J. Mycopathol, Res, 50(1) : 61-65 (2012)

Epidemiological morphological and enzymatic studies on *Rhizoctonia solani* Kühn causing damping off of fenugreek

V.K. YADAV
Department of Plant Pathology, JNKVV, College of Agriculture, Tikamgarh (M.P.)

Received : 24.07.2010 Accepted : 24.09. 2011 Published : 30.04.2012

A survey was conducted to know the effect of prevailing weather conditions on damping off disease of fenugreek. During the study period weekly observations were recorded from 13th August to 29th October. Maximum (77.06%) disease intensity was recorded in the 3rd week of September, at that time minimum and maximum temperature (23.9°C and 28.2°C respectively), relative humidity (91.5 %) and rainfall (237.3 mm) were recorded. However least (7.78 %) disease intensity was noticed in last week of October where maximum temperature (30.6°C), minimum temperature (18°C), relative humidity (69.6%) and no rains were observed. *In vitro* studies were conducted to know the effect of culture filtrate of *R. solani* on seed germination and seedlings growth of fenugreek and the suitable liquid and solid media for the growth of the *R. solani* was also tested. In culture filtrate bio-assay, pure culture filtrate of *R. solani* inhibited seed germination, however none of the dilutions of the filtrate showed inhibition of seed germination. Whereas in the case of seedlings growth, pure culture filtrate shows the collar rot symptom on the seedlings, but no such symptoms were observed in different dilutions, on the contrary these dilutions exhibited stimulatory effect on seedlings as compared with control. Nine liquid and solid media were tested for the growth of *R. solani*. Fenugreek medium was found poor but Richard's medium proved best (in both liquid and solid condition) for the mycelial growth and sclerotial production of *R. solani*.

Key words: Fenugreek, damping-off, *Rhizoctonia solani*, epidemiology, culture filtrate, culture media

INTRODUCTION

Fenugreek (*Trigonella foenum- graecum* Linn.) is a cool season crop commonly known as methi, It is an important vegetable and spice crop. It can tolerate, frost and freezing weather for short period. India is one of the major producer and exporter of fenugreek. In India, Rajasthan, M.P., Gujarat, U.P., Maharashtra and Punjab are the leading states of fenugreek production, yielding about 55,780 tones, of which only 8,500 tones are exported during 2006-07. Damping off is an important disease of fenugreek caused by *Rhizoctonia solani*, in almost all the fenugreek growing areas. *R. solani* is a common soil borne pathogen and in absence of host it survives for longer period in soil either in the form of rhizomorph or sclerotia (Palo, 1927; Naiki and Ui, 1972). During the survey work, damping off of fenugreek was observed in the fields on 10-15 days old seedlings nearby Jabalpur (M.P.), almost in all the locations. Looking to the severity of disease problem, effect of atmospheric weather factors on disease development, effect of culture filtrate of *R. solani* on seeds and seedlings growth and suitable media for the growth of the fungus under *in vitro* conditions have been studied.

email: vijay3426@yahoo.com

MATERIALS AND METHODS

The weather data on rainfall, relative humidity and maximum-minimum temperature were obtained from the Department of Statistics, College of Agriculture, J.N.K.V.V., Jabalpur.

Fenugreek was cultivated as relay crop and sown nearly every month during Aug.-Oct., by the farmers neared Jabalpur. Survey was conducted in grower's fields on seven locations viz., Adhartal, Maharajpur, Ranjhi, Gohalpur, Amkhera, Gorakhpur and Sadar, and incidence of damping off was recorded. The intensity of damping off of fenugreek was studied in relation to influence of different prevailing atmospheric weather factors such as maximum-minimum temperature, relative humidity and rainfall. For this purpose the fenugreek fields were regularly visited to record the number of healthy and diseased plants in a quadrate of 1x1 sq. feet,

normally in ten replications, from August to October 1999. The actual disease intensity per week was correlated with prevailing weather factors during that week and correlation coefficient (r) was calculated in each case. For study of regression analysis the following formula were used given as under:

$$r_{xy} = \frac{\Sigma^n X_i Y_i - \frac{(\Sigma^n X_i \Sigma^n Y_i)}{n}}{\sqrt{\left(\Sigma x_i^2 - \frac{(\Sigma x_i)^2}{n}\right)\left(\Sigma y_i^2 - \frac{(\Sigma y_i)^2}{n}\right)}}$$

For testing the significance between the two variables (disease and weather factor) students 't' test was calculated as :

$$t = \frac{|r_{xy}|\sqrt{(n-2)}}{\sqrt{(1-r_{xy}^2)}} \sim t_{(n-1)}$$

Regression line :

$$Y\hat{} = y_n + b_{yx}\ (x^{--} - x_n)$$

where,

r_{xy} = Is the correlation coefficient between two characteristics x (independent), y (dependent)

t_{n-1} = Student's t static with (n-1) degree of freedom and level of significance (α%) has been taken as 5%.

b_{yx} = Is the regression coefficient of the characteristic y on the characteristic x.

To know the influence of culture filtrate of *R. solani* on seed germination and on the growth of fenugreek seedlings, infected plants collected from various locations of Jabalpur viz., Maharajpur, Adhartal, Amkhera, Gohalpur, Sadar, Ranjhi, and Gorakhpur were used for making isolations in the laboratory. The associated fungi (*R. solani*) were purified for further studies. The culture filtrate was obtained by growing *R. solani* on Richard's solution. Five mm disc from seven days old culture of *R. solani* was placed in Richard's solution and flasks were incubated at 25±2°C for 15 days. Mycelial mat was separated by filtering the content of flask through Whatman filter paper No.1. The culture filtrate so obtained was stored in refrigerator till use. The culture filtrate was diluted in different concentrations viz., 10^{-1}, 10^{-2} and 10^{-3} by adding sterilized water. The surface sterilized hundred seeds of fenugreek were soaked separately in stock solution and in different concentrations of culture filtrate (10^{-1}, 10^{-2} and 10^{-3}) for 24 hrs. Twenty seeds were placed in each 100 mm plastic Petridish on moist blotter. Five replications were maintained for each treatment. Additional 5 ml of culture filtrate were added in each to ensure adequate amount of the culture filtrate. Suitable controls were maintained using sterilized distilled water. The percentage seed germination was recorded after seven days.

To know the effect of culture filtrate on fenugreek seedlings, seeds of fenugreek were sown in the plastic pots. Seedlings of uniform length (80 mm) were washed in sterilized distilled water and transferred to vials containing serially diluted culture filtrate at the rate of 5 seedlings per vial. Suitable controls were maintained in sterilized distilled water. One drop of toluene was added in each vial to avoid bacterial contamination. Each treatment was replicated five times. Vials were incubated at 25±2°C. Length of seedlings was measured after an interval of 24 hrs.

To know the suitable growth medium and variation in growth characters of *R. solani* under *in vitro* condition, fungus was grown on nine solid media in Petriplates. The media used were Czapek's agar, Oat meal agar, Czapeks's Dox agar, Potato dextrose agar, Richard's agar, Soil extract agar, Rice agar, Fenugreek agar and Malt agar. Media were autoclaved at 121°C for 15 min and 15 ml was poured in each Petriplate. Five mm disc of seven days old culture was cut from the periphery with the help of sterilized cork borer and placed in the centre of each plate. Three replications were maintained for each medium. After inoculation, the Petriplates were incubated at 25±2°C. Colony diameter was measured after 24 hrs. interval and finally after 7th day. Type of growth and colour of colony were also recorded after 10th day and sclerotial production was observed up to 32 days. For determining the best liquid medium for fungal growth nine liquid media were also tested. The media were Rice broth, Fenugreek broth, Malt extract, Czapek's, Czapek's Dox, Asthana and Hawker's, Martin's Rose Bengal Streptomycin, Potato dextrose broth and Richard's solution. Flask containing 30 ml of each medium were sterilized at 121°C for 15 min and inoculated with 5 mm disc of 7-day old culture of *R. solani* cut out aseptically with a sterilized cork borer. Three replications were maintained in each case. The inoculated flasks were incubated at 25±2°C for ten days after which the content of each flask was filtered through Whatman filter paper No. 1. The mycelial mats were dried in

oven at 60°C. After thorough drying the mycelial mats were weighed.

RESULTS AND DISCUSSION

Data presented in the table shows that the incidence of damping off ranges approximately from 5-80% during the month of August to October (Table 1). It was noticed that the incidence of damping off in fenugreek was high (78.74 and 80.22%) at Gohalpur during August and September, whereas, it was low (5.11%) at Sadar during October. Intensity of damping off of fenugreek was recorded every week from its initiation from August to October and the data were correlated with prevailing atmospheric weather factors (Table 2). The disease was first 9.77% observed on 13th August, which increased under the influence of prevailing weather conditions as 48.21% (August 3rd week), 66.66% (August 4th week), 69.08% (September 1st week) and so on. The maximum disease intensity (77.06%)

Table 1: Survey of disease incidence of damping-off on fenugreek

Area surveyed	*Plant mortality (%)		
	August	September	October
Adhartal	58.21	76.66	8.20
Maharajpur	62.29	70.12	8.01
Ranjhi	67.70	79.20	8.29
Gohalpur	78.74	80.22	8.96
Amkhera	73.01	77.06	9.49
Gorakhpur	65.33	79.88	6.82
Sadar	69.06	69.34	5.11

* = Mean of weekly observations

was recorded in the 3rd week of September when the maximum temperature was 28.2°C, minimum 23.9°C, relative humidity 91.5% and 237.3 mm rainfall. Thereafter the disease intensity was reduced and the minimum disease intensity (7.78%) was recorded in October last week, when the maximum temperature was 30.6°C, minimum 18°C, relative humidity 69.5% and no rains. In the second half of October (15th–22nd Oct.), disease intensity was very low when the maximum temperature remained around 30°C, minimum (16°C -20°C) relative humidity was i.e. 68-77.5 % and no rains. This clearly shows that the prevailing weather factors have influenced the intensity of damping off of fenugreek. The influence of weather factors in relation to damping off of fenugreek has been studied by Komaraiah & Reddy (1986), who reported complete inhibition of seed germination above 70% relative humidity. High relative humidity (100%) has been found to favour growth of *R. solani* (Schneider, 1953; Schmiederknecht, 1960), therefore designated *R. solani* as a hygrophyle, that it is moisture-loving fungi. The correlation between wither tip disease of citrus and relative humidity and temperature was reported by Rehman and Khan (2000). Sharma and Verma (2007) also reported that moderate temperature, high relative humidity and rainfall favour the mango anthracnose disease *in vivo*.

Table- 2: Influence of weather factors on disease incidence

Date of observation	Disease intensity (%)**	Mean			Rainfall (mm)
		Temperature		Relative humidity	
		Max	Min.		
13 Aug.	9.77	29.3	24.2	84.5	4.5
20	48.21	28.6	24.1	84.5	51.8
27	66.66	30.1	23.1	87.0	81.3
3 Sep.	69.08	27.9	23.8	88.0	54.2
10	72.56	28.9	23.3	91.5	221.4
17	77.06	28.2	23.9	91.5	237.3
24	54.18	29.9	23.5	83.5	43.9
1 Oct.	53.01	30.7	23.0	81.0	52.0
8	29.90	30.0	22.6	81.0	17.3
15	11.01	29.2	20.0	77.5	0.0
22	8.30	30.1	16.8	68.0	0.0
29	7.78	30.6	18.0	69.5	0.0
	r_{tmx}	r_{tmn}		r_{rh}	r_{rf}
	-0.4613	+0.6873		+0.8335	+0.7853
	N.S.	*		*	*

N.S.= Non significant

** = Mean of ten replications

* = Significant at P=0.05

Statistical analysis of the data (Table 2) to correlate disease intensity with weather factors showed that there was negative correlation with maximum temperature. While it was positive with minimum temperature, relative humidity and rainfall. Analyzing influence of each factors on the disease, it was found that with the increase in minimum temperature, relative humidity and rainfall, there was also increase in disease intensity. Whereas with the decrease in maximum temperature there was increase in disease intensity.

It is clear from the data presented in Table 3, that there is hundred per cent inhibition of seed germi-

nation in the pure culture filtrate of *R. solani*, however none of the dilutions of the filtrate showed inhibition of seed germination. Germination in all the dilutions (10^{-1}, 10^{-2} and 10^{-3}) was more or less similar to control (without filtrate). Similarly Prasad and Hiremath (1983) who also reported hundred per cent inhibition of seed germination by stock solution of *R. solani* in fenugreek.

Table 3: Influence of culture filtrate of *Rhizoctonia solani* on seed germination and seedlings growth of fenugreek

Dilutions	Per cent seed germination *		Increase in seedlings length (mm) *	
	24hrs	48 hrs	24 hrs	48 hrs
Stock solution	0	0	0	0
10^{-1}	90	100	6	8
10^{-2}	95	100	5	6
10^{-3}	95	100	4.5	5
Control (Sterilized water)	100	100	3	5

* Mean of five replications

It was observed that the seedlings were killed due to collar rot in case of pure filtrate. But no such symptoms were observed in different dilutions. On the contrary dilutions exhibited stimulatory effect on seedlings causing seedlings elongation as compared with control. Much of the elongation occurred within 24 hrs in 10^{-1} dilution (6 mm) followed by 10^{-2} (5 mm) and 10^{-3} (4.5 mm). It is clear from the data that there is stimulatory effect of culture filtrate dilutions on seedling growth. It may be due to the presence of phenyl acetic acid in culture filtrate of *R. solani* which has growth regulating properties (Prasad and Hiremath,1983). The production of typical collar rot symptoms and decrease in seedling length observed as effect of pure culture filtrate may be because of the toxic effect at very high concentrations that resulted in shrinkage of tissue causing reduced seedling growth.

The cultural and morphological characters of *R. solani* on different solid media are presented in Table 4. The data shows that there are variations in cultural characters on different culture solid media, such as Potato dextrose agar, Czapek's Dox, Richard's agar, Rice agar, Czapek's agar and Soil

Table 4: Growth of *R. solani* on different media (Solid / Liquid)

Media Solid	Colony diameter after 4th day	Type of growth and colony colour after 10 days	Days taken for sclerotial production	Sclerotial production/size and shape of sclerotia	Media Liquid	Mycelial dry weight (g)
PDA	90	Sm and A Creamy white	32	+++ Big and irregular in shape	Potato dextrose broth	0.3787
Czapek's Dox agar	90	Msm and F Brown	20	+++ Big, oval to round	Czapek's Dox	0.0974
Malt agar	55.87	Msm Yellowish brown	29	+ Very small, oval to round	Malt extract	0.1112
Oat meal agar	78.50	Sm Brown	16	++ Small, Oval to round	Richard's	0.5042
Richards agar	90	Sm Whitish brown	6	++++ Big, oval to round	Rice broth	0.0493
Rice agar	90	Msm and A Creamy white	-	-	Czapek's medium	0.3734
Czapeks agar	90	Sm Whitish brown	-	-	Fenugreek broth	0.0143
Soil extract agar	90	Msm and F Whitish brown	26	++ Small, oval to round	Asthana and Hawkers	0.1632
Fenugreek agar	50.62	MSmt and S Pale white	-	-	Rose Bengal Streptomycin	0.3012

Sm and A = Submerged growth in the centre and Aerial at margin
Msm and F = Mostly submerged growth and margin fluffy
Msm = Mostly submerged growth
Sm = Submerged growth
Msm and A = Mostly submerged and Aerial at margin
MSmt and S = Mostly submerged thin and sparse growth

\- = Absent
\+ = Poor
++ = Fair
+++ = Good
++++ = Excellent

extract agar, these media were good for mycelial growth covering entire Petriplate (90 mm) within 4 days. However, minimum growth was recorded in fenugreek agar medium (50.62 mm) and malt agar (55.87mm) in the same period. As regards growth pattern submerged growth was observed on Oat meal agar, Czapek's agar, mostly submerged growth on Malt agar, mostly submerged with fluffy growth at margin was observed in Czapek's Dox agar and Soil extract agar, submerged growth in the centre and aerial at margin on Potato dextrose agar, mostly submerged and aerial at margin developed on Rice agar, mostly submerged, thin and sparse growth was observed in Fenugreek agar medium. The colour of the colony was brown on Czapek's Dox agar and Oat meal agar, whitish brown on Richard's agar, Czapek's agar and Soil extract agar, Creamy white on PDA, Rice agar, Yellowish brown on Malt agar and pale white on Fenugreek agar medium. Out of nine media, sclerotia did not develop on Rice agar, Czapek's agar and Fenugreek agar, whereas they were formed in 6 days on Richard's agar, in 16 days on Oat meal agar, in 20 days on Czapek's Dox agar, in 26 days on Soil extract agar, in 29 days on Malt agar and in 32 days on PDA. Size of sclerotia big and shapes were oval to round in Czapek's Dox agar and Richard's agar, small, oval to round on Oat meal agar and Soil extract agar, big and irregular in shape on PDA, very small, oval to round in Malt agar. Sclerotial production was poor on Malt agar, fair on Oat meal agar and Soil extract agar, good on PDA and Czapek's Dox agar and it was excellent on Richard's agar and no sclerotia formed on Fenugreek agar , rice agar and Czapek's agar.

The growth of *R. solani* on nine liquid media (Table 4) showed that the maximum mycelial dry weight was recorded in case of Richard's medium (0.5042 g) followed by Potato dextrose broth (0.3787 g), Czapek's medium (0.3734 g), Rose bengal streptomycin (0.3012 g), Asthana and Hawker's (0.1632 gm) and Malt extract medium (0.1112 g). Czapek's Dox, Rice broth and Fenugreek broth medium proved to be poor as they yielded minimum mycelial dry weight (0.0974, 0.0493 and 0.0143 g) respectively.

REFERENCES

Komaraiah, M. and Reddy, S.M.1986. Influence of humidity on seed deterioration of methi (*Trigonella - foenum graceum*) seeds by some seed borne fungi. *Indian J. Mycol. Pl. Pathol.* **16** (1): 77-79.

Naiki, T. and Ui, T. 1972. The micro-organisms associated with the sclerotia of *Rhizoctonia solani* Kuhn. In soil and their effects on the viability of the pathogens. *Mem. Fac. Agric. Hokkaido Univ.* **8** : 252-265.

Palo, M.A. 1927.Rhizoctonia disease of rice1: A study of disease and the certain conditions upon the variability of the sclerotial bodies of the causal fungus. *Phillip. Agric.* **15**: 361-375

Prasad, C.K.P.S. and Hiremath, P.C. 1983. Effect of culture filtrate of *Rhizoctonia solani* on seed germination, seedling growth and loss of electrolytes from the root of fenugreek. *Indian Phytopath.* **36**: 95-99.

Rehman, U.S. and Khan, M. A. 2000. Citrus withertip disease severity in relation of environmental conditions in Faisabad. *Pakistan J. Phytopath.***12**: 101-105.

Schmiederknecht, M.1960. Feuchtigkeit als standortfaktor fur mikroskopische pilze. *Z. Pilzk (NF)* **25**: 69-77.

Schneider, R.1953. Un ter suchungen uber fenchtigkeitunspruche parasitischar pilze. *Phtopathology*. **21** : 63-68.

Sharma, A. and Verma, K.S. 2007. Epidemiology of *Colletotrichum gloeosporioides* causing anthracnose of mango. *J. Mycol. Pl. Pathol.* **37(2)**:248-250