constant weight and dry weight was recorded. Leaves were cut into small circular pieces for easy handling. The procedure of estimation was done using the method given by Barrs and Weatherley (1962) [4].

$$\text{Relative water content (RWC)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \text{x}100$$

Leaf trichome density
The observations on trichomes were recorded under microscope on abaxial leaf surface as per Maiti and Bidinger (1979) [9].
The observations on trichomes were recorded under stereo zoom binocular microscope. The leaf sample was kept overnight in acetic acid: alcohol (2:1) solution for removal of chlorophyll and easy observation of trichomes. After one night when the chlorophyll was completely removed the leaves were kept under binocular microscope and number of trichomes per mm2 was counted for three leaf samples (fig 1).

Plant height
Plant height was measured from ground level to the tip of the main shoot of the five random plants using a ruler. The mean of the five plants was considered as plant height in cm.

Total Chlorophyll content
Leaf chlorophyll content was measured by using SPAD Chlorophyll meter on lower, middle and upper leaves of all germplasms.

Leaf shape
Germplasms were classified based on their leaf shape as lanceolate, pointed ovate and round ovate given by Ramteke and Pooja (2012) [14]. Based on per cent damage, preference was observed (fig 4).

Bio chemical parameters
For analysis of phenols and reducing sugars leaf samples was collected after 45 DAS. Leaf sample was grinded with alcohol and the mixture is subjected to centrifugation, later the supernatant is collected and used for analysis.

Phenol estimation
Estimation of total phenols present in plant samples was determined by following Folin- Ciocalteau Reagent (FCR) method given by Ainsworth and Gillespie (2007) [2].
One hundred microliters of standard and sample extract solution were each reacted with 750 µl of Folin–Ciocalteou's reagent for 5 min. After addition of 750 µl of 7.5% Na2CO3, the mixture was allowed to stand in the dark for 30 min, transferred to test tubes and end phase was known by development of blue coloured compound. The blue coloured samples were subjected to absorbance measurement at 765 nm using a spectrophotometer (fig 3). Gallic acid was used for construction of a standard curve with a concentration range of 100–500 microgram/ml. using this standard curve, total phenolic content was calculated and expressed as mg/gram of soybean leaf sample.

Reducing sugars estimation
For the estimation of reducing sugar, Dinitro salicylic acid method (Miller, 1972) [10] was used. DNS reagent was prepared by adding 1gm of dinitro salicylic acid, 200mg of crystalline phenol, 50 mg of sodium sulphite, 100 ml of 1% sodium hydroxide solution. Along with DNS 40% solution of Rochelle salt (sodium potassium tartarate) was prepared. Then 3 ml of plant aliquot was pippeted into a test tube. In this 3 ml of DNS reagent was added and the mixture was heated for 5 minutes in boiling water bath. After the development of brownish orange compound, added 1 ml of 40% Rochelle salt solution and cooled the test tubes under running water. These coloured samples were subjected to absorbance at 540 nm and concentration was expressed as mg/g of plant sample (fig 3). Glucose was used for construction of a standard curve with a concentration range of 200–1000 microgram/ml. Based on the standard curve the concentration of reducing sugars in the sample was calibrated.

Results and Discussion
Per cent leaf damage and data of different physio-morphological and biochemical parameters was presented in table 1. Correlation studies between per cent leaf damage and different physio-morphological and biochemical parameters was presented in table 2.

Trichome density with per cent leaf damage
The correlational studies between trichomes and per cent leaf damage revealed that there was significant and highly negative correlation (r = -0.905**). The regression equation being y = -1.0788x + 78.504 indicated that with an increase of one trichome there will be reduction in per cent leaf damage by 1.0788% (graph 1).
The findings in the present investigation were similar with Ihsan-ul-Haq *et al.* (2003) [6], Anchala *et al.* (2015) [3], Sasane *et al.* (2018) [15] who found leaf hair density on the abaxial surface of the leaf had a strongly negative correlation with percent infestation in different crops.

Leaf succulency with per cent leaf damage

The correlational studies between leaf succulency and per cent leaf damage revealed that there is significant and highly positive correlation (r=0.84**). The regression equation being y = 1.887x1 - 39.529 indicated that with an increase of one per cent leaf succulency there will be increase in per cent leaf damage by 1.887% (graph 2).

The findings are similar with Ihsan-ul-Haq *et al.* (2003) [6], Mohammad *et al*., (2019) [11], Sasane *et al.* (2018) [15], who found a strong positive association between relative water content and percent infestation by different pests.

Chlorophyll (SPAD) with per cent leaf damage.

The correlational studies between Chlorophyll (SPAD) and per cent leaf damage revealed that there was significant and positive correlation (r =0.580**). The regression equation being y = 1.5847x2 - 19.727 indicated that with an increase of one unit reading in chlorophyll content there will be increase in per cent leaf damage by 1.5847% (graph 3).

Similarly, Haralu *et al.* (2018) [5] found chickpea pod borer, *Helicoverpa armigera* (Hubner) pod borer infestation percentage and total chlorophyll content were positively correlated.

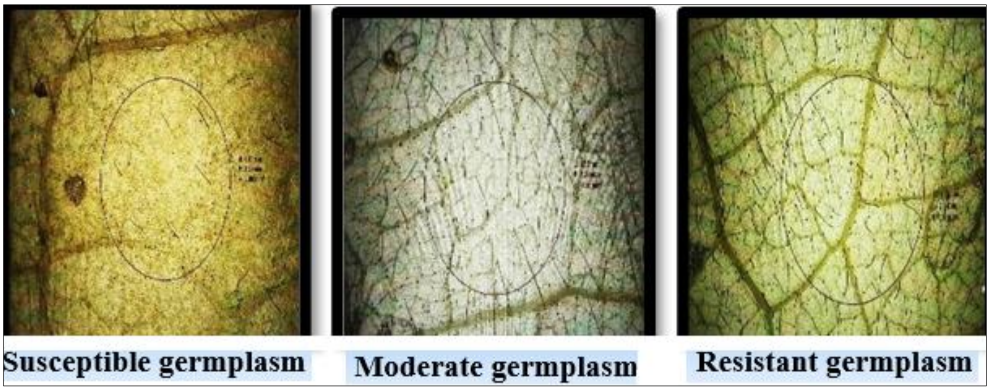


Fig 1: Trichome density/mm2 of different germplasms

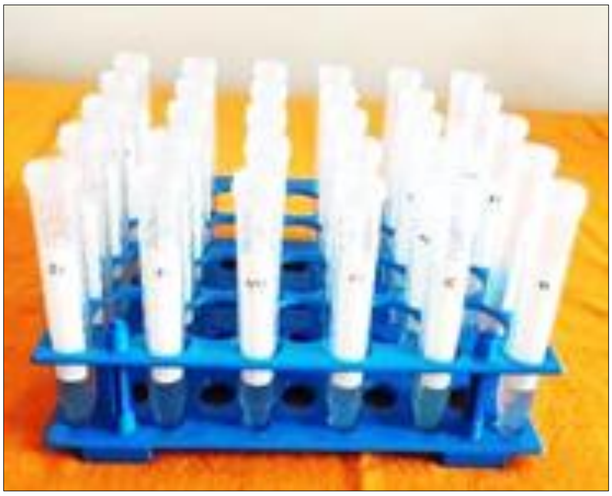


Fig 2: Samples of germplasms for phenol estimation

Fig 3: Samples of germplasms for reducing sugars estimation

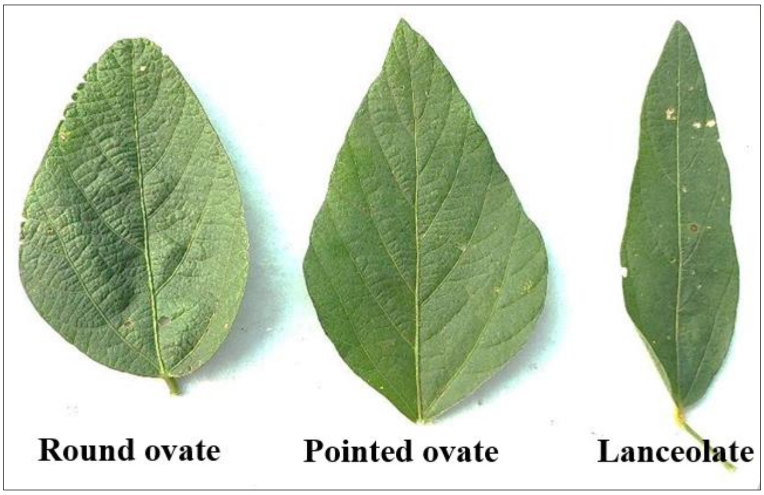


Fig 4: Different leaf shapes in soybean germplasms

Table 1: Per cent leaf damage and physio-morphological, biochemical parameters in soybean

S. No	Name of germplasm	Per cent leaf damage	Trichome density/mm2	Chlorophyll (SPAD)	Leaf succulency (%)	Plant height in cm	Leaf shape	Phenol content (mg/g)	Reducing sugar(mg/g)
1	VLS 104	50.98	23.67	43.57	46.64	85.00	Pointed ovate	0.23	6.30
2	NRCSL 5	48.96	28.00	42.07	44.48	72.40	Lanceolate	0.25	5.80
3	JS 24-26	49.16	39.00	42.80	45.25	34.80	Pointed ovate	0.24	6.12
4	NRCSL 7	54.96	19.67	44.37	48.70	69.60	Lanceolate	0.22	6.49
5	JS 20-116(C)	24.69	50.00	37.47	50.65	51.60	Pointed ovate	0.33	3.67
6	SKAUS 3	69.87	10.67	50.27	58.17	38.60	Pointed ovate	0.12	8.56
7	RVS 12-8	58.44	19.67	44.83	50.04	49.40	Round ovate	0.22	7.04
8	KDS 1203	49.35	26.33	43.17	45.47	36.40	Round ovate	0.24	6.25
9	NRC 253	61.11	19.33	46.03	51.01	34.20	Pointed ovate	0.19	7.51
10	MACS 1756	69.44	19.00	53.10	58.12	71.60	Round ovate	0.08	8.86
11	Lok Soya-2	41.13	33.00	40.90	43.32	58.80	Pointed ovate	0.25	4.85
12	AMS 2021-3	44.01	32.00	41.33	43.60	61.60	Pointed ovate	0.25	8.52
13	Himso 1695	48.97	26.67	42.30	44.83	42.60	Pointed ovate	0.24	6.00
14	TS - 156	61.72	10.67	46.53	52.01	30.00	Pointed ovate	0.17	4.82
15	NRCSL 8	25.37	46.33	37.80	47.18	48.40	Pointed ovate	0.31	3.98
16	JS 24-34	59.57	19.33	45.23	38.00	54.80	Pointed ovate	0.21	7.33
17	RSC 10-46 (C)	22.74	54.00	37.03	35.49	59.20	Pointed ovate	0.36	2.61
18	DS 1510	46.16	28.67	47.33	44.01	72.00	Round ovate	0.25	5.65
19	KSS 213	51.04	23.33	43.60	46.97	77.20	Pointed ovate	0.23	4.44
20	MAUS 824	35.75	35.67	40.10	42.29	66.80	Pointed ovate	0.26	4.65
21	NRC 254	59.42	19.67	45.23	50.25	75.40	Pointed ovate	0.21	7.07
22	AMS 2021-4	37.52	35.33	40.43	42.65	58.60	Pointed ovate	0.26	4.78
23	Himso 1696	41.25	33.00	40.93	43.48	56.20	Pointed ovate	0.25	5.08
24	DS 1529	31.27	37.00	39.43	39.84	59.20	Round ovate	0.27	4.56
25	KDS 1188	65.46	16.00	41.83	53.33	42.40	Pointed ovate	0.16	8.15
26	MACS 1745	64.32	16.67	41.80	53.31	46.60	Round ovate	0.16	8.15
27	NRC 255	53.14	20.00	44.03	48.34	71.20	Pointed ovate	0.22	6.48
28	Asb 93	64.02	28.67	40.77	52.84	31.20	Pointed ovate	0.27	8.06
29	VLS 105	52.55	22.67	43.60	38.43	71.00	Pointed ovate	0.23	6.40
30	NRCSL 4	29.68	26.67	39.03	39.61	62.00	Pointed ovate	0.27	6.38
31	NRC 257	56.04	19.67	44.40	48.90	44.20	Pointed ovate	0.22	6.66
32	MAUS 814	40.31	46.67	41.80	43.05	77.00	Round ovate	0.26	4.84
33	SL 1311	45.79	29.00	47.23	43.89	89.60	Pointed ovate	0.25	5.50
34	Asb 85	69.65	10.33	51.43	63.44	49.60	Pointed ovate	0.12	8.88
35	PS 1693	43.72	32.33	41.20	43.53	67.20	Round ovate	0.25	5.09
36	NRC 256	53.04	20.67	43.73	47.61	71.20	Pointed ovate	0.22	6.42
37	RSC 1165	63.20	18.33	47.03	52.59	39.80	Pointed ovate	0.16	7.59
38	BAUS 124	26.15	46.00	38.60	38.47	56.00	Lanceolate	0.27	4.00
39	DLSb 40	69.22	14.00	38.90	57.53	67.40	Pointed ovate	0.15	9.01
40	NRC 258	20.37	61.33	21.13	31.65	58.00	Pointed ovate	0.34	3.50
41	PusaSipani BS9	69.09	14.00	49.73	55.36	56.80	Pointed ovate	0.15	8.54
42	PS 1696	49.49	24.33	43.17	46.63	52.80	Round ovate	0.24	6.30
43	CAUMS 3	67.84	15.00	37.13	55.25	96.20	Round ovate	0.16	8.52
44	AUKS 212	28.36	39.00	50.20	39.38	53.40	Pointed ovate	0.16	4.31
45	RVSM 12-21	60.14	19.33	45.70	50.81	26.20	Pointed ovate	0.20	7.47
46	NRC 259	34.16	36.00	39.80	40.95	68.20	Pointed ovate	0.26	4.58
47	AS 34	48.31	18.33	41.93	44.45	85.40	Round ovate	0.25	5.75
48	NRC 128 (C)	36.05	35.33	40.17	42.46	95.00	Pointed ovate	0.26	4.77
49	RSC 1172	23.09	52.00	49.13	36.58	64.80	Lanceolate	0.34	3.51
50	AS 55	38.77	33.67	40.50	42.65	41.80	Round ovate	0.26	7.54
51	TS-208	67.21	15.67	47.40	54.83	46.60	Round ovate	0.16	5.25
52	NRC 260	45.76	29.33	47.20	43.71	69.00	Pointed ovate	0.25	5.36
53	NRC 196	25.29	33.67	37.70	38.36	55.20	Pointed ovate	0.31	3.74
54	Pusa Sipani-SPS-433	21.19	58.67	24.53	34.96	61.80	Pointed ovate	0.35	3.29

Table 2: Correlation between tobacco caterpillar damage and physio-morpho and bio- chemical parameters

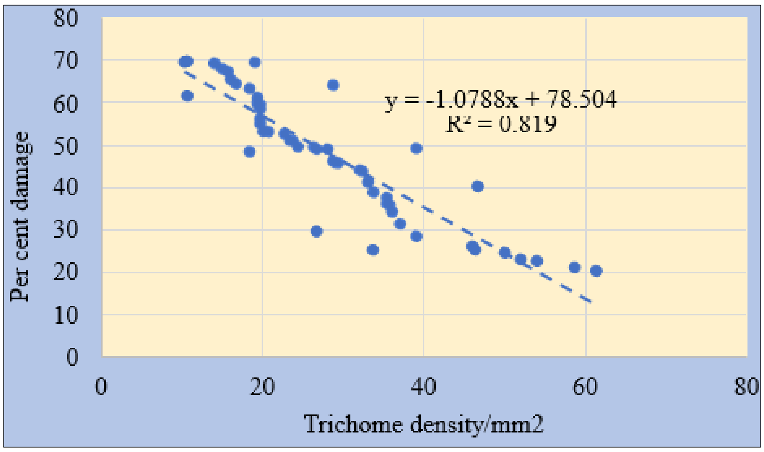
Traits	r value
Trichome density	-0.905**
Leaf succulency	0.841**
Chlorophyll (SPAD)	0.580**
Plant height	-0.168
Phenol	-0.866**
Reducing sugars	0.850**

Note: ** Correlation coefficients significance at 1% (r = 0.354) -ve sign: Negatively correlated No sign: positively correlated.

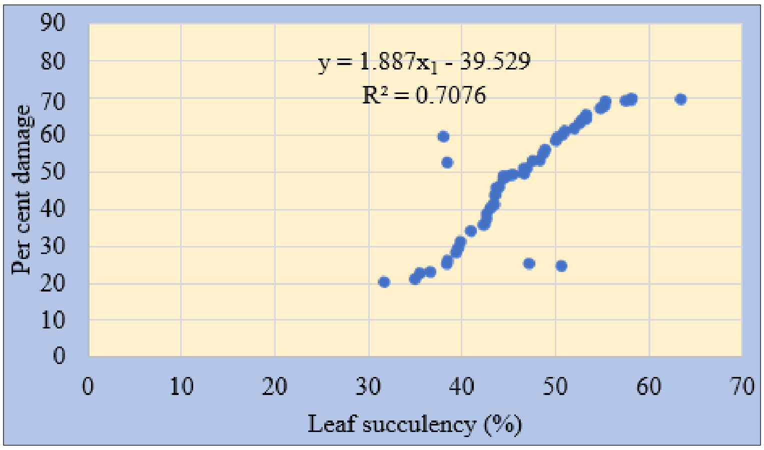
Plant height with per cent leaf damage.
The correlational studies between plant height and per cent leaf damage revealed that there was slightly negative correlation but it was non-significant (r = -0.168).
Similarly, Anchala *et al.* (2015) [3] who screened ten soybean varieties revealed that for *S. obliqua*, *S. litura*, and *T. orichalcea* there was non-significant correlation between plant height and per cent infestation and on contrary she found positive correlation (r =0.122).

Leaf shape with per cent leaf damage
Among fifty-four germplasms studied, four germplasms contained lanceolate leaves, thirteen germplasms contained round ovate leaves and the remaining germplasms contained pointed ovate leaves. Further based on percent damage it was observed that leaf shape did not show significant effect on the pest damage.

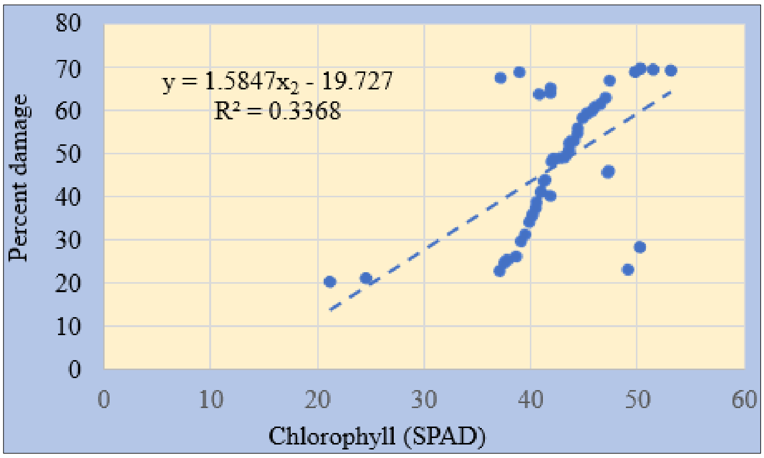
Phenol with per cent leaf damage
The correlational studies between phenol and per cent leaf damage revealed that there was significant and highly negative correlation (r = -0.866**). The regression equation being y = -216.47x3 + 97.797 indicated that with an increase of one unit phenol content there will be reduction in per cent leaf damage by 216.47% (graph 4).



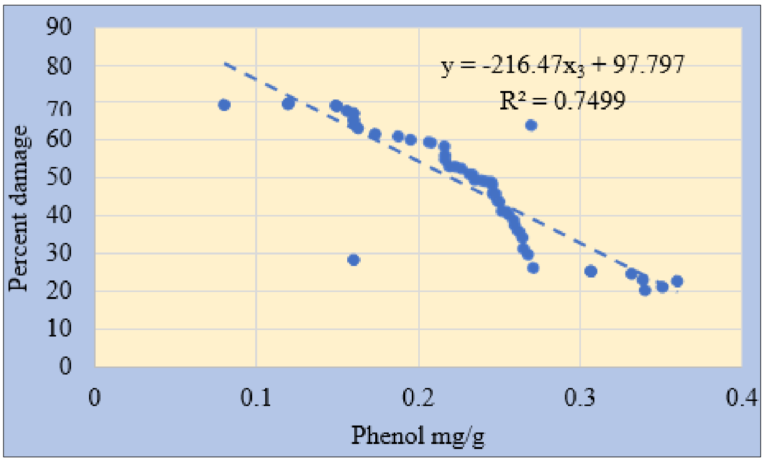
Graph 1: Regression of percent leaf damage on Trichome density



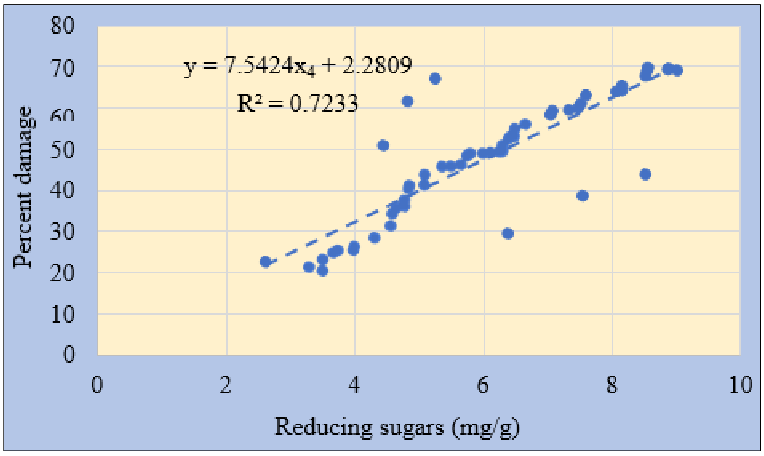
Graph 2: Regression of percent leaf damage on leaf succulency



Graph 3: Regression of percent leaf damage on chlorophyll (SPAD)



Graph 4: Regression of percent leaf damage on phenol



Graph 5: Regression of percent leaf damage on reducing sugars

Similarly, Jinsa *et al*. (2012) [7], Haralu *et al.* (2018) [5], Rahman *et al.* (2021) [13], Mohammad *et al.* (2019) [11], who studied biochemical basis of resistance in different crops found that total phenol concentration showed significant and negative correlation with pest infestation in different crops.

Reducing sugars with per cent leaf damage

The correlational studies between reducing sugars and per cent leaf damage revealed that there was significant and highly positive correlation (r =0.850**). The regression equation being y = 7.5424x4 + 2.2809 indicated that with an increase of one unit reading in reducing sugar content there will be increase in per cent leaf damage by 7.5424% (graph 5).

Similarly, Mohammad *et al*. (2019) [11], Rahman *et al.* (2021) [13] worked on *Spodoptera litura* in found that there was a statistically significant positive correlation between *S. litura* damage and reducing sugar.

Conclusion

Thus, the study revealed that per cent leaf damage had significant and positive correlation with different parameters like leaf succulency (r = 0.841**), chlorophyll content (r = 0.580**) and reducing sugars (r = 0.850**). Whereas significant and negative correlation was found between per cent leaf damage and trichome density (r = -0.905**), phenol content (r = -0.866**). For plant height (r = -0.168), Leaf shape there was non-significant correlation with per cent leaf damage.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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