

**STUDY OF FUNGI FOUND IN THE SOIL OF
POTATO PLANTATION AREAS**

N. Bijaya Devi

Botany Department, G.P. Women's College,
Dhanamanjritri University, Imphal

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Abstract

The Rhizosphere soil and non-rhizosphere soil mycoflora of potato plantation field in Imphal East area were carried out by using dilution plate method for one crop season. Non-infected rhizosphere and rhizosphere soil mycoflora analysis revealed 10 fungal species. Out of which 2 belongs to Zygomycotina and 8 belongs to Deuteromycotina. The maximum number of fungal species were contributed by deuteromycotina. Fusarium solan showed highest population in non-rhizosphere soil whereas Alternaria solan dominated the fungal population in the rhizosphere soil. The maximum concentration of fungal population was recorded in the month of February. The lowest fungal population was recorded in the month of November both in non-rhizosphere and rhizosphere soil. The maximum number of soil microbial population was recorded in the diseased rhizosphere soil whereas the least population was found in non-rhizosphere soil.

Keywords: *Rhizosphere soil, Zygomycotina, Deuteromycotina.*

Soil is the storage house for certain elements and compounds used by plants as well as the home of their roots. Soil microflora are not the same and is varied from place to place and from season to season. Their requirement of nutrition, interaction among themselves, different environmental factor and biochemical activities makes the soil a dynamic population. The micro organism has most diversified characters. The microbial equilibrium of the soil is subjected to certain variation as the kind of crop, the age of the plant, soil types, soil treatment, seasonal temperature and moisture condition (Alex and Hendrix, 1978). The moisture content, soil temperature, hydrogen ion concentration, other temperature and moisture condition (Alex and Hendrix, 1978). The moisture content, soil temperature, hydrogen ion concentration, other organic matter, etc which might be influenced in the quantitative nature of microbial population in the soil. Soil is a complex system where several micro organisms survive together affecting growth of plant, rhizosphere of diseased as well as healthy plants harbour several fungi and bacteria. Ecological investigations in relation to plants have explained that it is the root of the plant which is in a state of continuous interaction with soil microbial population. The struggle for existence among the members of the microbial component in the soil is in the vicinity of the root because of the large number of organisms in this zone and excretions of certain chemical, compounds into the surrounding root systems. These microorganisms formed a living part of soil complex using organo-mineral as a substrate (Chetia, 1965). The fungi were found to be much more active in relatively dry soil (Griffin, 1966). The colonization of the roots by fungi might be by a range of soil fungi and that their mixed population rapidly gave rise to a stable and typically root surface mycoflora (Parkinson et al, 1963). Garrett (1956) emphasized the importance of root region microbial population in developing root diseases. Hiltner (1904) introduced the term Rhizosphere to denote this region of increase microbial activity. The rhizosphere can be defined as a zone of intense biological and chemical activity in the soil that

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surrounds the root. The study of soil fungi and their ecology paved the way in understanding the mechanism, soil survival of root-borne pathogens.

In view of these facts, investigations have been carried out with the following objectives: To study fungi found in the soil of potato plantation areas; To study the Co-relation between meteorological parameters and fungal population in the soil of potato plantation field

Materials and Methods

During the investigation period, isolation of fungal species was carried out from rhizosphere soil (healthy and diseased) and non-rhizosphere. Non-rhizosphere soil samples were collected from the areas of free plant growth. At each sampling, at least 5 samples were collected from different areas of the field so as to represent the whole field. These samples were than brought to the laboratory in sterile polythene bags and mixed together thoroughly. Soil dilution plate technique (Waksman and Fred, 1922) was employed for the isolation of the fungi. Three samples (5-10gm) were weighed in previously weighed metal containers and dried overnight in a hot air oven at 105°C. The dried samples were than reweighed and moisture content of the soil sample calculated. 10g sample of the soil (determined on a dry soil basis) was placed in an Erlenmeyer flask containing 100ml sterile water to make the stock solution. The flask containing the suspension was shaken on a mechanical shaker for 15 minutes. 10ml of these suspensions was immediately drawn (while in motion) into a sterile 10 ml pipette and transferred into a 90ml sterile water blank. 10ml samples were then transferred to 90ml sterile water blanks until the desired final dilution is reached. Each suspension was shaken by hand for a few seconds. Plating was done using Rose Bengal agar medium. 1 (one) ml of the desired dilution was transferred aseptically into each of Petri dishes (5 replicated) and 15 ml of Martin's medium, cooled down to just above the solidifying temperature was added to each inoculated Petri dish. The dishes were rotated by hand in a broad, swirling motion so that the diluted soil was dispersed in the medium. These inoculated plates were than incubated at 27°C±1°C for 7-10 days. Fungi developing from the inoculated plates were isolated in pure culture, identified and recorded. The average number of fungal colonies per dish was multiplied by the dilution factor to obtain the number of fungal propagules per gram in the original soil sample. For rhizosphere soils of both healthy and infected potato plants, an estimation of population of fungi could be obtained by dilution plate technique. The dilution procedure for rhizosphere soil was similar to that used for non-rhizosphere soil except for obtaining soil samples and for method of determining weights or amount of soil used in the dilution series. To determine weight of rhizosphere soil, the roots were removed from the original dilution flask and washed. The washed water was collected in the original flask. The water was evaporated on a water bath and the soil residue was dried to constant weight in an hot air oven at 105°C. The flask containing dry soil was weighed and dilution factors were calculated. Further process of incubation and identification were similar as that of non- rhizosphere soil.

The total fungal population was calculated by the following formula:-

$$\text{Total number of fungal population} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Dry weight of soil/g}}$$

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Where dilution factor = Dilution x Amount of inoculums taken.

Results and Discussion: A groupwise list of the identified fungi from the soil rhizosphere and non-rhizosphere is given below:-

Zygomycotina: *Mucor racemosus*; *Rhizopus stolonifers*

Deuteromycotina: *Aspergillus niger*; *Aspergillus clavatus*; *Alternaria alternata*; *Alternaria solatia*; *Fusarium oxysporum*; *Fusarium solani*; *Fusarium roseum* and *Trichoderma viridae*

Table 1 - Total number of fungal (CFUg⁻¹soil^{0.3}) isolated from the rhizosphere soil (healthy and infected plants) and non-rhizosphere soil of potato plantation area of Imphal East district (Nov. 2014 to Mar. 2015).

Fungal Types	Total No. of Fungal			Percentage Contribution		
	RS	DRS	NRS	RS	DRS	NRS
1. <i>Mucor racemosus</i>	101	83	74	5.83	4.63	4.64
2. <i>Rhizopus sto/onifers</i>	119	106	105	6.87	5.92	6.59
3. <i>Aspergillus niger</i>	114	133	87	6.58	7.43	5.47
4. <i>Aspergillus clavatus</i>	152	140	126	8.78	7.82	7.91
5. <i>Alternaria a/ternata</i>	151	124	124	8.72	6.92	7.78
6. <i>Alternaria solani</i>	355	400	248	20.52	22.34	15.57
7. <i>Fusarium oxysporum</i>	197	265	161	11.38	14.80	10.11
8. <i>Fusarium solani</i>	300	245	371	17.34	13.68	23.30
9. <i>Fusarium roseum</i>	175	214	210	10.11	11.95	13.19
10. <i>Trichoderma viridae</i>	66	80	86	3.81	4.46	5.40
Grand Total	1730	1790	1592			

Table I. Reveals that *Alternaria Solani* dominated the fungal population in the rhizosphere soil (20.52%) and Disease Rhizosphere soil (23.34%) respectively. *Fusarium solani* (23.30%) showed highest population in Non-rhizosphere soil.

Table 2 - Monthwise total fungal types and their contribution (%) of the potato plantation field areas and meteorological parameters compared. (Crop season Nov. 2014 – March 2015).

Month	Meteorological Parameters						Total Number of Fungal Spore Types and Percentage					
	Temp °C (Max)	Temp °C (Min)	R.H. (%)	Rainfall (mm)	Wind speed (Km/h)		RS	%	DRS	%	NRS	%
Nov.	27.57	12.97	81.3	1.74	3.10	100	5.83	“			72	4.47
Dec.	24.76	6.50	78.35	2.23	2.90	137	7.99	-	-		122	7.57
Jan.	24.28	4.39	69.74	-	3.58	410	23.92	493	26.50		457	28.38
Feb.	28.98	8.07	58.43		3.67	697	40.66	943	50.69		635	39.44
Mar	29.74	13.05	57.77	5.33	5.54	370	21.58	424	22.79		324	20.12
Total:							1714		1860		1610	

Table II. reveals the comparison between monthwise total number of fungal population and meteorological parameters. The highest fungal population of healthy rhizosphere soil (40.66% with 697CFU), diseased rhizosphere soil (50.69% with 943CFU) and non-rhizosphere soil (39.44% with 635CFU) were recorded in February 2015. The corresponding meteorological parameters recorded were temperature (Max. 28.98°C), relative humidity (58.43%) rainfall (nil) and wind speed (3.67km/h). The lowest fungal population was recorded in the month of November, 2014 in non-rhizosphere soil (4.47% with 72CFU). The corresponding meteorological parameters recorded were temperature (Max 27.57°C, Min. 12.97°C), relative humidity (81.3%), rainfall (nil) and wind speed (3.10km/h).

A total of 10 fungal types were identified from rhizosphere soils of healthy, diseased

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and non-rhizosphere potato plantation field in Imphal areas (crop season Nov. 2014 to March 2015). The maximum number of fungal types were contributed by Deuteromycotina was followed by Zygomycotina. *Fusarium solani* was having highest population for both rhizosphere (Healthy) and non rhizosphere soils. O'Brien and Rich (1976) stated that *Fusarium* wilt was caused by *Fusarium oxysporum* and *Fusarium solani*. *Fusarium oxysporum* was more widespread but less pathogenic than *Fusarium solani*.

Snyder and Toussoun (1965) opined that *Fusarium* was responsible for vascular wilt, while *F. solani* produces a cortical decay. The fungi which causes *Fusarium* wilt was common soil pathogens with wide host ranges. They were recorded in infected seed potatoes or in infested soil adhering to potato tubers. *Alternaria solani* was dominant fungal species for the rhizosphere (diseased) soil. *Alternaria solani* causes the early blight of Potato. It also attacks tubers. Konger and Baruah (1958) analyzed the soil microflora of Potato and detected *Fusarium solani* and *Alternariasolani*. Satpute and Dutta (1987) reported *Trichoderma viride*, *Alternaria alternata*, *Fusarium solani*, *Alternaria solanietc* from the soil of potato plantation fields. The present investigation agrees with these previous reports.

The pattern of species distribution, composition and population density could be explained by the fact that in rhizosphere region specific groups of fungal species, more adapted to root region, were stimulated by the rhizosphere effect. This rhizosphere effect had been assigned to a number of factors. The most important factors were the annulations of fungistasis and bacteriostasis (Jackson, 1958; Brown, 1973), the selective stimulation of microbial activities rather than its quantitative enrichment (Vander Drift, 1957), the O₂/CO₂ ratio in the root region (Green wood, 1970), the presence of different energy sources in the rhizosphere and non-rhizosphere soils (Brown, 1975) and the different generation times of microorganisms in the rhizosphere and non-rhizosphere soils (Bowen and Rovira, 1976; Bowen, 1980). Themucilageneous layers which covers the root hairs was believed to serve as a substrate for the microorganisms (Darbyshire and Greens, 1971; Mosse, 1975). The fungal population increases in rhizosphere and non-rhizosphere soil in general from December to February. It might be due to soil temperature which increases from December to February and at moderate temperature, microbial activity increases than cool temperature (Pandey and Upadhyaya, 2000). In the present study, the lowest fungal population in soils (healthy rhizosphere and non-rhizosphere) were recorded in the month of November. It was also observed that the sudden fall of total fungal population in the month of November and December. Such decline in the occurrence of mycoflora, might be due to the low temperature during the winter months. During the investigation period the correlation between weather parameter and concentration of spores were positively correlated with temperature and Humidity whereas it is negatively correlated with rainfall and wind speed. During the investigation period, the maximum microorganisms were the found in the unhygienic storage rooms. So it is recommended to clean the storage rooms with proper ventilators and sanitation.

Summary and Conclusion

The rhizosphere soil and non-rhizosphere soil mycoflora of potato plantation field in Imphal areas were carried out by using dilution plate method for one crop season

(Nov 2014-nov 2015); In both Rhizosphere and Non-rhizosphere soils, mycoflora of potato plantation field using dilution plate method (Nov. 2014 to March, 2015) revealed 10 fungal species. Out of which 2 belongs to Zygomycotina and 8 belongs to Deuteromycotina; *Alternaria solani* showed highest fungal population in rhizosphere soil whereas *Fusarium solani* was dominated in the non-rhizosphere soil; In case of rhizosphere and non-rhizosphere soils, the highest fungal population was found in the month of February; In case of non-rhizosphere and rhizosphere (healthy) soils the lowest fungal population was found in the month of November

The successful infection by a pathogen to the root and development of disease will be greatly influenced by the adaptability of the pathogen in the root region, ability to compete with the root region microflora and finally its inherent virulence. Thus, study of root region microbial population is of great importance for understanding disease development and subsequently for controlling them.

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