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Effects of Sulphate and Salt Sources on the Development of *Alternaria alternata* Causing Root Rot Disease to Fenugreek

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Abstract: ***Fenugreek (Trigonella foenum- graecum L.) is an important plant grown on different regions of India for its vegetable, medicine and fodder source. It get infected by many fungi where Alternaria alternata (Fr.)Keissler, was found to be dominant among the diseased samples collected during investigation. From thesesamples of Alternaria alternata, wild sensitive (Aa-2) and highly resistant (Aa-8) isolates were identified using carbendazim. Different sulphate and salt sources were used to examine their response to pathogen either stimulant or inhibitory during culture of the pathogen (in vitro). The aim of present investigation was to evaluate sulphate and salt sources on disease development of fenugreek caused by Alternaria alternata. The linear growth of pathogen was examined for its response to these sources. The use of sulphate and salt sources when grown on Czapek Dox agar medium, showed variable response during cultural conditions. The response was either stimulant or inhibitory. But certain source was proved to be a beneficial candidate against the test fungus which is beneficial for cultivar.***

Keywords: Fenugreek, *Alternaria alternata*, root rot, sulphate and salt sources

1. Introduction

Fenugreek (*Trigonella foenum - graecum* L.), is an important vegetable and medicinal source for humans and fodder for animals. Fenugreek get diseased due to some pathogens, among them *Alternaria alternata* (Fr.) Keissler, was observed to be dominant during a survey in Maharashtra state of India (Khandare and Kamble, 2013). The infection of *Alternaria alternata* causes severe damage in quality and yield as well as biochemical loss (Khandare, 2014). From the isolated samples of *Alternaria alternata*, resistant and sensitive isolates was identified against carbendazim and the sensitive isolate was abbreviated as Aa-2 and resistant isolate Aa-8. The efficacy of carbendazim along with other fungicides was also tested against the sensitive and resistant pathogen (Khandare, 2014).Sulphate (0.05%) and Salt sources(0.05%) were used to study the effect on the growth of sensitive and resistant isolates of *Alternaria alternata* amending them in the Czapek's dox agar medium and was compared with control. Sulphate sources like magnesium sulphate, potassium hydrogen sulphate, copper sulphate and ammonium sulphate as well as salt sources like magnesium chloride, potassium chloride, sodium chloride, calcium chloride and cobalt chloride were used to access their role in the growth and development of the pathogen. The use of sulphate and salt sources were seen to be either stimulant or inhibitory role in the development of disease due to *Alternaria alternata*. The aim of present investigation was to determine the role of sulphate and salt sources in disease development (*in vitro*)due to *Alternaria alternata* causing root rot disease to fenugreek.

2. Material and Methods

2.1. Materials

Diseased(root rot)samples of fenugreek (*Trigonella foenum-graecum*. L.), infected by *Alternaria alternata*. Different sulphate (0.05%)and salt sources (0.05%),for further investigation.

2.2. Methods

A Survey of the diseases of fenugreek (*Trigonella foenum - graecum*. L.) in Maharashtra state of India was carried out. In the survey, the samples of infected fenugreek were collected and pathogens were isolated by direct plating analysis (Pitt and Hocking, 1997). The isolated pathogens were identified (Subramanian, 1971; Barnett and Hunter, 1972). The sensitivity of *Alternaria alternata* was identified using food poisoning method (Dekker and Geinning,1979) and the sensitive(Aa-2) and resistant (Aa-8), isolates were identified by abbreviating them from Aa-1 to Aa-12 (Khandare and Kamble, 2013). These isolates were evaluated for assessment of growth response of Sulphate (0.05%) and Salt sources (0.05%), (*in vitro*) by observing linear growth of pathogen. The procedure was carried out as follows.

2.2.1. Sulphate Nutrition

Magnesium sulphate, potassium hydrogen sulphate, copper sulphate andammonium sulphate were used for this study. All sulphate sources were incorporated in Czapek Dox agar medium at 0.05%. The sensitive (Aa - 2) and resistant (Aa - 8) isolates were inoculated on the agar plates and the plates were incubated at 28 ± 2°C. Plates without sulphate source served as control. The linear growth was measured at different intervals.

2.2.2. Salt Nutrition

Sodium chloride, potassium chloride, magnesium chloride, calcium chloride and cobalt chloride were used for this study. These salts were incorporated in medium at 0.05% in Czapek Dox agar medium. The sensitive (Aa-2) and resistant (Aa-8) isolates were inoculated and plates were incubated at 28 ±2ºC. Plates without salt source served as control. The linear growth was measured at different intervals.

2.3. Statistical Calculations

Statistical calculation was calculated by using two way analysis of variance using Anova.

3. Results

3.1. Sulphate Nutrition

Magnesium sulphate, potassium sulphate and ammonium sulphate were incorporated with medium at 0.05%. There was significant variation in the growth of both the sensitive and resistant isolates between various sulphate sources and various incubation periods. Among these sulphate sources it was found that Magnesium sulphate and Ammonium sulphate show high growth while potassium hydrogen sulphate stimulated the growth of both the sensitive and resistant isolates. (Table.1)

3.2. Salt Nutrition

Altogether 5 salts were used in this study. Potassium chloride, magnesium chloride, sodium chloride, calcium chloride and cobalt chloride were incorporated in medium at 0.05 % concentration and discs (8 mm) of sensitive and resistant isolates were inoculated on the plates. There was variation in growth of both the isolates between various salts sources and various incubation periods. Growth of resistant isolate was found to be higher than the sensitive one. Magnesium chloride and potassium chloride show higher growth than other salt sources. Sodium chloride and Calcium chloride appeared to be stimulant while Cobalt chloride inhibited the growth of sensitive and resistant isolates of *Alternaria alternata* (Table.2).

Table 1: Effect of different Sulphate sources on the linear growth (mm) of*Alternaria alternata* isolate Sensitive and Resistant to carbendazim on Czapek Dox agar medium

	Sensitive isolate						*Resistant isolate*					
Sulphate sources 0.05%	*Days*						*Days*					
	2	4	6	8	10	12	2	4	6	8	10	12
Magnesium sulphate	14.33	20.00	30.33	42.66	56.00	69.33	15.00	22.33	32.66	44.66	59.00	74.33
Potassium hydrogen sulphate	12.33	17.66	26.66	37.00	49.66	64.00	13.66	19.00	28.33	39.33	54.33	67.33
Ammonium sulphate	13.66	18.33	28.00	39.66	52.33	66.33	14.33	20.66	30.33	42.66	55.66	70.66
Control	11.33	16.33	24.66	34.33	47.00	62.66	13.00	17.66	26.33	36.33	51.33	64.66

Sensitive isolate- P = 0.0003 , Resistant isolate- P=0.9585.

Table 2: Effect of different Salt sources on the linear growth (mm) of*Alternaria alternata* isolate sensitive and resistant to carbendazim on Czapek Dox agar medium

	Sensitive isolate						Resistant isolate					
Salt source 0.05%	Days						Days					
	2	4	6	8	10	12	2	4	6	8	10	12
Magnesium Chloride	13.66	19.33	28.66	39.66	54.66	67.33	13.66	20.33	30.33	41.66	56.33	69.66
Potassium Chloride	14.00	21.66	31.00	42.33	57.66	70.33	14.33	23.00	33.66	44.66	59.66	72.66
Sodium Chloride	11.66	17.00	24.66	35.66	50.33	63.66	13.33	19.33	26.66	37.33	52.33	65.00
Calcium Chloride	12.33	17.66	26.00	36.66	51.66	64.66	13.66	19.66	28.00	38.66	53.66	67.66
Cobalt Chloride	10.33	14.66	21.33	30.33	43.66	55.33	12.33	16.66	22.33	32.66	46.33	57.66
Control	11.00	16.33	24.33	34.66	49.33	60.33	13.00	18.66	26.33	36.33	51.33	62.66

Sensitive isolate- P = 0.9810, Resistant isolate P = 0.991

4. Discussion

Root rot disease of Fenugreekis caused by pathogen *Alternaria alternata* (Dwivedi *et al*.,1982). The experiment was carried out to understandphysiological needs of nutrients and its consequences in the development of disease.The requirement of the sulphate and salt nutrient sources (*in vitro*) for development of growth of pathogen are examined so that to overcome disease development in the host. The study will enable the cultivar to know the possible sources of development of pathogen so that those can be avoided during cultural practices. There are many workers who studied physiology of fungi. Taber *et.al* (1968), made a comparative nutritional study of *Alternaria raphani, A. brassicae*, and *A. brassicicola* with special reference to *A. raphani*. Lilly and Barnett, (1951); Hasija (1970), as well as Cochrane (1951), studied physiology of the fungi Reddy *et.al* (1965) did physiological studies of *Alternaria ricini*, causative agent of *Alternaria* blight of castor bean. Thind and Madan (1969), observed effect of various nitrogen sources on the growth and sporulation of *Cephalohecium roseum*, causing pink rot of apple. Growth and nutrition study of pathogenic fungi was also supported by Rajdekar (1966), Sami Saad (1970) and Khilare (2011).

In present studies Sulphate showed significant variation in the growth of both the sensitive and resistant isolates between various sulphate sources and various incubation periods. Among the sulphate sources used it was found that magnesium sulphate and ammonium sulphate show high growth while potassium hydrogen sulphate stimulated the growth of both the sensitive and resistant isolates.

There was variation in growth of both the isolates between various salts sources and various incubation periods. Growth of resistant isolate was found to be higher than the sensitive one. Magnesium chloride and potassium chloride show higher growth than other salt sources. Sodium chloride and calcium chloride appeared to be stimulant while cobalt chloride inhibited both the growth of sensitive and resistant isolates of *Alternaria alternata.*

Similar studies were carried out by many workers. Khandare (2014), studied,response of nitrogen and amino acid sources on development of *Alternariaalternata*causing root rot to fenugreek. Kumara and Rawal (2008), studied influence of carbon, nitrogen, temperature and pH on the growth and sporulation of some Indian isolates of *Colletotrichum gloeosporioides*causing anthracnose disease of papaya (Carrica papaya L). Naim, and Sharoubeem (1963), described Carbon and nitrogen requirements of *Fusarium oxysporum* causing cotton wilt. B.P. Singh (1976), did nutritional study, on two species of *Curvularia* causing leaf spot disease.Vakeeshan (2014), studied some carbon and nitrogen sources for growth of *Cercospora beticola.* An attempt was made in the present study to find out sulphate and salt nutritional requirement of *Alternaria alternata* in the cultural conditions. Natrajan and Govindrajan (1974), studied on the physiology and control of *Alternaria cymopsidis,* the incitant of blight disease of guar. (Lilly and Barnett, 1951). Bais *et. al* (1970), studied effect of different carbon and nitrogen sources on the growth and sporulation of *Curvularia pallescens.*

5. Conclusions

From above results it was concluded that

5.1. All sulphatesources used were stimulant for the growth of pathogen*Alternariaalternata.*

5.2. The source selected among salts showed that Cobalt Chloride inhibited the growth of sensitive and resistant isolates of *Alternaria alternata* and becameabeneficialcandidate among all sources used.

5.3. The observations are useful for cultivar during management of root rot diseaseof Fenugreek.

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