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Effect of organic treatments on total phenol, Saponin and flavonoids in leaf spot infected fenugreek leaves

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Abstract
Fenugreek (*Trigonella foenum graecum* L.) is an important medicinal crops cultivated throughout the world. Increasing demand of medicinal plants for the pharmaceutical industry, is required to boost the production of fenugreek with minimized the infection of diseases which reduces the huge yields. therefore present study was conducted to determine the effect of organic treatments on secondary metabolites. The total phenol in healthy leaves was maximum in seed treatment with cow urine@10 %(12.24µg/g) followed by foliar spray of cow urine (11.47 µg/g) and panchgavya (11.37 µg/g). Flavonoid content was observed highest in cow urine (4.11 µg/g) and lowest in vanaspativash (2.17 µg/g). Whereas saponin content was recorded higher in seed treatment withcow urine (15.33 µg/g) and lower in foliar spray of vanaspativash (13.06 µg/g). Percent disease intensity of Cercospora leaf spot was 33.29% in seed treatment with mixture of vanaspativash, cow urine and cow dung slurry and maximum in control 41.34%.

Keywords: Fenugreek, *Cercospora*, leaf spot, organics, biochemicals

Introduction
Cercospora traversiana is only species of the *Cercospora* that can infect fenugreek (Ryley, 1989) [11]. The disease is uniformly distributed throughout the field grown for seed production. In severely infected plants only a few upper leaves remained survive. The sign of disease symptoms were surrounded by yellowish halo on older leaves stem and pods *(*Mishra and pandey, 2013) [6]. Fenugreek leaves and seeds have been used extensively to prepare extract and powder for medicinal uses (Basu *et. al.*, 2003). The medicinal value lies in the form of secondary metabolites produced in plants are low molecular weight in natural products. Saponin, flavonoids and total phenolics compound are widely distributed in fenugreek plants that have exerted multiple biological effects including antioxidant, free radical scavenging, anti-inflammatory and anti carcinogenic. The accumulation and oxidation of secondary metabolites are associated with defence mechanisms in plants are also known to increase especially during fungal infections (Gasper *et al.*, 1982) [4]. The activities of phenolics related enzymes and the accumulation of phenolics have been correlated with resistance to biotic stresses (Mohammad and Kazemi**,** 2002) [7]. Investigation in disease physiology may provide in depth understanding of host pathogen interaction. This study was therefore designed to biochemically analyse with *Cercospora* leaf spot infected fenugreek plants at maturity stage in comparison to healthy ones.

Materials and Methods
The experiment was conducted at main experiment station (MES), Department of Vegetables Science, Narendra Deva University of Agriculture & Technology, Kumarganj Faizabad during 2015-16 to 2016-17. Experimental field was sandy loam, slightly alkaline soil (pH 8.0), low in organic carbon and nitrogen, medium in phosphorus and potassium. The mechanical composition of soil constituted 64.4 per cent sand, 27.8 per cent silt and 11.3 per cent clay. Trial was conducted in a randomized block design with three replication, 11 treatments and fenugreek variety Hisar sonali was sown @ 50 g seed/plot. Seeds were sown in 3.0 X 2.4m plot size on 30 November each year with 20 X 10 cm spacing. The layout of the experiment in detail is T_1: Seed treatment with cow urine @10% concentration,T_2: Foliar spray of cow urine @10% concentration at 60 and 90 days after sowing (DAS),T_3: Seed treatment with cow dung slurry @10% concentration,T_4: Foliar spray of cow dung slurry @10% concentration at 60 and 90 days after sowing (DAS),T_5: Seed treatment with panchgavya@10% concentration,T_6: Foliar spray of panchgavya@10% concentration at 60 and 90 days after sowing (DAS), T_7:

Seed treatment with Vanaspativash @10% concentration,T_8: Foliar spray of vanaspativash@10% concentration at 60 and 90 days after sowing (DAS),T_9: Seed treatment with Vanaspativash + cow dung slurry+ cow urine @10% concentration,T_{10}: Foliar spray of Vanaspativash+ cow dung slurry+ cow urine @10% concentration at 60 and 90 days after sowing (DAS) . Recommended package of cultural practices were followed to raise the crop and to promote natural infection. Ten plants in each plot were randomly selected and tagged for visual observations on symptoms appearance till 90 days after sowing. The leaf spot disease intensity noted on each treatment with scale 0 - 5. Percent Disease Intensity (PDI) was calculated by formula as given-

$$\text{Per cent disease intensity (PDI)} = \frac{\text{Sum of total numerical ratings}}{\text{Total no. of leaves examined x maximum disease grade}} \text{x100}$$

Biochemical Study

Leaves of healthy and infected plant were collected from *Cercospora* leaf spot disease infected fenugreek field to study the biochemical changes such as total phenol, flavonoids and saponins. The healthy and infected leaves were directly selected from the field for the estimation of total phenol. One gram of dried sample was weighted, cut in to small pieces and then placed in smearing methanol until the green colour was extracted. Leaves tissues were homogenized after decanting the methanol. These homogenized tissues were again boiled in methanol for further 5 minute and then filtered. Residual material was washed with 80% acidified (0.1HCL conc.) methanol. Methanol was evaporated using a rotavapour and the aqueous layer was collected to adjust the final volume as ml/g of weight with distilled water. The aqueous portion of the extract was then washed with n-hexane to remove the green colour. Total phenols were estimated using Folin-Ciolcalteu reagent, according to the modified method Bray and Thorpe (1954) [2]. Total flavonoids were determined according to the methods of Nabavi *et al.,* (2008) [8]. The homogenous samples of each of the samples of the leaves were used for qualitative determination of saponins according to the methods described by Nyam *et al.,* (2009) [9]. The process was repeated two more times to get an average. Saponins content was determined by difference and calculated as a percentage of the original sample as described by Harbone (1973) [5]

Statistical analysis

The data of each character in three replication of each sample were used for statistical analysis. Data were reported as mean. Analysis of variance and least significant difference tests were conducted to identify differences among mean. Statistical significance was declared at $P<0.05$. The per cent values were converted into Arc sin transformed values and statistically analyzed (Fisher and Yate's 1968) [3].

$$\text{Sin}^{-1}\sqrt{\text{PDI before statistica l analysis}}$$

Results and Discussion

The quantitative contents of total phenol, flavonoids and saponins and percent leaf spot intensity in healthy and infected leaves of fenugreek was presented in table-1. The total phenol, flavonoids and saponin contents were higher in healthy leaves as compared to infected leaves. Data of the results revealed that the organic treatments increased all the secondary metabolites in healthy and infected leaves of fenugreek. The total phenol content in healthy leaves was maximum in seed treatment with cow urine @10% (12.24µg/g) followed by foliar spray of cow urine (11.47 µg/g), seed treatment with vanaspativash (11.47 µg/g) and seed treatment with panchgavya (11.37µg/g) as compared to infected leaves. Flavonoids contents was observed highest 4.11 µg/g in seed treatments with cow urine and lowest2.17 µg/g in foliar spray with vanaspativash. Saponin contents was 15.33 µg/g in seed treatment with cow urine and 13.06 µg/g in foliar spray of vanaspativash. The percent disease intensity was minimum 33.29% in seed treatment with vanaspativash+ cow dung slurry + cow urine@10% and maximum in control 41.34%. Among the treatment, effects were observed at par. The phenolic compounds are widely distributed in hi*gher plants and are involved in disease resistance* (Schlosser and Schonbeck, 1976; Scarpari, *etal.*, 2005) [15, 14]. The phenolic compounds act as hydrogen donars/acceptors in host parasite interaction during host pathogen interaction (Parihar, 2012) [10]. Total phenolic, flavonoid and saponin content were recorded highest in which treatments the percent disease intensity was lowest. Results is supported with Sahoo, *et al*; (2009) [12] and Saxena, *et al;* (2013) [13]. It is because of fungal development is inhibited due to physiological changes in plant tissues. In conclusion, the organic treatment induce the systemic defence mechanism which resist the growth and development of pathogen in plants.

Table 1: Effects of organic treatments on total phenol, Flavonoid and Saponins contents in *Cercospora* leaf spot infected leaves of fenugreek plant

S. No.	Treatments	Total phenol contents (µg. /g.)		Flavonoid contents (µg./g.)		Saponins contents (µg. /5g.)		Percent leaf spot intensity
		Healthy Leaves	Infected Leaves	Healthy Leaves	Infected Leaves	Healthy Leaves	Infected Leaves	
1.	Seed treatment with cow urine @10% concentration	12.24 (36.0)	8.76 (22.68)	4.11 (77.92)	2.81 (36.40)	15.33 (23.3)	12.33 (46.09)	33.31 (35.24)
2.	Foliar spray of cow urine @10% concentration	11.47 (27.44)	7.65 (7.14)	3.49 (51.08)	2.19 (6.31)	13.66 (9.63)	12.73 (50.82)	33.47 (35.30)
3.	Seed treatment with cow dung slurry @10% concentration	10.08 (12)	6.89 (9.48)	3.80 (64.50)	1.93 (7.31)	14.66 (17.65)	10.09 (19.54)	35.24 (36.51)
4.	Foliar spray of cow dung slurry @10% concentration	9.58 (6.44)	9.48 (32.77)	3.61 (56.27)	2.65 (28.64)	14.67 (49.83)	13.33 (57.93)	35.15 (36.33)
5.	Seed treatment with panchgavya@10% concentration	11.39 (26.55)	7.54 (5.60)	2.53 (9.52)	2.17 (5.33)	14.66 (17.65)	14.03 (66.23)	39.33 (38.82)
6.	Foliar spray of panchgavya@10% concentration	9.86 (9.55)	7.57 (6.02)	2.91 (25.97)	2.25 (9.22)	13.60 (9.14)	12.76 (51.18)	39.76 (39.06)
7.	Seed treatment with Vanaspativash @10% concentration	11.47	9.34	3.17	3.02	14.00	9.46	40.21

		(27.44)	(30.81)	(37.22)	(93.68)	(12.35)	(12.08)	(39.25)
8.	Foliar spray of vanaspativash@10% concentration	9.36 (4.0)	9.08 (27.17)	2.17 (7.06)	1.80 (13.62)	13.06 (4.81)	9.07 (7.46)	40.31 (39.41)
9.	Seed treatment with Vanaspativash + cow dung slurry+ cow urine @10% concentration	9.46 (5.11)	10.29 (44.11)	2.62 (13.41)	2.38 (15.53)	15.00 (20.38)	9.60 (13.74)	33.29 (35.24)
10.	Foliar spray of Vanaspativash+ cow dung slurry+ cow urine @10% concentration	9.73 (8.11)	9.87 (38.23)	2.35 (1.73)	2.13 (3.39)	13.33 (6.98)	8.42 (5.23)	35.97 (36.59)
11.	Control	9.00	7.14	2.31	2.06	12.46	8.44	41.34 (39.99)
12.	SEm±	1.53	1.58	0.78	0.44	0.89	0.76	1.95
13.	CD (P= 0.05%)	4.59	4.65	2.34	1.35	2.67	2.28	5.74

Figures in parenthesis are representing percent increased values over control in biochemical and arc sine transformed value in leaf spot intensity

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