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Isolation and identification of fungi from soil samples of banana field

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Abstract
The soil samples were collected from Mariyamman kovil, Marungulam and Thirukattupalli East and West of Thanjavur district. The isolation of fungi were performed from soil samples of banana field. The Physico-chemical and mycofloral diversity were analysed from respective soils of Banana field. In the present study the physiochemical parameters such as soil temperature and extractable iron and manganese contents were positively correlated with disease severity whereas pH, nitrate, organic carbon, nitrogen, organic matter and extractable calcium, zinc, potassium and phosphorus contents were negatively correlated, but less consensus exists for bananas. There are numerous incompletely understood interactions between soil abiotic attributes and disease severity were made including those between pH- and redox-controlled micronutrient availability, buffering by organic matter clay, and effects of nutrients on plant defence mechanisms.

Keywords: isolation and identification of fungi, physicochemical parameter

Introduction

Banana is a major food staple and an important cash crop in highland areas of Tanzania (Pedersen, 2012) [21], normally grown in association with the common bean in homestead gardens with little or no fertilizer input due to limited access to financial credit. Banana ranks fourth after maize, cassava, and sweet potato in terms of the quantities produced (FAOSTAT. 2017) [10] and is estimated to feed up to 30% of the total human population in the country. Approximately 30% of the total produce is consumed at the homestead while the remaining 70% is sold in the local market (Kalyebara, 2007) [6]. Hence contributing significantly in food security and income stability 80% of bananas cultivate (Perrier, 2019). The banana fruit yields under the farmer's conditions are low only 10% of the potential yield in East Africa (Maruo, 2002) [19]. Reported that soil N deficiency was amongst the main constraints to crop production in most areas of the country inclusive of the study area. This nutrient is required by banana plants in large amounts, only second to K, and is a constituent of many plant cell components including amino and nucleic acids (Irizarry, 2002) [14]. Therefore, in order to increase banana fruit yields, the current soil N levels need to be improved. Crop nutrient requirements in banana-based farming systems are currently addressed via cattle manure only. However, in most farms, the quantity of manure produced by stall-fed dairy cows is not enough to maintain the soil fertility of the farms.

Banana (*Musa paradisiaca* L.) is popular, important and commercial fruit crop of many tropical and sub-tropical regions of India. Banana fruits can play significant role in human nutrition by supplying the important growth factors such as vitamins and essential minerals (Muhammad *et al*., 2018) [20]. In India it is largely cultivated in Maharashtra, Tamil Nadu, Gujarat, Andhra Pradesh and Karnataka (Salve *et al*., 2019) [25]. It is cultivated in India an area of 830.5 thousand ha and total production is around thousand tons (Gnanase karan *et al.,* 2015) [13]. Wide variety of bacteria, fungi, viruses and nematodes affected banana crops and causes hazardous diseases. Among these the fungal diseases are most destructive once for example *Fusarium* wilt (Panama disease) is caused by the soil fungus *Fusarium oxysporum* Anthracnose is caused by *Colletotrichum musae* (Thangavelu *et al*., 2004) [28], black sigatoka by *Mycosphaerella fijiensis* (Churchill, 2011) [8], fruit rot by Botryosphaeria ribis, black root rot by Rosellinia bunodes and leaf spot caused by *Curvularia eragrostidis* (Jones, 1997) [15]. Fungal infection affected on yellowing, rusting and wilting of leaves, rotting of root, stem and fruits, decaying of fruits and ultimately affected on shelf life, nutritional profile and economy of fruits.

Fusarium wilt a vascular wilt disease, is caused by soil-borne pathogenic strains of *Fusarium oxysporum*, a diverse species complex of fungus including both pathogenic and non-pathogenic forms. The pathogenic strains are largely host specific and are responsible for yield losses in a variety of important crops such as tomato, cucumber, melons, flax, lettuce, strawberry, oil palm, tobacco, carnation, cotton, Banana and can even affect humans. *F. oxysporum* is currently subdivided into forma specialis based on host plant species rather than taxonomic distinctness (Kang *et al.,* 2014) [17]. As a hemibiotroph, *F. oxysporum* attacks susceptible hosts, but it is a facultative saprophyte, able to survive on dead organic material for extended periods of time. It can also live as an endophyte in symptomless host plants including common weed species (Altinok, 2013) [1].

The inconsistency amongst results demonstrates the complexity of the response to changes in pH and the importance of directly versus indirectly manipulated variables. Particularly redox-sensitive micronutrients such as iron, manganese, zinc and copper. The micronutrients many organisms produce siderophores with stability constants differing in magnitude and pH dependence (Rousk *et al.,* 2010) [24] the soil pH range for most crops. Increased diversity of the bacterial population enhances general suppression and broadens the array of nutrient. Increased competition, combined with reduced availability of

nutrients, means *F. oxysporum* is less likely to meet its metabolic requirements. The soil pH can be altered by inputs and losses of materials, nitrogen and carbon; this is particularly important in agricultural systems in which nitrogen fertilizer and organic matter are applied. Application of ammonium-based fertilizers or urea, or generation of ammonium from organic matter breakdown, leads to acidification of the rhizosphere due to excretion of protons by roots when ammonium is taken up, and to nitrification and leaching loss of nitrate. On the other hand, application of nitrate-based fertilizers tends to increase soil pH (Tinker and Nye, 2000) [29]. Organic matter, either produced in situ or imported from elsewhere, has a large cation exchange capacity, which buffers soil pH through adsorption of ions. (Weinert and Simpson, 2016) [31].

The characterization of organic matter, and variability of results, makes it difficult to identify consistent trends. Reviews linking organic matter characteristics to have had mixed success at identifying unifying trends (Bonanomi *et al.,* 2007) [5]. The organic matter application in 74% of cases involving *Fusarium* across more than 150 studies. Suppression of wilt associated with organic matter is generally attributable to a combination of interlinked biotic and abiotic characteristics (Baum *et al.,* 2015) [3]. The biotic controls include total microbial community size, which creates competition for organic carbon, nutrients and space. The importance of microbial populations to disease suppression has been demonstrated through a loss of suppressiveness when soil is sterilised (Cotxarrera *et al.,* 2002) [9].

Banana is affected by a wide number of diseases, of which, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. cubense (Foc) race 1 has played a major role in devastating Gros Michel banana plantations. Since 1960s, the pathogen Foc race 4 has threatened the survival and existence of the Cavendish group of bananas, which has necessitated detailed on *Fusarium* wilt, the causal organism Foc, its biology, dispersal, diversity and detection and its management. The recently developed technique of transferring the gene encoding green fluorescent protein into Foc has assisted in visualizing and analysing the colonization and infection of banana plants by the pathogen. Studies on the pathogenicity secreted in xylem genes have helped in rapid detection of the pathogen in planta and techniques such as real-time fluorescence loop-mediated isothermal amplification assay have facilitated rapid and direct quantitative detection of Foc in soil. Several management practices and biological control methods are available for the effective management of this deadly disease. Strict quarantine procedures and reduction of Foc inoculum are the methods undertaken to limit the spread of the disease to other un-infected regions. This review summarizes the recent developments of *Fusarium* wilt in banana and its management. (Thangavelu *et al.,* 2020) [27].

Materials and Methods

Collection of soil sample

The soil sample was collected from Mariyamman kovil, Marungulam and Thirukkattupalli (Este and West) area of banana field of Tanjavur district. Rhizosphere soil mycoflora were isolated by soil dilution method on PDA medium

Physicochemical analysis of soil samples: (**APHA, 1989**).
The physico-chemical parameters of collected soil samples were analyzed by standard methods. The analysis of physico- chemical parameters of the soil samples were done at soil testing laboratory, Department of Agriculture, Government of Tamilnadu.

Isolation and identification of fungi from Banana cultivated soil sample (Warcup, 1950)
Serial dilution technique was used for isolation of fungal colonies from banana cultivated soil sample in which each sample of 1 g soil were suspended in 10 ml of sterile distilled water and prepared stock. From this stock, various dilutions were prepared from 10^{-3} to 10^{-6}, using sterile distilled water. One milliliters of the diluted sample was poured into petriplates containing Potato Dextrose agar medium. Triplicates were maintained for each dilution. Streptomycin was added to the molten medium after autoclave and the plates were incubated at 30° C for 3- 5 days to identify the fungi (Gilman, 1957). Distinct fungal colonies with different morphological form were sub-cultured to purity and were preserved on potato dextrose agar slants under refrigeration conditions.

The colonies growing on PDA plates with different colour and morphology were counted separately. A portion of the growing edge of the colony was picked up with the help of a pair of needles and mounted on a clean slide with lacto phenol cotton blue stain. The slide was gently heated in a spirit lamp so as to facilitate the staining and remove air bubbles if any. The excess stain was removed with the help of tissue paper and then the cover slip was sealed with transparent nail polish. The slide was observed under a compound microscope.

Microphotography of the individual fungal species was also taken using Olympus light microscope, Indian Biotrack Research Institute Thanjavur.

Results and Discussion

Fungal flora of agricultural soils is affected by various physico-chemical properties of soil. Organic content, moisture content and pH of soil have great impact on fungal diversity of soil. The fungal diversity of soils affected positively as well as negatively on growth of crop plants. (Ratna Kumar *et al.,* 2015) [11]. The soil mycoflora of banana field at Manachanallur, Tiruchirappalli, Tamil Nadu recorded 65 fungal species belonging to 26 genera. Furthermore they reported that *Aspergillus*, *Penicillium* and *Trichoderma* were dominant genera. Fungal diversity from rhizosphere soil of banana fields at Jalgaon district of Maharashtra was studied by Salve sadhana *et al.,* (2019) [25]. They analyzed eighteen banana field's rhizosphere soils and reported 26 fungal colonies belonging to 35 fungal species and 21 genera. They found *Cladosporium*, *Fusarium* and *Aspergillus* as dominant species (Kumar and Saxena 2015) [18]. Isolated the fungi from infected banana stem and banana field soils and reported 19 fungal species belonging to 13 genera. The *Fusarium* showed maximum percentage (83.4%) contribution in rhizosphere soil. The fungal diversity of rhizosphere soils from paddy, pulses, ragi, sugarcane, vegetables and banana fields of Nanjangud taluk

of Mysore district, Karnataka were studied by Chandrashekar *et al.*, (2014) [6].

In the present study the physico chemical factor including soil texture, pH, electrical conductivity, organic carbon, organic matter, nitrogen, phosphorus and potassium. Iron, manganese, zinc, copper, calcium, magnesium, sodium and cat ions exchange capacity recorded respectively. (Table - 1).

Soil samples were analysed for their physicochemical properties and their results were represented. However, soil texture in terms of percentage of sand, silt, clay in test soils, respectively. Higher water holding capacity was observed in test soil. Increased water holding capacity and electrical conductivity in contaminated soil of agriculture field may be due to the accumulation of organic waste. EC is an important indicator of soil health, it effects crop yields, crop suitability, plant nutrient availability and activity of soil microorganisms which influence key soil processes including the emission of greenhouse gases, clay soil dominated by such as amino acid residues, acids and alkalis in the industrial soils (Seema Meena *et al.*, 2020) [26]. The increased electrical conductivity in soil contaminated by the effluents industries. The parameters like organic matter percentage, total chloride, calcium, nitrogen, magnesium were higher in test soil. Higher organic matter of the polluted soil may be due to the discharge of waste water, this increased organic matter enhanced soil enzyme activity. This is due to organic waste that may contribute to maintain or increase the organic matter and nutrient content in the soil (Bollag *et al.*, 2002) [4]. The fungal species were noticed from rhizosphere soil samples of banana field. Among these fungi of *Aspergillus* 03 species *Rhizopus* 02 species, *Trichoderma* 01 species then the *Pythium, Mucor, Chaetomium, Alternaria, Botrytis, Cladosporium, Drechslera, Curvularia, Fusarium, Helminthosporium, Nigrospora, Penicillium* and *Rhizoctonia* were recorded. The percentage contribution of Deuteromycotina was maximum 63.63% followed by Zygomycotina 18.18%, Ascomycotina 13.63% and Mastigomycotina 4.54%. *Aspergillus, Rhizopus, Trichoderma, Mucor, Alternaria, Curvularia, Fusarium, Penicillium* and *Rhizoctonia* were found frequently during the study period and the other hand *Pythium, Chaetomium, Botrytis, Cladosporium, Drechslera, Helminthosporium* and *Nigrospora* were found occasionally. Soils are extremely complex systems with many components playing varied functions mainly due to the activity of soil organisms (Chiang and Soudi, 1994) [7]. Fungi are vital component of soils present very rich in amount compared to bacteria and other microorganisms (Gnanasekaran *et al.*, 2015) [13]. The microorganisms isolated from Marungulam east banana soil samples together with their frequency of occurrences are shown in Table - 2. The isolated fungi are *Aspergillus awamori, A.candidus, A.chevalieri, A.flavipes, Aflavus, A.fumigatus, A.nidulans, A.niger, A.ochraceus, A.ruber, A.sydowii, A.terreus, A.versicolor, Curvularia lunata, Fusarium moniliforme, F.solani, F. oxysporum, Memnoniella* sp, *Helminthsporium oryzae, Penicillium janthinellum, P.citrinum, Trichoderma viride, T.harzianum,* and *Verticillium* sp., were recorded respectively. The potential fungi like *Aspergillus awamori A.flavus, A.terreus, A. niger, Curvularia lunata, Memnoniella* sp, *Penicillium citrinum, Trichoderma viride* and *T.harzianum* was most frequently isolated. They described total of 10 fungal species belonging to 7 genera from studied agricultural fields and found that *Aspergillus, Penicillium* species were dominant throughout the investigation. Isolation and identification of soil mycoflora from paddy, corn, ragi, red gram, Cotton and sugarcane crop fields at Salur Mandal were investigated by (Gaddeyya *et al.*, 2012) [11]. They observed total of 15 fungal species belonging to 6 genera and found *Aspergillus flavus, A. fumigatus, A. niger, A. nidulans, A. terreus, P. chrysogenum, P. frequentans, P. funiculosum, Trichoderma viride, T. harzianum, Fusarium oxysporum, F. solani, Curvularia clavata, C. lunata* and *Rhizopus stolonifer* as most dominant genera during the study.

Table 1: Physico-chemical properties of the soil of banana field

S.No	Parameters	Study site					
		Mariyamman kovil		Marungulam		Thirukattupalli	
		East	West	East	West	East	West
1.	pH	7.91	7.88	7.98	7.92	7.95	7.91
2.	Electrical conductivity (dsm^{-1})	0.38	0.43	0.51	0.42	0.40	0.26
3.	Organic Carbon (%)	0.25	0.22	0.32	0.23	0.30	0.16
4.	Organic Matter (%)	0.50	0.64	0.65	0.56	0.44	0.32
5.	Available Nitrogen (mg/kg)	112.0	114.6	117.6	110.1	110.6	97.8
6.	Available Phosphorus (mg/kg)	4.25	3.65	4.85	4.53	2.82	4.00
7.	Available Potassium(mg/kg)	116.5	114.6	126.6	113.3	112.4	105.4
8.	Available Zinc (ppm)	0.84	0.87	1.22	0.81	0.79	0.89
9.	Available Copper (ppm)	0.42	0.45	0.59	0.41	0.56	0.52
10.	Available Iron (ppm)	4.13	4.23	4.65	4.00	4.23	4.32
11.	Available Manganese (ppm)	2.36	2.84	2.89	2.01	2.86	1.84
12.	Calcium (mg/kg)	12.6	12.1	13.2	11.7	13.3	11.3
13.	Magnesium (mg/kg)	7.6	7.6	7.9	7.4	7.2	6.5
14.	Sodium (mg/kg)	1.26	1.20	1.29	1.23	1.20	1.28
15.	Potassium (mg/kg)	0.13	0.15	0.16	0.15	0.16	0.23

Table 2: Population of soil fungi (number of colonies $x10^3$ g^{-1} dry wt of soil) in the banana filed

S.No	Name of the fungi	Study site					
		Mariyamman kovil		Marungulam		Thirukattupalli	
		East	West	East	West	East	West
1	*Aspergillus awamori*	03	02	01	02	05	03

2	*A.candidus*	02	03	-	03	04	-
3	*A.chevalieri*	01	09	04	07	-	05
4	*A.flavipes*	02	04	12	03	06	02
5	*A.flavus*	04	-	02	-	06	05
6	*A.fumigatus*	10	01	05	10	07	04
7	*A.nidulans*	03	04	03	02	-	06
8	*A.niger*	-	-	01	04	05	05
9	*A.ochraceus*	-	01	01	-	03	06
10	*A.ruber*	05	07	06	05	-	06
11	*A.sydowii*	-	-	02	05	03	02
12	*A.terrreus*	05	01	04	-	01	06
13	*A.versicolor*	04	05	03	06	03	02
14	*Curvularia lunata*	02	-	-	-	04	-
15	*Fusarium moniliforme*	-	03	01	02	-	01
16	*F.solani*	04	-	01	05	03	01
17	*F. oxyporum*	06	04	05	06	03	04
18	*Memnoniella* sp.,	04	02	04	03	-	-
19	*Helminthsporium oryzae*	05	03	01	01	01	01
20	*Penicillium janthinellum*	04	01	04	03	03	01
21	*P. citrinum*	-	02	04	03	02	05
22	*Trichoderma harzianum*	04	07	08	05	04	04
23	*T. viride*	-	-	02	01	04	02
24	*Verticillium* sp.,	-	03	02	-	-	-
Total no of colonies		68	62	76	71	68	69
Total no of species		17	18	22	20	19	21

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