SHORT COMMUNICATION

Studies on soil mycoflora of fruit orchards of Darbhanga Raj (Presently,L.N.M.U.Campus)

S.K. SINGH, SHEELA AND S.N. CHOUDHARY

J. Mycopathol, Res, 54(2) : 291 -293, 2016;
ISSN 0971-3719

Department of Botany,
University of Calcutta,
Kolkata 700 019, India

J. Mycopathol. Res. ***54(2)*** *: 291-293, 2016;* (ISSN 0971-3719)

SHORT COMMUNICATION

Studies on soil mycoflora of fruit orchards of Darbhanga Raj (Presently,L.N.M.U.Campus)

S.K. SINGH*, SHEELA AND S.N. CHOUDHARY
University Department of Botany, L.N. Mithila University, Darbhanga 846008, Bihar
** D.S. College, Katihar, Bihar*

Received : 04.01.2016 RMS Accepted : 16.03.2016 Published : 25.07.2016

Soil mycoflora plays a great role on fruit breeding, shape, size and taste of the fruit. Especially in mango orchard of Darbhanga Raj campus spread in forty acres followed by Litchi orchard. Due to recurrent flood and soil erosion, quality and quantity of these two fruits received adverse effects. A total of 15 species belonging to 6 genera of fungi were isolated from superficial soil beds of two orchards during the September 2014 to March 2015. The mycoflora were isolated by using soil dilution techniques and soil plate technique on Potato Dextrose Agar and Czepeks Dox Agar medium supplemented by suitable antibiotics such as penicillin and streptomycin. Identification and characterisation of the mycoflora were made with the help of authentic mannual of fungi. The most common fungi viz. *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus nidulans, Aspergillus terreus, Penicillium chrysogenum, Penicillium frequentans, Penicillium funiculosum, Trichoderma viride, Trichoderma harzianum, Fusarium oxysporum, Fusarium solani, Curvularia clavata, Curvularia lunata* and *Rhizopus stolonifer* were isolated and characterized. The seasonal variation and percentage frequency of the mycoflora were statistically analyzed. These isolates has great bearing on qualitative restoration of taste and fragrance.

Key words : Raj Darbhanga, orchard, mycoflora, fruits, Deuteromycetes

Soil mycoflora plays a pivotal role in evaluation of soil condition and in stimulating plant growth, flowering, fruiting, shape and size of fruits, taste and fragrance of fruit etc. Microorganism are beneficial in increasing the soil fertility and plant growth as they are involved in several biochemical transformation and mineralization activities in soil. Fungi and bacteria are fundamental for soil ecosystem functioning. Especially in orchard, they play a key role in many essential processes such as organic matter decompositon and elemental release by mineralization Fungi play a vital role in nutrient cycling by regulating soil biological activities.

L.N. Mithila University, Kameshwaranagar, Darbhanga, Bihar has an area of three hundred acre which was handed over by Raj Darbhanga through the State Government in 1972. Out of three hundred acres,Charles Maries had developed some exclusive variety of mangos inside private orchard of mango specially Durgabhog (Red orange in colour), Lakshmeshwarbhog (Green and golden colour), Shahpasand and Sundarpasand (golden yellow and Red in colour). There was a

*Corresponding author : sksps1987@gmail.com

late variety named as 'Bhopali' (small bright red colour mango) besides local varieties. Maries not only developed mango orchard but he planted Vermillion and Rudraksha tree alongwith twenty variety of sandal wood trees.

Maries was recommended by Sir Joseph Hooker and the then Maharaja Laxmeshwar Singh employed him as garden superintendent in 1882. It is revealed by the literature that Maries work "Cultivated mangoes in India"is kept in Royal Botanic Garden archives at Kew (London). It is also revealed that Mr. Maries brought rich quantity of soil along with mango samplings. This exotic soil mycoflora helped the sampling to survive and thrive.

Mango decline is the most destructive disease of mango. The whole plant dies just in a few months to year. The disease had worldwide distribution. An increasing number of mangoes are dying from MSDS rather than gradual collapse or decline. Mango decline is of great economic importance, in India affecting 30 – 40% of plantation and Mango Sudden Death Syndrome (MSDS) is a complex disease and different fungi are reported as casual organism. Mango decline in present, it is required to isolate the pathogens associated with rhizosphere soil, their physiology and *in vitro* control for effective management strategies in the field.

Investigation of mango and Litchi decline was carried out on following lines:

· Collection of soil samples from six zones of orchards.
· Isolation, purification and identification of causal organisms.
· Physiological studies of isolate fungi
· To find out the efficacy of different fungicide against isolated fungi

Darbhang Raj (Presently L.N. Mithila University) campus covers almost 250 acres of area. The same Raj campus is also housed K.S.D.S. University, having 105 acres of land. Mango orchards/Litchi orchards mostly managed and maintained by L.N. Mithila University, Darbhanga. The temperature of the area ranges from 24°C to 36°C and relative humidity 66% to 90% in raining season. As Darbhanga is the nearer to the Himalayas range, hence prolonged rainy season is experienced condition is more suitable for mycoflora growth and sustainability.

Entire orchard was divided into 6 zones, 3 zones for mango and 3 zone for litchi according to canopy of vegetation. Almost 25 trees were taken in one group in each of mango orchard and ten tree were bunched for litchi orchard.

Potato Dextrose Agar medium was used for the isolation of fungi from the infested soil. Needle method and Dilution plate method were applied for the isolation of fungi associated with rhizosphere soil of mango.

In needle method, an inoculating needle was sterilized by dipping in methylated spirit and flaming several times. A small quantity of soil was transferred with the inoculating needle to 90 mm Petri plates containing PDA medium and these Petri plates were incubated at 25°C with 12 hrs alternate period of light and darkness to isolate the fungi. The fungi which colonized on agar from soil samples were purified on PDA slants and identified under microscope

A soil dilution plate technique was used for isolating the fungi from infested rhizosphere soil of mango. In this technique, the air dried soil samples were ground with a mortar and pestle and mixed thoroughly. Ten g sub sample of rhizosphere soil of mango was transferred to 100 ml of sterile water in a bottle, to give a dilution of 1:10. From that sample, 10 ml of suspension was transferred to a second bottle containing 90 ml of water to give a dilution of 1:100 and mixed well. This step was repeated to give 1:1000 dilutions. 1ml of soil suspension was dispersed across the medium in 90 mm diameter Petri dish .

All Petri plates were incubated at 25°C with 12 hrs alternate periods of light and darkenss for 7 days in an incubator SANYO MIR254 and were observed on daily basis. The fungi which developed from the on soil dilutions was purified on PDA slants and identified under microscope.

Pathogenecity tests were carried out in glass house in the Department of Plant Pathology, University of Agriculture, Faisalabad. Ten months old grafted seedlings of mango were planted in earthen pots filled with sterilized soil, inoculations were made following completely randomized design (CRD) with four treatments. Specimen plants were confirmed for the absence of pathogens before inoculation. The viable cultures of test fungi were mixed in the soil to inoculate the healthy mango plants. Plants were monitored for the development of symptoms and data were recorded.

In physiological studies, effect of different temperature and pH levels were observed.

The isolated fungi were inoculated in Petri plates containing PDA medium and incubated at 15°C, 20°C, 25°C,

30°C and 35°C with 12 hrs. alternate periods of light and dark period for 1 week and colony diameter was measured daily to know the suitable temperature for the growth of pathogen.

The isolated fungi were inoculated in Petri plates containing PDA medium and incubated at optimum temperature which was determined by temperature studies. The pH of PDA was adjusted 4, 5, 6, 7, 8 and 9 using 0.1N NaOH or HCL with the pH meter. Optimum pH was determined by calculating colony diameter daily for one week.

Fungal diversity of any soil depends on a large number of factors of the soil such as pH, organic content, and moisture. Physioco-chemical analysis of soil showed that pH range of soil conditions ranging from 5.1 to 7.5 and soil textures were determined the fungal population and their diversity in agricultural fields of Salur. During the investigation period 173 fungal colonies of 15 fungal species were observed. The maximum fungal species belongs to Deuteromycotina (169 colonies) and Zygomycotina (4 colonies) were observed. Among the isolates the genera *Aspergillus* and *Penicillium* were dominant (Table I).

The soil pH, organic content and water are the main factors affecting the fungal population and diversity. The organic carbon, nitrogen, phosphorus and potassium are important for fungi. In the absence of any of these the growth and sporulation of moulds as well as other microorganisms are hampered a lot. It has been reported that the density of fungal population occurred during the monsoon (rainy) season when the soil moisture was significantly high and environmental factors such as pH, moisture, temperature, organic carbon, organic nitrogen play an important role in the distribution of mycoflora.

The number of colonies per plate in 1g of soil was calculated. The percent contribution of each isolate was calculated by using the following formula :

$$\% \text{ contribution} = \frac{\text{Total no. of CFU of an individual specie}}{\text{Total no.of CFU of all species}} \times 100$$

**CFU -Colony Forming Unit*

Table 1 : Frequency of mycoflora in different zone of Mango and Litchi orchard

Fruit Orchard	Average no of total colonies	*Aspergillus*				*Penicillium*				*Fusarium*		*Curvulari*		*Trichoderma*		*Rhizopus*
		An	Afl	Afu	Ani	At	Pch	Pf	Pfu	Fo	Fs	Ccl	Clu	Tv	Th	Rs.
M-A	34	3	4	4	-	2	3	2	2	2	1	2	3	3	2	1
M-B	32	4	3	5	2	3	2	3	2	1	1	1	2	3	2	-
M-C	21	2	2	2	2	2	2	2	-	2	1	1	-	1	2	-
L-1	30	2	4	3	2	4	3	2	-	1	-	2	3	2	1	-
L-2	28	3	2	-	3	2	2	2	2	1	3	1	1	1	2	2
L-3	28	4	4	3	2	3	-	2	-	2	2	-	1	2	2	1
Total :	173	18	19	17	11	16	12	13	6	9	8	7	10	12	11	4
% Contribution		10.4	10.9	9.8	6.3	9.2	6.9	7.5	3.4	5.2	4.6	4.0	5.7	6.9	6.3	2.3

01	An	-	*Aspergillus niger*	02	Pch	-	*Penicillium chrysogenum*	03	Fo	-	*Fusarium oxysporum*
	Afl	-	*Aspergillus flavus*		Pf	-	*Penicillium frequentans*		Fs	-	*Fusarium solani*
	Afu	-	*Aspergillus fumigatus*		Pfu	-	*Penicillium funiculosum*				
	Ani	-	*Aspergillus nidulans*								
	At	-	*Curvularia clavata*								
04	Ccl	-	*Curvularia clavata*	05	Tv	-	*Trichoderma viride*	06	Rs	-	*Rhizopus stolanifer*
	Clu	-	*Curvularia lunata*		Th	-	*Trichoderma harzianum*				