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ND More
M.Sc. Student, Department of Plant Pathology, College of Agriculture, Pune, Maharashtra, India

SR Lohate
Assistant Professor, Department of Plant Pathology, ZARS, Ganeshkhind, Pune, Maharashtra, India

AA Bhagat
Assistant Professor, Department of Statistics, ZARS, Ganeshkhind, Pune, Maharashtra, India

Corresponding Author:
AA Bhagat
Assistant Professor, Department of Statistics, ZARS, Ganeshkhind, Pune, Maharashtra, India

Correlation of chemical and biochemical parameters with powdery mildew disease of fenugreek (*Trigonella foenum-graecum* L.)

ND More, SR Lohate and AA Bhagat

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Abstract
The present investigation was undertaken to study the chemical and biochemical parameters of selected powdery mildew resistant and susceptible genotypes of fenugreek. It is observed that the Kasuri selection - 2 was found immune, Purandar local-1 found moderately susceptible and Lasalgaon as highly susceptible. Rest of fifteen varieties were found susceptible. It is observed that the activity of polyphenol oxidase (PPO) and peroxidase (PO) enzymes were higher in immune variety (Kasuri selection-2) as compared to susceptible varieties before and after incidence of powdery mildew and has shown negative significant correlation (-0.766) for (PPO) and (-0.569) for (PO) after 80 DAS with PDI.
It is concluded that total chlorophyll content in immune variety was higher than susceptible varieties, before (40 DAS) and after (80 DAS) incidence of powdery mildew and has shown negative significant correlation (-0.654) and (-0.741) at 40 DAS and 80 DAS, respectively with PDI. The activity of polyphenol oxidase (PPO) and peroxidase (PO) enzymes were higher in immune variety (Kasuri selection-2) as compared to susceptible varieties before and after incidence of powdery mildew. The nitrogen concentration has direct relation with powdery mildew of fenugreek. However, there was no concrete relation with other macro or micronutrient concentration in fenugreek with powdery mildew disease.

Keywords: Fenugreek, correlation, chemical parameters, biochemical parameters and powdery mildew

Introduction
Fenugreek (*Trigonella foenum-graecum* Linn.) commonly called *methi* belongs to family Leguminosae. Fenugreek is cultivated in India and other parts of world for leafy vegetable, condiments medicinal as well as fodder purpose. In India fenugreek is cultivated in Rajasthan, Maharashtra, Madhya Pradesh, Gujarat, Uttar Pradesh and Punjab states. Rajasthan is considered as "fenugreek bowl" of the country and contributes about 90 per cent to the country's production. India is one of the major producers and exporter of fenugreek seeds. Among the different fungal disease powdery mildew incited by E. polygoni DC is considered (Saksena and Ahmed, 1983) [18] a serious pathogen specifically during flowering and pod formation stages of crop resulting in heavy yield losses. The losses in seed yield may go up to 50 per cent (Anonymous, 1990) [2]. Early sown varieties escape the infection, while late sown and late maturing varieties are affected by the disease (Sharma, 1999) [20].
The disease symptoms is present on the upper side of leaves, but it also affects the underside of leaves, young shoots, stems, buds, flowers and young fruit (Agrios, 2005) [1]. Som Prakash and Saharan (2002) [22] reported 27-30 per cent losses in fenugreek due to powdery mildew (E. polygoni). The resistance to powdery mildew is under genetic control regardless of the controversy regarding the nature and number of gene(s) controlling the resistance (Fondevilla *et al*., 2010; Azmat *et al*., 2010) [5, 3]. The development of powdery mildew resistant cultivar is usually a lengthy procedure taking 8-12 years (Poehlman and Sleper, 1995) [13]; the selection of resistant plants with confidence is therefore crucial. The nutrients play an important role in determining the yield potential in pulses. The nutrition status of a plant determines its resistance or susceptibility to diseases, its histological or morphological structure, the function of tissues to hasten or slow pathogens and the virulence and ability of pathogens to survive. The information on the role of nutrients *viz*., nitrogen, phosphorus, potassium, manganese, zinc, copper, Iron on disease resistance traits in fenugreek is meagre.

Hence, it is essential to study the influence of nutrients on powdery mildew disease. Recent studies have also shown that there are possibilities of resistance to this disease in certain fenugreek plants that express high levels of polyphenol oxidase, peroxidase and phenols. The present investigation was undertaken to study the chemical and biochemical parameters of selected powdery mildew resistant and susceptible genotypes of fenugreek.

Materials and Methods

The experiment was conducted on the experimental farm of Vegetable Improvement Project, Division No. 16 at National Agricultural Research Project (PZ), Ganeshkhind, Pune. Eighteen fenugreek varieties/cultivars were sown as Methi extra bold, asundhara, Jalgaon local-1,Lasalgaon, Rahuri local-1, Baramati local-1, Rahuri local -2,Jalgaon local-2, Jalgaon local-4, Jalgaon local-5, Purandar local-1, Pune local, Kasuri-1, Selection-1, IC-74, Kasuri selection -2, RTM-1and HM-57.

Sowing of eighteen varieties of fenugreek was done in the plots having size 3.00 x 2.40 m2 at 30 cm row length spacing on November 29, 2016. Recommended doses of fertilizers were applied and plots were irrigated lightly for better seed germination. Ten-fifteen days after each sowing, thinning and gap filling of plant was done to maintain uniform plant population. All intercultural operations were performed regularly as and when required. Observations on disease incidence were recorded date wise and per cent incidence was calculated. For recording observations on powdery mildew intensity 5 plants per plot were randomly selected and tagged before appearance of the disease.

Per cent disease incidence was calculated by following formula.

$$\text{Per cent disease incidence} = \frac{\text{No. of leaves infected}}{\text{Total No. of leaves observed}} \text{ x } 100$$

$$\text{PDI} = \frac{\text{Summation of numerical ratings}}{\text{No. of leaves/plants observed x maximum rating}} \text{ x } 100$$

Description of fenugreek powdery mildew Grade/Scale (Mayee and Datar, 1986) [10].

0-No any symptoms on leaves, 1-Small powdery spots on leaves covering less than 1% of leaf area, 3- Powdery lesions on leaves small, scattered, covering 1-10% of leaf area, 5-Powdery lesions bigger, covering 11-25% leaf area, 7 - Powdery patches bigger, coalescing and covering 26-50% of leaf area also on petioles, flowers and pods, 9-Powdery growth covering 51% or more of leaf area, white coating on petioles, flowers and pods resulting in its shading. Reduced pod set.

Grouping of genotypes based on reaction type (Khare and Lakpale, 1997) [8].

Immune (I)- No symptom of powdery mildew on leaves, Highly resistance (HR)- Small scattered powdery mildew specks covering 1% or less leaf area, Resistance (R)- Small powdery lesions covering 1-10% of leaf area, Moderately susceptible (MS)- Powdery lesions enlarged covering 11-25% of leaf area, Susceptible (S)- Powdery lesions coalesce to form big patches covering 26-50% of leaf area, Highly susceptible (HS)- Big powdery patches covering 51% or more of leaf area and defoliation occur.

Statistical analysis

Correlation analysis was performed to determine whether the observed difference between the sample means was statistically significant or not at 0.01 or 0.05 probability levels.

$$r = \frac{\sum X_i Y_i - \frac{(\sum X_i)(\sum Y_i)}{n}}{\sqrt{\left(\sum X_i^2 - \frac{(\sum X_i)^2}{n}\right)} \cdot \sqrt{\left(\sum Y_i^2 - \frac{(\sum Y_i)^2}{n}\right)}}$$

Where,

r = Coefficient of correlation, $\sum X_i$ = Total of the first variable value, $\sum Y_i$ = Total of the second variable value, $\sum X_i Y_i$ = Sum of the product of first and second value, $\sum X_i^2$ = Sum of the square of the first value, $\sum Y_i^2$ = Sum of the square of the Second value and n = Total number of observations.

Results and Discussion

The results obtained in respect of disease symptoms first observed among varieties (Table 1) ranged between 44 to 60 days after sowing. Firstly symptoms were observed in Lasalgaon variety (44 DAS) and late symptoms were observed in Purandar local-1 variety (60 DAS). Disease incidence among varieties was in range of 0% to 100%. Higher disease incidence i.e. 100% observed in Lasalgaon, Baramati local-1 and Selection-1, while in Kasuri selection-2 no disease incidence was observed. Eighteen varieties were screened under natural field conditions for resistance to powdery mildew fungus *E. polygoni*. Kasuri selection-2 recorded least powdery mildew severity (PDI 0.00%) as compared with other varieties and was resistant to powdery mildew. The maximum disease severity (66.67%) was recorded in Lasalgaon variety which was highly susceptible to powdery mildew. Whereas, other varieties like Purandar local-1 showed minimum disease severity (14.07%) and were found moderately susceptible to powdery mildew. While, other fifteen genotypes were recorded as susceptible. The present investigation is in agreement with the earlier work done by Rathore and Rathore (1995) [15] who reported that all the ten genotypes were susceptible to powdery mildew pathogen. The results are also tallying with Gupta *et al*. (1997) [7] who observed all the genotypes of fenugreek infected with powdery mildew. Som Prakash and Saharan (1999) [21] screened 44 germplasm lines of fenugreek against powdery mildew.

The observations of different varieties of biochemical parameters in leaves before and after incidence of powdery mildew are presented in (Table 2).The concentration of total chlorophyll (mg/g) had significant variation both before (40 DAS) and after (80 DAS) powdery mildew infection. Higher amount of total chlorophyll was found in Kasuri selection-2 (0.87 mg/g) and lower amount of total chlorophyll was found in Lasalgaon (0.53 mg/g) before incidence (40 DAS) of powdery mildew.

After infection with powdery mildew the chlorophyll content has found decreasing in all the varieties except the resistant variety Kasuri selection-2, in which increase in chlorophyll content was observed. Higher amount of total chlorophyll was found in Kasuri selection-2 (0.94 mg/g) and lower amount of total chlorophyll was found in Vasundhara (0.47 mg/g) after incidence (80 DAS) of powdery mildew. After the infection of powdery mildew the total chlorophyll content was drastically reduced in susceptible varieties. The correlation coefficient have shown that disease severity has highly

significant negative correlation with total chlorophyll content at 40 DAS and 80 DAS. Similar results were reported by Scholes and Farrar (1986) [19] who studied the cause of reduction in chlorophyll concentration as one of them is powdery mildew infection. Sabri *et al.* (1997) [17] found that the chlorophyll content in leaves of oat is progressively declined as the leaves become older, but those of infected leaves powdery mildew showed the greatest reductions. Xu *et al.*, (2005) [24]; Dinesh, 2009 [4] reported that the considerable lower chlorophyll content in powdery mildew infected lucern leaves. Tirupathiswamy *et al.*, (2014) [23]; Muhammad Abubakkar *et al.*, (2016) [12] reported the decrease in chlorophyll content after the powdery mildew infection.

The polyphenol oxidase (PPO) content was increased in immune variety Kasuri selection-2 than other susceptible varieties before the incidence of disease (40DAS). In immune variety Kasuri selection-2 it was 8.2 units/min/g of sample and lower PO activity found in Rahuri local-2 and that was 7.0 units/min/g of sample (40 DAS). After the incidence of disease the activities of PPO found to be decreased in all susceptible varieties except in immune variety which further shown an increase in activity (80 DAS). In immune variety Kasuri selection-2 it was increased up to 10.9 units/min/g of sample while it was drastically reduced in highly susceptible variety Lasalgaon (4.8 units/min/g of sample). In moderately susceptible variety Purandar local-1 enzyme activity was (7.3 units/min/g of sample) more than susceptible variety but less than immune variety (80 DAS). The correlation coefficient have shown that disease severity has highly significant negative correlation (-0.766**) with PPO activity at 80 DAS. Peroxidase content was more in immune variety Kasuri selection-2 than other susceptible varieties before the incidence of disease (40DAS). In immune variety Kasuri selection-2 it was 11.8 units/min/g of sample and lower PO activity found in Vasundhara and that was 8.6 units/min/g of sample (40 DAS). After the incidence of disease the activities of PO found to be decreased in all susceptible varieties except in immune variety which further shown an increase in activity (80 DAS). In immune variety Kasuri selection-2 it was increased up to 16.2 units/min/g of sample. It was drastically reduced in highly susceptible variety Lasalgaon (3.0 units/min/g of sample). The correlation coefficient had shown that significant negative correlation (-0.569*) with PO activity at 80 DAS (Table 4). Melo *et al*. (2006), Malti and Basu (2009), reported the different pathways and role of enzymes in plant defense against pathogen. The total chlorophyll content in immune variety was higher than susceptible varieties, before (40 DAS) and after (80 DAS) incidence of powdery mildew and has shown negative significant correlation (-0.654) and (-0.741) at 40 DAS and 80 DAS, respectively with PDI. The activity of polyphenol oxidase (PPO) and peroxidase (PO) enzymes were higher in immune variety (Kasuri selection-2) as compared to susceptible varieties before and after incidence of powdery mildew.

The nitrogen concentration before the incidence of disease (40 DAS) was ranged from 2.24% to 6.72% (Table 3). The N content was shown decreasing trend after incidence of powdery mildew (80 DAS) in all varieties except in resistant variety, where it was found increased from 2.24 to 3.36 per cent. The results are in agreement with the findings by Dordas (2009).

Phosphorus (P) concentration before incidence of disease (40 DAS) was found more than after the incidence of disease (80 DAS). P content was reduced after incidence of disease in all varieties including immune one. At 40 DAS the P content was in range of 0.14% to 0.53%. At 80 DAS the P content reduced and was in range of 0.09% to 0.19%. Similar results were reported by Reuveni and Reuveni (1998) [16]. Potassium (K) concentration has shown similar trend like nitrogen and phosphorus. K content before incidence of disease (40 DAS) was found more than after the incidence of disease (80 DAS). K content was reduced after incidence of disease in all varieties. At 40 DAS the K content was in range of 2.79% to 4.60%. At 80 DAS the K content reduced was in range of 1.57% to 2.09%. Similar results were reported by Debarati Bhaduri *et al.*, (2014).

Zinc (Zn) concentration before incidence of disease (40 DAS) was found less than after the incidence of disease (80 DAS). Zn concentration was found to be increased in all varieties after incidence of disease. At 40 DAS the Zn content was in range of 20 to 30 mg/kg. At 80 DAS with increase in Zn content it was found to be in the range of 25 to 40 mg/kg. Iron (Fe) concentration before incidence of disease (40 DAS) was found more than after the incidence of disease (80 DAS). Fe content was reduced after incidence of disease. At 40 DAS the Fe content was in range of 330 to 410 mg/kg. At 80 DAS the Fe content was in range of 65 to 150 mg/kg.

Copper (Cu) concentration before incidence of disease (40 DAS) was found more than after the incidence of disease (80 DAS). Cu content was found to be decreased in all varieties after incidence of disease. At 40 DAS the Cu content was in range of 10 to 13 mg/kg. At 80 DAS with decrease in Cu content it was found to be in the range of 1 to 3 mg/kg. Manganese (Mn) concentration before incidence of disease (40 DAS) was found less than after the incidence of disease (80 DAS). Mn content was increased after incidence of disease. At 40 DAS the Mn content was in range of 20 to 60 mg/kg. At 80 DAS the Mn content was in range of 35 to 75 mg/kg. The findings are in agreement with Grewal *et al*., (1996) [6] and Rathi *et al*. (1998) [14].

Table 1: Symptoms first observed, severity/intensity of powdery mildew on different fenugreek varieties/cultivars under natural field condition.

Sr. No.	Variety	Symptoms first observed (DAS)	Disease incidence (%)	PDI	Disease reaction
1	Methi extra bold	49	80	43.70	S
2	Vasundhara	47	80	32.59	S
3	Jalgaon local-1	50	60	29.63	S
4	Lasalgaon	44	100	66.67	HS
5	Rahuri local-1	47	80	34.07	S
6	Baramati local-1	53	100	46.67	S
7	Rahuri local-2	56	60	37.78	S
8	Jalgaon local-2	51	80	43.70	S
9	Jalgaon local-4	54	60	28.89	S
10	Jalgaon local-5	52	80	37.04	S
11	Purandar local-1	60	60	14.07	MS
12	Pune local	48	60	28.15	S
13	Kasuri-1	50	80	28.15	S

14	Selection-1	45	100	49.63	S
15	IC-74	48	80	40.74	S
16	Kasuri selection-2	-	0	0.00	I
17	RTM-1	51	80	33.33	S
18	HM-57	53	80	32.59	S

HS = Highly susceptible, S = Susceptible, MS = Moderately susceptible, I = Immune / resistant

Table 2: Observations of different biochemical parameters in leaves before and after incidence of powdery mildew.

Sr. No.	Variety	Total chlorophyll (mg/g) (40 DAS)	Total chlorophyll (mg/g) (80 DAS)	Polyphenol oxidase (units/minute/gram of sample)		Peroxidase (units/minute/gram of sample)	
				40 DAS	80 DAS	40 DAS	80 DAS
1	Methi extra bold	0.55	0.49	7.8	7.0	10.9	5.6
2	Vasundhara	0.63	0.47	7.8	7.1	8.6	6.4
3	Jalgaon local-1	0.61	0.54	7.5	6.9	9.2	3.9
4	Lasalgaon	0.53	0.39	7.9	4.8	9.5	3.0
5	Rahuri local-1	0.70	0.64	7.9	7.2	11.4	3.2
6	Baramati local-1	0.71	0.62	7.8	6.9	11.4	5.3
7	Rahuri local-2	0.71	0.64	7.0	6.5	10.3	2.3
8	Jalgaon local-2	0.71	0.63	7.2	6.3	11.6	5.6
9	Jalgaon local-4	0.81	0.59	7.8	7.1	10.0	6.0
10	Jalgaon local-5	0.59	0.54	7.7	6.8	9.2	5.4
11	Purandar local-1	0.83	0.76	8.0	7.3	9.8	9.0
12	Pune local	0.69	0.57	7.6	7	10.0	6.3
13	Kasuri-1	0.76	0.71	7.8	7.1	10.2	6.6
14	Selection-1	0.73	0.59	7.9	6.8	10.9	8.5
15	IC-74	0.78	0.69	7.6	5.6	10.3	6.5
16	Kasuri selection-2	0.87	0.94	8.2	10.9	11.8	16.2
17	RTM-1	0.78	0.72	7.8	6.9	10.8	7.1
18	HM-57	0.79	0.74	7.9	7.1	10.6	7.8

Table 3: Observations of different chemical and micronutrients (Zn, Fe, Cu, Mn) concentration in leaves before and after incidence of powdery mildew

Sr. No.	Variety	Nitrogen (%)		Phosphorus (%)		Potassium (%)		Zinc (mg/kg)		Iron (mg/kg)		Copper (mg/kg)		Manganese (mg/kg)	
		40 DAS	80 DAS	40 DAS	80 DAS	40 DAS	80 DAS	40 DAS	80 DAS	40 DAS	80 DAS	40 DAS	80 DAS	40 DAS	80 DAS
1	Methi extra bold	5.32	1.96	0.53	0.17	3.59	1.82	20	30	350	90	11	2	40	65
2	Vasundhara	5.32	3.92	0.53	0.11	3.47	1.78	30	35	340	100	12	2	40	45
3	Jalgaon local-1	6.72	3.92	0.38	0.19	3.03	1.81	20	30	330	105	11	1	50	60
4	Lasalgaon	6.16	3.64	0.53	0.19	3.72	1.97	20	30	370	65	11	2	60	65
5	Rahuri local-1	5.88	4.2	0.38	0.09	3.14	2.02	20	30	410	135	10	1	30	35
6	Baramati local-1	4.20	3.36	0.33	0.11	2.79	1.78	30	40	340	80	11	2	40	55
7	Rahuri local-2	4.20	3.22	0.28	0.17	3.19	1.57	20	25	340	80	11	2	50	60
8	Jalgaon local-2	5.04	3.5	0.23	0.14	3.47	1.85	30	35	350	90	11	1	50	60
9	Jalgaon local-4	5.60	3.22	0.19	0.14	3.30	1.73	20	25	350	65	11	2	50	55
10	Jalgaon local-5	4.48	3.5	0.19	0.14	4.03	1.80	20	25	370	65	11	2	60	70
11	Purandar local-1	4.76	4.06	0.19	0.14	3.37	1.65	30	40	370	150	10	1	40	45
12	Pune local	7.84	3.92	0.23	0.17	4.60	1.62	30	40	410	115	13	2	50	60
13	Kasuri-1	4.48	3.22	0.14	0.11	3.27	1.76	20	30	360	130	11	1	20	55
14	Selection-1	3.92	2.8	0.23	0.07	4.12	1.75	20	25	350	80	12	1	40	45
15	I C-74	2.80	1.96	0.23	0.11	3.51	1.85	30	30	360	80	13	3	40	50
16	Kasuri selection-2	2.24	3.36	0.23	0.09	4.60	2.09	20	30	350	85	11	3	40	70
17	RTM-1	3.92	2.8	0.19	0.11	3.37	1.57	20	25	360	115	11	2	50	65
18	HM-57	5.24	3.5	0.19	0.17	3.30	1.67	30	35	370	85	12	2	60	75

Table 4: Correlation coefficients among PDI of powdery mildew and different chemical and biochemical parameters.

Sr. No.	Parameter	40 DAS	80 DAS
1	Total chlorophyll	-0.654**	-0.741**
2	Polyphenol oxidase (PPO)	-0.184	-0.766**
3	Peroxidase (PO)	-0.072	-0.569*
4	Nitrogen (N)	0.239	-0.176
5	Phosphorus (P)	0.447	0.211
6	Potassium (K)	-0.362	-0.095
7	Zinc (Zn)	0.145	0.148
8	Iron (Fe)	-0.053	-0.129
9	Copper (Cu)	0.036	-0.319
10	Manganese (Mn)	0.096	-0.259

** significant at 5% and *significant at 1% levels.

Conclusions

It is concluded that total chlorophyll content in immune variety was higher than susceptible varieties, before (40 DAS) and after (80 DAS) incidence of powdery mildew and has shown negative significant correlation (-0.654) and (-0.741) at 40 DAS and 80 DAS, respectively with PDI. The activity of polyphenol oxidase (PPO) and peroxidase (PO) enzymes were higher in immune variety (Kasuri selection-2) as compared to susceptible varieties before and after incidence of powdery mildew. The nitrogen concentration has direct relation with powdery mildew of fenugreek. However, there was no concrete relation with other macro or micronutrient concentration in fenugreek with powdery mildew disease.

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