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Genotypic reaction and biochemical basis of resistance in sunflower against leafhopper

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Abstract
Field experiment was conducted during *Rabi* 2024-2025 to screen 23 sunflower genotypes along with susceptible check, Morden to evaluate their reaction against sunflower leafhopper at the field of Oilseeds Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. Field screening of 23 sunflower genotypes revealed that, three genotypes AKSFI-6R, LH/AR/PM-7-8 and CFSI 5082-2 were categorized as 'Resistant' genotypes with Mean Scale Index ranging from 0.8 to 1.0. Fifteen genotype GPN 219-2, ID-1079, GMU-490, AKSFI-148, LH-23-41, AKSFI 21-13, AKSFI-218, AKSFI-214, RHF 138-2R, 6-D-1R, 189/1R, RB -9-1, LH/AR/PM-1, RB-7-1and LH/AR/PM-2, were found as 'Moderately Resistant' with Mean Scale Index ranging from 1.2 to 2.4 and two genotypes GP6442 and RB-4-2 were found susceptible with Mean Scale Index ranging from 2.6 to 3.5. The biochemical constituents phenol (r=- 0.744) and tannin (r=-0.871) showed negative significant correlation with leafhopper infestation, suggesting their defensive role. In contrast, chlorophyll (r=0.836), nitrogen (r=0.808) and protein (r=0.807) showed a positive significant correlation with leafhopper infestation, implying a possible susceptibility factor, therefore, phenolic, tannin, chlorophyll and nitrogen compounds could serve as key biochemical markers for identifying leafhopper resistance sunflower genotypes.

Keywords: Leafhopper, phenols, tannins, chlorophyll, nitrogen, protein

Introduction

Sunflower (*Helianthus annus* L.) is an important oilseed crop in India popularly known 'Surajmukhi'. It is one of the important oilseed crop in the world, which ranks third in area after soybean and groundnut (Ghante *et al.*, 2019) [4]. It is an important source of high quality edible oil with wider adaptability to season and soils. Sunflower oil is light in colour, bland flavour, high smoke point and good nutritional quality. The oil content varies from 30 to 48 per cent (Tilak *et al.*, 2016) [15]. Indian farmers started large scale cultivation of sunflower in 1972 with the introduction of high yielding Russian varieties. In India during 2017-18 sunflower was grown on an area of 0.33 million hectares with 0.23 million tonnes of production and 0.70 metric tons per hectare of productivity.

Amrasca bigutulla bigutulla (Ishida) is the major pest in sunflower. *A. bigutulla bigutulla* has a broad host range including cotton, okra, brinjal, eggplant, jute and sunflower. This pest is more serious in the tropics and subtropics because of the favourable environmental conditions for its growth and development around the year (Amandeep, 2016) [12]. In sunflowers, leafhopper population is predominantly observed during the *kharif* and *Rabi* seasons. Both nymphs and adults of leafhopper suck the cell sap from the leaves and shows, symptoms like stunted growth, burning of leaf margins, coupled and crinkled leaves. Infestation of sucking insect pest is becoming a major concern in obtaining the expected yield from sunflower crop because it is incidence starts from the seeding stage and prevail through the entire plant life. Yield level of the country is the lowest in the world due to several biotic and abiotic factor and yet the potential of the crop is, far from being exploited.

Severe infestation of leafhopper cause crop loss up to 46 per cent. Reducing the cost of production is one of the important crop technologies in sunflower to increase the profitability of the crop. Many insecticides are being used to control the pest complex of sunflower, which pose health hazards.

Host resistance is a potential alternate management strategy to reduce such pest's damage. Host plant resistance in crop plants is an important component of integrated pest management (IPM). It is considered as non-monetary input use of resistant or less susceptible cultivars is one of the most significant methods of keeping insect population below economic threshold levels. It is most helpful when carefully utilizes with other components of pest management. However, there are no durable resistant lines against leafhopper in sunflower. Keeping all these things in mind, the present investigation was undertaken to screen out the sunflower genotypes for resistance against leaf hopper under field conditions.

Materials and methods

Screening of sunflower genotypes: Field screening experiment was conducted during *kharif* 2024-25. Total 23 genotypes were screen out along with check in the field under natural infestation of leafhopper pests to identify the resistant genotype. Sunflower genotypes were sown in two replications at 60 x 30 cm between rows and plants respectively, and all the recommended package of practices were followed except plant protection measures. For recording leaf hopper population, five plants of each entry were selected and labelled for observations at both seedling and star bud stage. Observations were recorded on two upper leaves, two middle leaves and two bottom leaves of plant canopy and later it was expressed as mean number per plant (mean no./six leaves / plant) nymph was counted.

Table 1: Leafhopper injury grade

Injury grade	Scoring of leafhopper injury
0	Free from leafhopper injury
1	Slight yellowish on the edges up to 30 per cent
2	Yellowing and curling up to 40 per cent
3	Yellowing and curling up to 60 per cent
4	Yellowing and curling up to 80 per cent
5	Maximum, yellowing, cupping and curling up to 100 per cent

Leafhopper injury on five randomly selected plants was scored as per Ingale *et al.*, (2019) [5] and grade was awarded as follows

On the basis of injury grade, Mean Scale Index (MSI) was determined as under:

$$MSI=\frac{(G0 \times P) + (G1xP) + (G2xP) + (G3 \times P) + (G4 \times P) + (G5xP)}{TP}$$

Where, G-Leafhopper Injury Grade (0 to 5)

P-The number of plants under the grade for each category

TP-Total number of plants taken for observation

Table 2: Categorization of genotypes

MSI	Resistant category
0.0	Highly Resistant
0.1-1.0	Resistant
1.1-2.5	Moderately resistant
2.6-3.5	Susceptible
3.6-5.0	Highly susceptible

Table 5: Sunflower genotypes, reaction against leafhopper and associated biochemical constituents**.**

Sr No.	Genotypes	MSI	Reaction	Phenols mg/g	Tannins mg/g	Chl'a 'mg/g	Chl'b' mg/g	Total chl mg/g	Leaf Nitrogen %	Protein (%)
1	GPN 219-2	2.0	Moderately resistant	1.89	3.28	28.49	24.91	24.72	2.87	17.93
2	ID-1079	1.5	Moderately resistant	1.93	2.98	29.64	24.73	24.68	2.63	16.43
3	GMU-490	2.1	Moderately resistant	2.01	3.00	26.40	24.41	24.09	2.34	14.62
4	GP6442	2.7	susceptible	1.00	2.04	30.39	29.19	27.14	3.72	23.25
5	AKSFI-148	1.5	Moderately resistant	1.90	3.16	30.11	24.69	24.69	2.67	16.68
6	LH-23-41	2.0	Moderately resistant	1.78	3.18	29.96	24.60	24.60	2.54	15.87
7	AKSFI 21-13	2.4	Moderately resistant	1.59	3.12	29.82	24.79	24.75	2.79	17.43
8	AKSFI-6R	1.0	Resistant	4.02	4.27	26.24	23.96	23.67	1.99	8.93
9	AKSFI-218	1.4	Moderately resistant	1.68	3.08	30.29	24.82	24.82	2.23	13.93
10	AKSFI-214	2.0	Moderately resistant	1.97	2.92	30.15	24.78	24.77	2.43	15.18
11	RHF 138-2R	1.5	Moderately resistant	1.92	3.06	30.34	24.70	24.72	3.02	18.87
12	RB-4-2	2.6	Susceptible	0.85	1.98	31.18	28.88	28.69	3.46	23.81
13	6-D-1R	1.6	Moderately resistant	1.74	3.22	30.53	24.90	24.91	2.35	14.68
14	LH/AR/PM-7-8	0.8	Resistant	2.43	3.97	30.85	22.49	22.81	1.43	12.43
15	189/1R	1.9	Moderately resistant	1.59	2.98	30.38	25.46	25.39	2.53	15.81
16	RB -9-1	1.4	Moderately resistant	1.83	3.37	30.44	24.65	24.69	2.25	14.06
17	LH/AR/PM-1	1.6	Moderately resistant	1.47	2.97	30.06	25.17	25.11	2.85	17.81
18	CFSI 5082-2	1.0	Resistant	3.73	3.86	30.21	22.90	23.12	1.57	9.81
19	RB-7-1	1.6	Moderately resistant	2.07	3.08	30.31	25.18	25.14	2.45	15.31
20	LH/AR/PM-2	1.2	Moderately resistant	1.62	2.87	30.13	25.26	25.19	2.94	18.37
21	Morden	3.2	susceptible	0.89	1.97	29.90	27.49	28.49	3.81	23.50
22	KBSH-44	2.9	susceptible	1.03	2.00	30.55	27.37	27.10	3.26	20.37
23	NDCMS	3.4	susceptible	0.97	2.13	31.92	28.79	28.48	3.37	21.06

Biochemical parameters estimation:

Collection of samples

The leaf samples were collected from sunflower genotypes. The collected leaves were oven dried. Each sample was analysed for total phenol, total tannin, chlorophyll, leaf nitrogen and protein content. The data were correlated with insect pest infesting for their significance

Preparation of sample for analysis

The dried leaves were powered separately in morter and pestle, so as to pass through 60 mesh size. The powered material was used for total phenol, total tannin, chlorophyll, nitrogen and protein estimation. The analysis was undertaken separately by using the following methods.

- **Total phenol**: Phenol from sunflower leaves was determined by method suggested by Bra and Thorpe (1954).
- **Total tannin**: Tannin from sunflower leaves was estimated by method of Sadashivam and Manickam (1992).
- **Chlorophyll:** Chlorophyll was estimated by method of Sadashivam and Manickam (1992)
- **Leaf nitrogen**: Estimation of total nitrogen content from the leaf was carried out using the Micro-Kjeldahl method, as described by Sadashivam and Manikam (1992).
- **Total protein**: Total protein can be estimated by converting nitrogen content to protein by factor of 6.25, assuming nitrogen content in protein as 16%.

Results and Discussions

The leafhopper incidence was observed in the range of 4.0 to 9.2 nymphs/6L/plant at seedling stage and 8.9 to 19.2 nymphs/6L/plant at star bud stage. Lowest leafhopper population was recorded on genotype CFSI 5082-2 i.e. 4.0 nymphs/6L/plant at seedling and 8.6 nymphs/6L/plant at star bud stage followed by AKSFI-6R (4.4 at seedling and 8.9 at star bud stage nymphs/6L/plant), LH/AR/PM-7-8 (4.8 at seedling and 9.7 at star bud stage nymphs/6L/plant). Highest number of leafhoppers were reported on susceptible check Morden (9.2 and 19.2 nymphs/6L/plant at seedling and star bud stage respectively), followed by NDCMS (9.10 and 18.65 nymphs/6L/plant at seedling and star bud stage).

leafhopper injury was observed in the ranged between 19.6 to 60 per cent. lower leafhopper injury 19.6 per cent was observed on LH/AR/PM-7-8 followed by AKSFI-6R (20%), CFSI 5082-2 (26 %).While the injury grade lies in between 1.0 to 4.0, lower injury grade i.e. 1 was observed in the genotypes AKSFI-6R, RHF138-2R, LH/AR/PM-7-8, 189/1R, CFSI 5082-2, followed by GPN 219-2, ID-1079, GMU-490, AKSFI-148, LH-23-41, AKSFI-21-13, AKSFI-218, AKSFI-214, 6-D-1R, RB-9-1, LH/AR/PM-1, RB-7-1, LH/AR/PM-2 having injury grade 2 Mean scale index lies between 0.8 to 3.5. Lowest mean scale index i.e. 0.8 was recorded in LH/AR/PM-7-8 followed by AKSFI-6R (1.0), CFSI 5082-2(1.0), LH/AR/PM-2(1.2), AKSFI-218(1.4) and RB-9.1(1.4). Among the tested genotypes GP6442 recorded highest i.e. 2.7 MSI The susceptible check Morden recorded highest 60 per cent leafhopper injury and 4 injury grade and 3.5 mean scale index.

Based on MSI AKSFI-6R, CFSI 5082-2 and LH/AR/PM-7-8 categorized into resistant reaction. GP6442 and RB-4-2 were categorized into susceptible reaction and remaining genotypes were categorized into moderately resistant reaction. The present findings helped in the identification of promising genotypes against *A. biguttula biguttula*. Earlier scientist from different institutes did the same line of work with different genotypes of them, Ghante *et al.*, (2019) [4] conducted an experiment with 28 sunflower genotypes six of the genotypes reacted as resistant (GMU-25, 339, 504, 922, 570 and GP9-472-4-13) and 16 genotypes as moderately resistant (GMU-1, 4, 116, 405, 595, 669, 703, 782, 914, 1029, 243, 327, 556, 696, 776 and AKSFI-46-2,) and six as susceptible (GMU-255, 713, 795, 1093, 112 and 343) to leafhopper pest. Tabassum *et al*., (2021) evaluated the elite sources of sunflower against leafhopper

Table 3: Reaction of sunflower genotypes against leafhopper damage

Sr. No.	Genotype	At seedling stage	At star bud stage	leafhopper injury	Injury grade	MSI	Reaction
1	GPN 219-2	5.00 (2.23)	13.12 (3.62)	34.50%	2	2.0	Moderately resistant
2	ID-1079	6.40 (2.53)	11.88 (3.45)	30.00%	2	1.5	Moderately resistant
3	GMU-490	5.50 (2.35)	15.39 (3.92)	38.00%	2	2.1	Moderately resistant
4	GP6442	8.70 (2.95)	17.15 (4.14)	42.50%	3	2.7	Susceptible
5	AKSFI-148	7.20 (2.68)	12.81 (3.57)	32.40%	2	1.5	Moderately resistant
6	LH-23-41	7.63 (2.76)	13.30 (3.64)	36.20%	2	2.0	Moderately resistant
7	AKSFI 21-13	5.40 (2.32)	11.27 (3.35)	41.70%	2	2.4	Moderately resistant
8	AKSFI-6R	4.40 (2.08)	8.98 (2.99)	20.00%	1	1.0	Resistant
9	AKSFI-218	6.60 (2.57)	14.53 (3.81)	37.20%	2	1.4	Moderately resistant
10	AKSFI-214	7.30 (2.70)	13.00 (3.60)	32.50%	2	2.0	Moderately resistant
11	RHF 138-2R	6.37 (2.52)	11.93 (3.45)	30.00%	1	1.5	Moderately resistant
12	RB-4-2	8.80 (2.97)	16.80 (4.10)	47.20%	3	2.6	Susceptible
13	6-D-1R	7.62 (2.76)	11.00 (3.31)	33.50%	2	1.6	Moderately resistant
14	LH/AR/PM-7-8	4.87 (2.20)	9.75 (3.12)	19.60%	1	0.8	Resistant
15	189/1R	6.50 (2.55)	12.60 (3.55)	30.00%	1	1.9	Moderately resistant
16	RB -9-1	5.90 (2.43)	12.89 (3.59)	35 4%	2	1.4	Moderately resistant
17	LH/AR/PM-1	6.70 (2.59)	13.63 (3.69)	43.10%	2	1.6	Moderately resistant
18	CFSI 5082-2	4.00 (2.00))	8.68 (2.94)	26.00%	1	1.0	Resistant
19	RB-7-1	5.80 (2.41)	12.45 (3.52)	32 60%	2	1.6	Moderately resistant
20	LH/AR/PM-2	7.80 (2.79)	13.46 (3.67)	40.00%	2	1.2	Moderately resistant
21	Morden	9.20 (3.03)	19.20 (4.38)	60.00%	4	3.5	Susceptible
22	KBSH-44	8.90 (2.98)	16.20 (4.02)	57.50%	3	2.9	Susceptible
23	NDCMS	9.10 (3.02)	18.65 (4.32)	59.30%	3	3.4	Susceptible
'F' test	-	Sig.	Sig.	-	-	-	-
SE (m)±	-	0.06	0.13	-	-	-	-
CD at 5 %	-	0.19	0.39	-	-	-	-
CV (%)	-	8.08	5.09	-	-	-	-

Table 4: Categorization of sunflower genotypes based on Mean Scale Index

Sunflower genotypes	MSI	Reaction
AKSFI-6R, CFSI 5082-2, LH/AR/PM-7-8	0.8-1.0	Resistant
Total=3		
GPN 219-2, ID-1079, GMU-490, AKSFI-148, LH-23-41, AKSFI 21-13, AKSFI-218, AKSFI-214, RHF 138-2R, 6-D-1R, 189/1R, RB -9-1, LH/AR/PM-1, RB-7-1, LH/AR/PM-2,	1.2-2.4	Moderately resistant
Total=15		
Morden, GP6442, RB-4-2, KBSH-44, NDCMS	2.6-3.5	Susceptible
Total=5		

The total phenol content on dry weight basis in leaves of sunflower genotypes varied from 0.83mg/g (RB-4-2) to 4.02 mg/g (AKSFI-6R). Genotypes who exhibited resistance reaction against sunflower leafhopper had higher phenol content as compare to susceptible genotypes. Present findings indicated that, the total phenol content was higher in resistance genotype as compared to susceptible genotype. The data in table.5 showed that, higher the total phenols in the genotypes, lesser the leafhopper attack. This might be due to the fact that the phenols act as antifeedant to insect herbivores. The findings are in line with Raju and Reddy (1982) who reported a negative and significant correlation of nymphal population of leafhopper in cotton with total phenols. Onkara and Somashekhar (2015) [10] reported that, thrips resistant groundnut varieties had higher concentration of phenols compared with the susceptible varieties.

Tannin content varied from 1.96 mg/g (Morden) to 4.27 mg/g (AKSFI-6R). Genotypes who exhibited resistance reaction against sunflower leafhopper, had higher tannin content. Present findings indicated that, the total tannin content was higher in resistance genotype as compared to the susceptible genotype. The data is given in table.5 showed that, higher tannin content in the genotypes, exhibited lesser leafhopper attack. The findings of Rahman *et al*., 2022 stated that, tannins showed strong negative relationship with leafhopper incidence. Reported that, the cotton entries with higher tannins exhibited resistance against jassids. Revealed that, genotypes with higher tannin levels experienced lower leafhopper infestations, suggesting that these compounds which may act as feeding deterrents Chlorophyll content in sunflower genotypes varied from 22.81mg/g (LH/AR/PM-7-8) to 28.69 mg/g (RB-4-2). The data presented in Table 5 showed that, lower chlorophyll content in the genotypes, exhibited the lower leafhopper injury grade. Manivannan *et al.*, (2020) [7] revealed that, higher level of chlorophyll favours the leafhopper infestation, in case of chlorophyll a content, susceptible check DCH 32 showed higher chlorophyll a (1.61 mg/g), however all other genotypes were less, total chlorophyll content was higher in susceptible check (2.30 mg/g) compared to other genotypes. Reported that, genotypes with lower level of chlorophyll exhibited resistance to the leafhopper.

The leaf nitrogen and protein content in sunflower genotypes varied from 1.43 % (AKSFI-6R) to 3.81 % (RB-4-2) and 8.93 % (AKSFI-6R) to 23.25 % (RB-4-2) respectively. The data presented in table. 5 showed that,
lower nitrogen and protein content in the genotypes, exhibited lower leafhopper infestation. Ghante *et al*., (2019) [4] reported that, leaf nitrogen content of different sunflower genotypes varied from 1.4 % (GMU-25) to 3.81 % (GMU-112). These contents were positively correlated with leafhopper pest population and their damage. Manivannan *et al.*, (2020) [7] revealed that, elevated levels of proteins in the plant also favour the incidence of leafhoppers in cotton which are built from amino acids and these amino acids are essential for the growth and development of insects. Murugesan and Kavitha (2010) [8] founded that, a non-significant correlation between protein content and leafhopper infection in cotton genotypes. Showed that, nitrogen and protein had significant positive association with incidence of leafhoppers.

Table 5: Sunflower genotypes, reaction against leafhopper and associated biochemical constituents**.**

Sr. No.	Genotypes	MSI	Reaction	Phenols mg/g	Tannins mg/g	Chl'a 'mg/g	Chl'b' mg/g	Total chl mg/g	Leaf Nitrogen %	Protein (%)
1	GPN 219-2	2.0	Moderately resistant	1.89	3.28	28.49	24.91	24.72	2.87	17.93
2	ID-1079	1.5	Moderately resistant	1.93	2.98	29.64	24.73	24.68	2.63	16.43
3	GMU-490	2.1	Moderately resistant	2.01	3.00	26.40	24.41	24.09	2.34	14.62
4	GP6442	2.7	susceptible	1.00	2.04	30.39	29.19	27.14	3.72	23.25
5	AKSFI-148	1.5	Moderately resistant	1.90	3.16	30.11	24.69	24.69	2.67	16.68
6	LH-23-41	2.0	Moderately resistant	1.78	3.18	29.96	24.60	24.60	2.54	15.87
7	AKSFI 21-13	2.4	Moderately resistant	1.59	3.12	29.82	24.79	24.75	2.79	17.43
8	AKSFI-6R	1.0	Resistant	4.02	4.27	26.24	23.96	23.67	1.99	8.93
9	AKSFI-218	1.4	Moderately resistant	1.68	3.08	30.29	24.82	24.82	2.23	13.93
10	AKSFI-214	2.0	Moderately resistant	1.97	2.92	30.15	24.78	24.77	2.43	15.18
11	RHF 138-2R	1.5	Moderately resistant	1.92	3.06	30.34	24.70	24.72	3.02	18.87
12	RB-4-2	2.6	Susceptible	0.85	1.98	31.18	28.88	28.69	3.46	23.81
13	6-D-1R	1.6	Moderately resistant	1.74	3.22	30.53	24.90	24.91	2.35	14.68
14	LH/AR/PM-7-8	0.8	Resistant	2.43	3.97	30.85	22.49	22.81	1.43	12.43
15	189/1R	1.9	Moderately resistant	1.59	2.98	30.38	25.46	25.39	2.53	15.81
16	RB -9-1	1.4	Moderately resistant	1.83	3.37	30.44	24.65	24.69	2.25	14.06
17	LH/AR/PM-1	1.6	Moderately resistant	1.47	2.97	30.06	25.17	25.11	2.85	17.81
18	CFSI 5082-2	1.0	Resistant	3.73	3.86	30.21	22.90	23.12	1.57	9.81
19	RB-7-1	1.6	Moderately resistant	2.07	3.08	30.31	25.18	25.14	2.45	15.31
20	LH/AR/PM-2	1.2	Moderately resistant	1.62	2.87	30.13	25.26	25.19	2.94	18.37
21	Morden	3.2	susceptible	0.89	1.97	29.90	27.49	28.49	3.81	23.50
22	KBSH-44	2.9	susceptible	1.03	2.00	30.55	27.37	27.10	3.26	20.37
23	NDCMS	3.4	susceptible	0.97	2.13	31.92	28.79	28.48	3.37	21.06

Correlation between biochemical parameter and leafhopper Mean Scale Index

The correlation between biochemical parameters and the leafhopper Mean Scale Index (MSI) is presented in (Table 6). The total phenol (r=-0.744) and tannin (r=-0.871) shows strongly negative significant correlation with MSI of leafhopper. It indicated that, increased phenol and tannin content contributed to reduced down leafhopper infestation, suggesting their defensive role. In contrast total chlorophyll (r=0.836), Nitrogen (r=0.808) and protein (r=0.807) shows a positive significant correlation with MSI of leafhopper.

Table 6. Correlation of biochemical parameter with leafhopper mean scale index

Biochemical parameter	Leafhopper (r values)
	MSI
Total phenol (mg/g)	- 0.744
Total tannins(mg/g)	- 0.871
Total chlorophyll (mg/g)	0.836
Leaf nitrogen (%)	0.808
Protein (%)	0.807

Conclusions

In the current investigation, 23 sunflower genotypes screened in the field experiment, three genotypes (AKSFI-6R, CFSI 5082-2, LH/AR/PM-7-8) are identified as resistant genotypes to leafhopper. The genotypes having high total phenol and tannin content compared to the susceptible genotypes were recorded as resistant genotypes. The genotypes having low chlorophyll, nitrogen and protein content as compare to susceptible genotypes were recorded as resistant genotype. Antibiosis type of mechanism was observed in tested sunflower genotype against the leafhopper

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