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A comparative study of the rhizosphere mycoflora of healthy and virus infected plants of Bhindi [*Abelmoschus esculentus* (L.) Moench]

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An analysis of the rhizophere mycoflora of both healthy and diseased Bhindi plants was carried out during the crop season from May 2009 to July 2009. Twenty one fungal species were isolated from the rhizosphere soil of healthy plants whereas rhizophere mycoflora of diseased plants harbour fifteenth fungal species. The fungal population was found more in healthy plants than in diseased one, both in quantity and quality. The species of *Aspergillus* was found to be the dominant fungus in the rhizophere soil of both the healthy and diseased plants. There was an overall increase in the fungal population with increase in plant age and maximum was reached at the 60th days (flowering) of growth of the healthy plants but in case of diseased plants it was recorded at the 45th days of growth of the plants. The rhizosphere mycoflora may be greatly changed due to change in C/N ratio. The differences in the physiology and morphology of healthy and diseased plants resulted in variation in the mycoflora associated with their root regions. The physico-chemical properties of the soil such as soil pH, temparature, moisture content, texture, phosphorus, potassium, organic carbon & total nitrogen were found to play an important role in the distribution of soil fungi in the rhizosphere of both the healthy and diseased plants.

Key words: Rhizosphere mycoflora, healthy and virus infected Bhindi plants, soil properties, fungal species.

INTRODUCTION

Bhindi or Ladies finger or Okra [*Abelmoschus esculentus* (L.) Moench] is one of the most important vegetable crop of the world. It is commonly cultivated for its immature fruit. The crop is grown in the plains in the almost all the states of India and also in some hills.

Soil is a complex system where several micro-organisms survive together affecting growth of plant. Microorganisms play an important role in restoring the physico-chemical and biological properties of soil. The microbial population and there activities in rhizosphere are always higher and more complex than in soil away from roots. The over all metabolic activities of the mocroorganisms in the rhizosphere are several folds higher than in the soil further away from the influence of root zone (Rangaswami, 1988). Anandapandian and Rajendra (2003) have studies extensively on the microbial diversity in rhizosphere of tree species and have reported that rhizosphere soil samples have more microbial population in comparison to non-rhizosphere soil samples. Rao and Reddy (1990) have made a comparative study in the rhizosphere of bhindi, sorghum and pigeonpea and have reported that the fungal population in the rhizosphere and non-rhizosphere soils varies with the types of crops and their age.

The rhizosphere mycoflora of virus infected plants have been studied by several workers (Sadasivan, 1963; Mishra and Kamal, 1970; Mishra *et al.*, 1970; Tyagi and Dublish, 1983; Bhagat, 2000). Ali (2000) and Rao and Reddy (1990) have studied the rhizosphere mycoflora of healthy bhindi plants but no in-

formation is available regarding the mycoflora associated with the roots of virus infected bhindi plants. Therefore, the present study has been carried out to make a comparative study of the rhizosphere mycoflora of healthy and diseased bhindi plants.

MATERIALS AND METHODS

The study was carried out during the crop season from May 2009 to July 2009. Bhidi seeds (variety-Parbhani Kranti) was taken from the Assam Seed Corporation, Guwahati, Assam in sterilized polythene bags. Surface sterilized seed were sown in the Botanical garden of Nabajyoti College, Kalgachia, Barpeta, Assam. After germination, some of the plants were mechanically inoculated by a virus of strain known as Bhindi Yellow Vein Mosaic Virus (BYVMV). The roots of diseased and healthy plants were removed with a sterilized spatula on the 15th, 30th, 45th, 60th, 75th and 90th days of growth. Soil samples from the experimental site were collected and the physico-chemical characteristics were also analysed. Soil pH was determined in soil water suspension (1:2.5) using a digital pH meter (Jackson, 1973), temperature was measured by inserting a soil thermometer into the soil, and soil moisture was estimated by Gravimetric method. Organic Carbon percentage was estimated by Walkley and Black Wet digestion method (Piper, 1966). Phosphorous was estimated colorimetrically employing Vanado-molybdate method. Potassium was estimated by using Flame photometer with neutral normal ammonium acetate solution (Standford and English, 1949). Nitrogen was determined by Kjeldahl method using Kjeltech autoanalyser 1030 (Piper, 1966).

The root systems of both healthy and diseased plants were suspended separately in 250 ml flask containing 100 ml sterilized water. The roots were subjected to constant washing in sterile water following the method described by Harley and Waid (1955). Suitable dilutions were prepared and for the isolation of fungi 10^{-4} dilution was used. 1 ml of soil suspension from the dilution was poured in Petriplate containing 20 ml of Czapeck's dox agar medium with streptomycin (5 mg/ 100 ml medium). The non-rhizosphere mycoflora of healthy and diseased plants were analysed by dilution plate method (Warcup, 1951). All the Petriplates were incubated at 27±1°C and the fungal colonies were counted on the 8th day of incubation. Subculture for each individual fungus was maintained on slants for microscopic examination and identification. Fungi were identified on the basis of morphological and reproductive characters and were confirmed as per keys and methods proposed by Gilman (1957) and Barnett and Hunter (1972).

RESULTS AND DISCUSSION

The experimental results (Table 1) revealed the number and species of fungi in the rhizosphere soil of both the healthy and diseased bhindi plants with the different ages of growth viz., 15, 30, 45, 60, 75 and 90 days. A total of 21 different fungal species belonging to 15 genera were isolated from the rhizosphere and non-rhizosphere soil of healthy bhindi plants while 15 different fungi belonging to 9 genera were isolated from the rhizosphere and non-rhizosphere soil of diseased bhindi plants at the different stages of growth. Some of the pre-dominantly occuring rhizosphere and non-rhizosphere fungi isolated from both the healthy and diseased bhindi plants were *Aspergillus fumigatus, A flavus, A. niger, Cladosporim cladosporoides, Curvularia lunata, Fusarium oxysporum moniliformae, Mucor hiemalis, Penicillium oxalicum, Rhizopus nigricans* and *Trichoderma viride*, while *Alternaria alternata, Chaetomium* sp., *Helmintnosporium* sp., *Nigrospora* sp., *Phoma* sp., and *Pythuim* sp. occured in lesser number in both the rhizosphere and non-rhizosphere soil of healthy plants but these fungi were not found in the rhizosphere and non-rhizosphere soil of diseased bhindi plants. It was observed from the results (Table 3) that comparatively higher number of fungal colonies (1455 no. of colonies) were isolated from both the rhizosphere and non-rhizosphere soil of healthy bhindi plants than diseased plants (496 no. of colonies). The results (Table 3) showed that the highest total fungal colonies (318 no.) was isolated from the rhizosphere soil of healthy plants where the age of the plants was 60 days while in case of diseased plants, the highest total fungal colonies (81 no.) was isolated when the plants age was 45 days. It was observed (Table 3) from the results that the frequency of occurrence of *Aspergillus niger* showed an increasing trend in healthy plant at the age of 60 days whose maximum frequency was 30 colonies whereas in diseased plant it was 8 colonies. The number and frequency of occurrence of fungi were found more at the maximum growth of the plant (60 days) in healthy plants while in case of diseased plants it was found more at the age of 45 days of the plants.

Table 1 : Number of fungal colonies in rhizosphere (R) and non-rhizosphere soil (S) of both healthy and diseased plants with respect to different ages of bhindi plant. (Results represent the average number of fungal colonies/g soil)

Fungal species isolated	Age of the plants (in days)																							
	15 days				30 days				45 days				60 days				75 days				90 days			
	Healthy plants		Diseased plants		Healthy plants		Diseased plants		Healthy plants		Diseased plants		Healthy plants		Diseased plants		Healthy plants		Diseased plants		Healthy plants		Diseased plants	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Alternaria alternata	2	-	-	-	3	1	-	-	4	2	-	-	4	1	-	-	1	1	-	-	2	-	-	-
Aspergillus sp	4	2	2	-	8	5	4	2	10	5	5	-	12	10	2	-	3	2	1	1	3	-	1	-
A. fumigatus	8	4	4	2	11	5	6	3	13	6	8	2	29	10	5	2	5	3	2	-	4	2	1	-
A. flavus	5	3	4	3	7	2	5	2	12	4	3	1	16	6	3	2	6	3	1	-	5	3	2	1
A. niger	12	6	8	4	17	7	8	3	23	8	12	4	30	9	8	4	10	6	6	2	8	3	4	2
Chaetomium sp.	-	-	-	-	3	1	-	-	3	2	-	-	4	1	-	-	2	-	-	-	-	2	-	-
Cladosporium cladosporoides	3	2	2	-	6	4	3	2	9	4	4	2	20	7	3	2	8	5	2	2	6	4	3	2
Curvularia lunata	7	3	5	3	8	5	4	2	15	10	6	3	25	10	3	1	7	5	3	1	6	3	1	2
Fusarium oxysporum	10	5	6	4	14	5	7	3	16	10	7	3	28	8	6	2	18	7	3	2	10	6	4	3
F. moniliformae	8	3	5	2	11	7	5	3	14	6	5	2	20	7	3	-	14	6	2	1	8	5	3	2
Helmintho sporium sp	2	1	-	-	2	1	-	-	2	1	-	-	4	-	-	-	1	1	-	-	1	-	-	-
Mucor hiemalis	6	3	5	2	8	3	4	3	12	5	6	3	19	6	3	2	5	3	2	-	4	3	1	-
Nigrospora sp.	1	1	-	-	2	-	-	-	3	1	-	-	2	-	-	-	1	-	-	-	2	-	-	-
Penicillium citrinum	10	4	6	2	13	3	7	3	18	6	6	3	21	10	4	-	9	5	2	-	6	3	2	-
P. oxalicum	12	5	5	3	13	4	7	4	14	5	3	3	18	9	2	2	7	3	1	2	4	2	2	1
Phoma sp.	2	1	-	-	4	2	-	-	8	3	-	-	12	6	-	-	7	3	-	-	3	2	-	-
Pythium sp.	2	-	-	-	1	1	-	-	2	1	-	-	2	1	-	-	2	-	-	-	2	-	-	
Rhizopus nigricans	6	3	3	1	7	3	4	2	9	4	4	1	15	6	2	2	4	1	3	2	2	1	2	-
Trichoderma viride	8	4	5	2	13	5	8	4	14	5	7	2	20	7	4	2	7	4	2	-	5	3	2	1
Dark sterile mycelia	3	2	2	3	5	2	3	-	4	3	2	1	5	3	2	-	1	-	2	1	2	1	-	1
Unclassified group	3	3	4	2	6	3	3	1	6	4	3	2	12	3	3	1	2	1	2	2	3	1	2	1
Total no. of fungal colonies	114	55	66	32	162	71	78	37	211	95	80	31	318	120	53	22	120	59	34	16	86	44	30	16

The experimental results (Table 2, Fig. 1) showed that the occurrence of fungal population in the rhizosphere soil of healthy plants was 1.95 to 2.65 times and in case of disease plants it was 1.87 to 2.61 times greater than the non-rhizosphere soil depending on the growth stages of the plants. The R/S values indicated the relative number of fungal population in the rhizosphere and non-rhizosphere soil. In case of healthy bhindi plants, the highest R/S value was 2.65 when the plants age was 60 days but in case of diseased plants it was 2.61 when the age of the plant was 45 days. The variation between the rhizosphere and non-rhizosphere mycoflora with the ageing of plants may be correlated to the oxygen uptake by rhizosphere soil sample which incresed with increasing plant age followed by a fall with old age of the plants (Parkinson and Thomas, 1969). In the present investigation, it was found that higher number of fungal population was recorded in rhizosphere soil than non-rhizosphere soil. The present findings are similar with the findings of Jayanthi *et al.*, (2001), Singh and Singh (1982), Pandy and Upadhaya (2000), Desai (1999) and Anandapandian and Rajendra (2003). A gradual increase and proliferation of fungal population was observed during the advancing growth stages of the plant which might be due to the gradual increase in exudation of some chemicals by the plant roots, which is in agreement with the findings of Venkentson and Rangaswami (1964) and Kagti (1966). The results showed the

Table 2 : Quantitative analysis of rhizosphere mycoflora of both healthy and diseased bhindi plants during the crop season. (R = rhizosphere soil, S = non-rhizosphere soil, Fungi are expressed as numbers/g soil)

		Age of the plants											
		15 days		30 days		45 days		60 days		75 days		90 days	
Micro organisms	Soil types	Healthy plants	Diseased plants	Healthy plants	Diseased plants	Healthy plants	Diseased plants	Healthy plants	Diseased plants	Healthy plants	Diseased plants	Healthy plants	Diseased plants
Fungi	R	114	66	162	78	211	81	318	53	120	34	86	30
(Thousands/g)	S	55	32	71	37	95	31	120	22	59	16	44	17
	R/S	2.07	2.06	2.28	2.1	2.22	2.61	2.65	2.4	2.03	2.12	1.95	1.87

Table 3 : Analysis of physico-chemical properties of the soil collected from the experimental site.

	Physical and chemical parameters							
Months	pH	Temp. (°C)	Moisture (%)	Texture	P_2O_5 content ($kgha^{-1}$)	K_2O content ($kgha^{-1}$)	Organic carbon (%)	Total nitrogen (%)
March' 2009	5.90	21.0	69.0					
April' 09	5.80	23.0	72.0					
May' 09	5.75	250	76.0	Sandy				
June' 09	5.40	27.0	79.0	loam	7.20	138.0	0.80	0.09
July' 09	5.15	29.0	85.5					
Aug.' 09	5.12	31.0	87.5					

species of *Aspergillus* to be the diminant fungi in the rhizosphere soil of both healthy and diseased plants. This is in agreement with the findings of Upadhaya and Rai (1983), Pandey and Upadhaya (2000) and Ali *et al.*, (2007). The variations of fungal population observed during the different ages of growth of the plants may be due to the variation in the physico-chemical properties of the soil (Table 3).

In the investigation, it was observed that quantitatively and qualitatively the mycoflora was higher in healthy plant than that of virus infected ones. It has been suggested by various workers that the metabolism of virus infected plants is different from the healthy ones. Stanley (1937) and Danlap (1930) reported that change in Carbohydrate/Nitrogen (C/N) ratio in the virus infected plants, some of the dis-

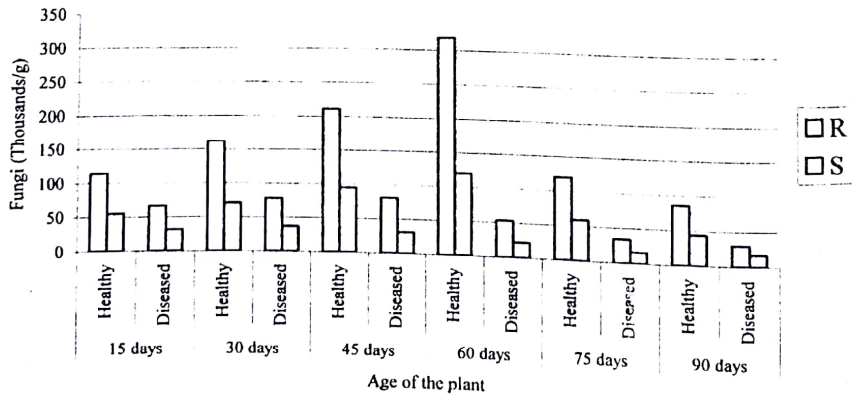

Fig 1. Graphical representation of quantitative analysis of rhizosphere (R) and non-rhizosphere (S) mycoflora of both healthy and diseased plants.

eases increase the C/N ratio and other decrease this ratio. Due to change in C/N ratio the root inhabiting fungi may be greatly changed. Due to the virus infection the root system is also less developed and so the root exudates and the microflora in root region may also be affected (Mishra *et al.,* 1970). Mishra *et al.,* (1970) extensively studied the rhizosphere mycoflora of virus infected and healthy plants of *Curcurbita maxima* and they reported that the mycoflora population was more in healthy plants than in diseased plants both in quantity and quality. They isolated 39 different fungal species from the rhizosphere soil of healthy plants and 29 fungal species from diseased one.

The results of the present investigation suggest that the differences in the physiology and morphology of healthy and diseased plants resulted in variation in the mycoflora associated with their root regions.

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