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Effect of temperature and humidity on the maximum concentration of fungal population in the potato plantation areas

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Potato (*Solanum tubersum* L.) cultivar Kufri Jyoti was grown about one hectare in a local farmer's field in Imphal ($24^0 44^/$ N latitude and $93^0 38^/$E longitude) East areas. The isolation of fungal species were carried out from the rhizosphere soil (healthy and diseased) and non-rhizosphere soil of potato plantation areas. The rhizosphere soil and non-rhizosphere soil mycoflora of potato plantation field in Imphal East areas were carried out by using dilution plate method for one crop season. Non-infected rhizosphere and rhizosphere soil mycroflora 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 salani (16.13%)* showed highest population in non-rhizosphere soil whereas *Alternaria solani* dominated the fungal population in the rhizosphere soil (19.28%) and disease Rhizosphere soil (19.32%) respectively. The maximum concentration of fungal population was recorded in the month of February. The corresponding meteorological parameters recorded were temperature (Max. 24.8^0C, Min 9.0^0 C), relative humidity (88%), rainfall (0.4 mm) and wind speed (3.7 Km/hr).

Key words : Potato field, *Fusarium salani,* rhizosphere soil, Deuteromycotina

INTRODUCTION

Potato *[Solanum tuberosum* L.) is an important vegetable crop of Manipur which belongs to the family of Solanaceae. Potato is susceptible to a number of diseases, some of which are widespread and others are localized. The causal agents of these diseases include bacteria, fungi, viruses, mycoplasmas, viroids and nematodes. In Manipur, Early blight and Late blight diseases of potato causes considerable damage to the crop thereby causing huge losses to the cultivators. The potato is agriculturally unique in that it is vegetatively propagated, meaning that a new plant can be grown from a potato or a piece of potato. In Manipur, cultivation of potato requires moderately cool temperature ranging between 18^0 C to 20^0 C. In general, it is a short duration crop, about $2^1/_2$ months to $4^1/_2$ months as compared to the main cereals like wheat, and rice. Manipur with its salubrious climate and soil type is suitable for potato cultivation. Potatoes contribute key nutrients to the diet including vitamin C, potassium, and dietary fibre. In Manipur, potato is very important food. Rice and potato is our main food. Women always demand potato for preparing "Yongchak iromba" (an item of food associated with potato). Manipur is a small state but women are very hardworking. They have given self employment to make potato fry, potato chop, pokora of potato, potato chutney, potato chips, potato sticks etc. From these small industries, they can earn money easily. Soil is a complex system where several microorganisms survive together affecting growth of plant, rhizosphere of diseased as well as healthy plants harbor several fungi and bacteria. Ecological investigations in relation of plants have explained that it is the root of the plant which is in a state of continuous interaction with soil microbial population. The rhizosphere can be defined as a zone of intense biological and chemical activity in the soil that 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 isolate pathogenic and non pathogenic fungi found in the rhizosphere and nonrhizosphere soil of potato plantation areas and To measure the co-relation between meteorological parameters

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and fungal population in the rhizosphere and nonrhizosphere soil of potato plantation field.

MATERIALS AND METHODS

Potato (*Solanum tuberosum* L.) cultivar Kufri Jyoti was grown in about one hectare in a local farmer's field in Imphal (24^{0}44$^/$ N latitude and 93^{0}38$^/$E longitude) East areas for one crop season (November, 2017 to March, 2018). During the **investigation** period, isolation of fungal species were 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 then brought to the laboratory in sterile polythene bags and mixed together thoroughly. Soil dilution plate technique was employed for the isolation of the fungi. Three samples (5-10 g) 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. IO g sample of the soil (determined on a dry soil basis) was placed in an Erlenmeyer flask containing 100 ml sterile water to make the stock solution. The flask containing the suspension was shaken on a mechanical shaker for 15 minutes. 10 ml of these suspension was immediately drawn (while in motion) into a sterile 10 ml pipette and transferred into a 90ml sterile water blank. 10 ml samples were than transferred to 90 ml 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 propagates 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:-=

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

Where dilution factor = Dilution x Amount of inoculums taken.

Collection of Weather data

Meteorological data viz, temperature, relative humidity, rainfall and wind speed were recorded for the investigating period. The data were collected from the meteorological section, ICAR Research Complex for N.E.H. Region, Manipur centre, Lamphelpat, Imphal.

RESULTS AND DISCUSSION

A total of 10 fungal types were identified from the soil rhizosphere and non-rhizosphere of potato plantation field in Imphal East areas for the crop season (November, 2017 to March, 2018) is given below:-

ZYGOMYCO TINA :

1. *Mucor racemosus* (Fig. 1)
2. *Rhizopus stolonifers* (Fig. 2)

DEUTEROMYCOTINA

3. *Aspergillus niger* (Fig. 3)
4. *Aspergillus clavatus* (Fig. 4)
5. *Alternaria solani* (Fig. 5)
6. *Cladosporium herbarum* (Fig. 6)
7. *Fusarium oxysporum*

8. *Fusarium solani* (Fig. 7)
9. *Fusarium roseum*
10. *Penicilium citrinum* (Fig. 8)

Table 1: Total number of fungal (CFUg'soillO^3) isolated from the rhizosphere soil (healthy and infected plants) and non-rhizosphere soil of potato plantation area of Imphal East district (Nov. 2017 to Mar. 2018).

	Total No. of Fungal types			Percentage Contribution		
Fungal Types	RS	DRS	NRS	RS	DRS	NRS
Mucor racemosus	135	120	98	5.23	4.25	5.27
Rhizopus stolonifers	165	200	140	6.40	7.09	7.53
Aspergillus niger	250	290	169	9.70	10.28	9.09
Aspergillus clavatus	205	191	150	7.95	6.77	8.06
Alternaria solani	497	545	230	19.28	19.32	12.37
Cladosporium herbarum	380	412	180	14.74	14.60	9.68
Fusarium oxysporum	255	307	201	9.89	10.88	10.81
Fusarium solani	260	285	300	10.08	10.10	16.13
Fusarium roseum	200	215	180	7.76	7.62	9.68
Penicilium citrinum	230	255	211	8.92	9.04	11.35
Grand Total	2577	2820	859			

Note : RS = Rhizosphere soil, DRS = Diseased rhizosphere soil, NRS = Non rhizosphere soil.

Table 2: Total number of colonies and their contribution to each colony type out of the total population of the rhizosphere soil (Health and diseased) and nonrhizosphere (Crop season Nov. 2017 to March 2018).

	Total No. of Fungal types			Percentage Contribution		
Colony Types	RS	DRS	NRS	RS	DRS	NRS
Zygomycotina	300	320	238	11.64	11.34	12.80
Deuteromycotina	2277	2500	1621	88.35	88.65	87.19
Grand Total	2577	2820	1859			

Table 1 revealed that total number of fungal population (CFU) isolated from rhizosphere soils (healthy and diseased) and nonrhizosphere plants of potato plantation field. *Alternaria solani* (19.28%), *Cladosporium herbarum* (14.74%), *Fusarium solani* (10.08%), *Aspergillus niger* (9.70%) etc were the dominant fungal population in healthy rhizosphere soil smaples. *Fusarium solani* (16.13%), *Alternaria solani* (12.37%), *Fusarium oxysporum* (10.81%), *Penicilium citrinum* (11.35%), *Cladosporium herbarum* (9.68%) etc. were dominant fungal types on the non-rhizosphere soil samples.

It was reported that *Alternaria solani, Alternaria alternata, Fusarium solani,* etc. from the soil of potato plantation fields in Shillong area. It was reported that three is trend in weather variables and concentrations of airborne conidia of *Alternaria solani,* from the potato field in South Africa during three potato growing seasons. Bijaya Devi (2019) showed that *Altenaria solani* dominated the fungal population in the rhizosphere soil and diseases rhizosphere soil from the soil of potato plantation field whereas *Fusarium solani* showed highest population in non-rhizosphere soil of the November 2014 to march 2015 crop season. The present investigation was in agreement with these previous workers.

Table 2 represents the total number of fungal colonies and percentage contribution of each colony type to the total population of the rhizosphere soil and nonrhizosphere soil. It reveals

Table 3: Monthwise total fungal types and their contribution (%) of the potato plantation field areas and meteorological parameters record. (Crop season Nov. 2017 – March 2018).

Month	Meteorological Parameters					Total Number of Fungal Spore Types and Percentage					
	Temp °C (Max)	Temp°C (Min)	R.H. (%)	Rainfall (mm)	Wind Speed(Km/hr)	RS	%	DRS	%	NRS	%
Nov. (2017)	26.6	13.5	93.3	0.3	2.3	300	12.0	-	-	238	13.0
Dec. (2017)	22.4	9.7	93.8	3.8	2.0	455	18.0	-	-	319	17.0
Jan. (2018)	21.8	6.5	89.4	0.3	2.6	515	20.0	755	27.0	410	22.0
Feb. (2018)	24.8	9.0	88	0.4	3.7	877	34.0	1346	48.0	501	27.0
Mar (2018)	27.0	12.0	87.2	2.3	4.8	430	17.0	719	25.0	391	21.0
Total:						2577	2820		1858		

Table 4: Statistical analysis of Meteorological parameters and Soil types

Meteorological parameters	Soil types		
	RS	DRS	NRS
Temperature (max.)	-0.209^{ns}	0.020^{ns}	-0.199^{ns}
Temperature (min.)	-0.538^{ns}	-0.474^{ns}	-0.630^{ns}
Relative Humidity	-0.540^{ns}	-0.880*	-0.812**
Rainfall	-0.252^{ns}	-0.439^{ns}	-0.213^{ns}
Wind speed	0.310^{ns}	0.646^{ns}	0.559^{ns}

ns = Not significant
* = significant at 5% level
** = significant at 10% level

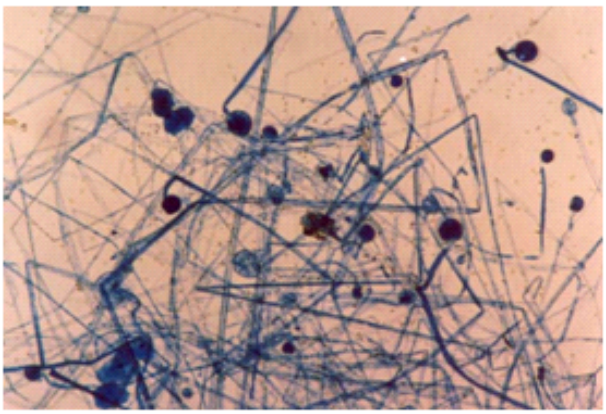

Fig. 1 : *Mucor racemosus* Fresenius (Mag. X 40)

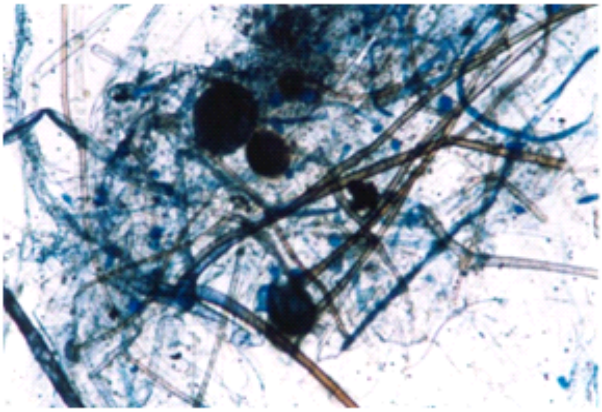

Fig. 2: *Rhizopus stolonifer* Ehrenberg (Mag. X 40)

Deuteromycotina dominated the fungal soil mycoflora of potato plantation field. The percentage contribution were 88.35% in rhizosphere soil, 88.65% in diseased rhizosphere soil and 87.18% in nonrhizosphere soil. *Zygomycotina* contributed 11.64% in healthy rhizosphere soil, 11.34% in diseased rhizosphere soil and 12.80% in nonrhizosphere soil. The distribution and dominance of Deuteromycetes fungi suggested that the fungi belonging to this class are strong colonizers of the decaying substrate with better and wider adaptability coupled with high competitive ability. Whereas,

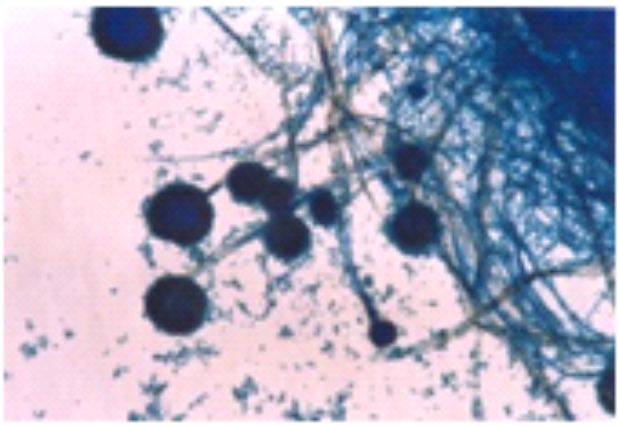

Fig. 3 : *Aspergillus niger* Van Tieghem (Mag. X 40)

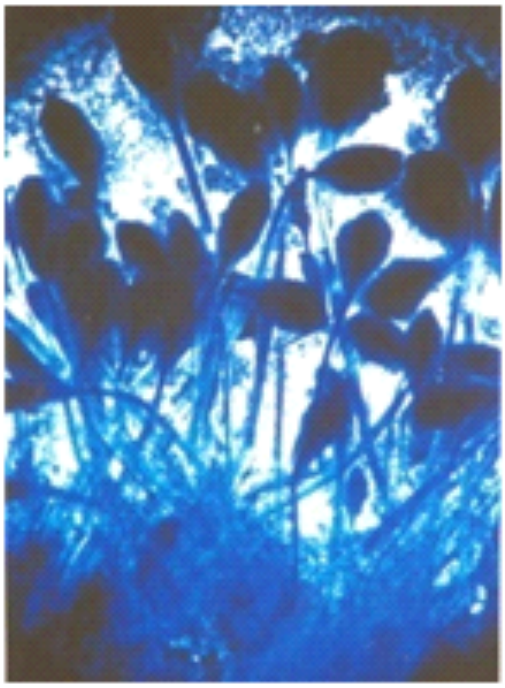

Fig. 4 : *Aspergillus clavatus* Desmazieres (Mag. X 40)

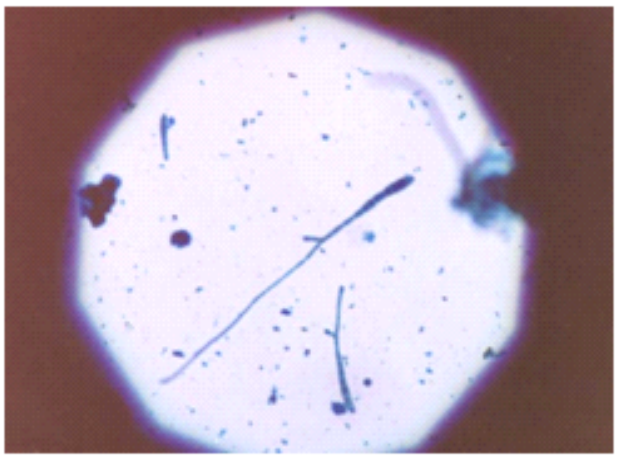

Fig. 5: *Alternaria solani* Ell and Mart (Mag. X 40)

those of Zygomycetes and Ascomycetes were weak colonizers (Kumar *et. al.* 2011) with narrow and /or poor adaptability.

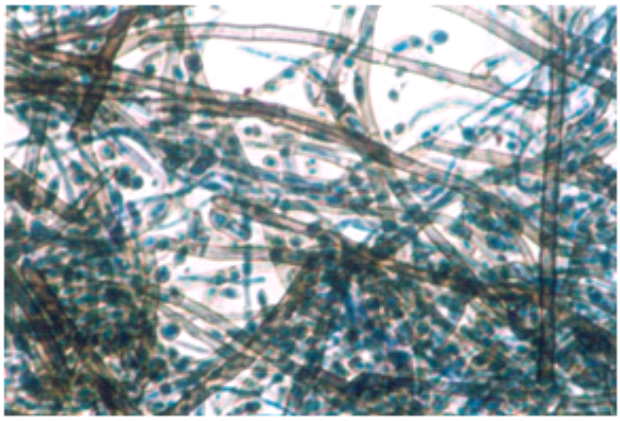

Fig. 6: *Cladosporium herbarum* Link ex Fries (Mag. X 40)

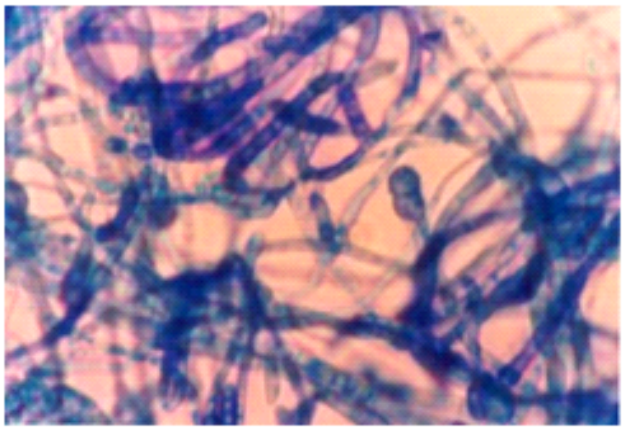

Fig. 7: *Fusarium solani* Appel and Wollenweber (Mag. X 40)

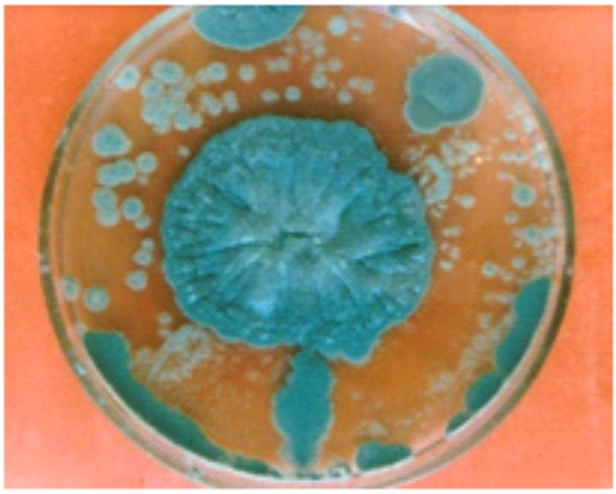

Fig. 8: Culture plate of *Penicillium*

Table 3. showed the correlation between monthwise total fungal types with the meteorological parameters recorded during the investigation period. The highest fungal population of healthy rhizosphere soil (34% with 877 CFU), diseased rhizosphere soil (48%, 1346 CFU) and non-rhizosphere soil (27% with 501 CFU) were recorded in February (2018). The corresponding meteorological parameters recorded were temperature (Max. 24.8^0 C, Min. 9.0^{0}C), relative humidity (88%), rainfall (0.4 mm) and wind speed (3.7 Km/hr.). The lowest fungal population of healthy rhizosphere soil (12%) and nonrhizosphere soil (13%) were recorded in November, 2017. The corresponding meteorological parameters recorded were temperature (Max. 26.6^{0}C, Min. 13.5^{0}C), relative humidity (93.3%), rainfall (0.3 mm) and win speed (2.3 Km./ hr.). The lowest fungal population of diseased rhizosphere soil (27%) was recorded in the month of January 2018. The corresponding meteorological parameters recorded were temperature (Max. 21.8^{0}C, Min. 6.5^{0}C), relative humidity (89.4%), rainfall (0.3 mm) and wind speed (2.6 km/hr). In the month of February maximum concentration of fungal population were recorded. The period was coincided with the vegetative and tuberization stage of the crop. In the month of January, there was less disease intensity in the field thereby lowest fungal populations were recorded. In the month of November, the available crop canopy was least because it records seedling stage of the plant. During this phase, the amount of substrates for the microorganism was low, resulting low fungal population were recorded. The field infection on the leave of the potato plant were noticed when the plants were 60 days. The corresponding meteorological parameter recorded on that day were temperature (Max. 21.3^0 C, min 3.7^0 C), relative humidity 92%, wind speed (2.6 Km/ hr.) and rainfall (nil). Diseased rhizosphere soil was not considered in the month of November and December, 2017 due to non appearance of diseases. The fungal population increased in rhizosphere and non-rhizosphere soil in general from December to February. It might be due to soil temperature which increase from December to February and at moderate temperature, microbial activity increases tha cool temperature.

Table 4 revealed that both positive and negative correlation were observed for soil types and meteorological parameters. The most important meteorological factor affecting fungal population was relative humidity; low humidity greatly influenced in increase fungal types in all the soil

types. Low temperature have moderate effect on RS and NRS negatively. Wind speed also have positive correlation effect on fungal types, and thus fungal population increases with increase in wind speed. High temperature slightly increased fungal concentration for RS and NRS but decreased for DRS. Decrease in amount of rainfall weakly correlates with the rise in fungal population. Biswas *et. al* (2013) revealed that the disease severity of late blight of potato correlated negatively with temperature but positively correlated with relative humidity during 2010-11 at Kanpur. Grinn–Gofron *et. al* (2018) reported that the hourly concentration of *Alternaria* and *Cladosporium* spores were similarly positively correlated with air temperature, however the relation was stronger with *Cladosporium* than with *Alternaria.* From the investigation, it was reported that relative humidity and precipitation negatively affect and wind speed positively affects hourly concentrations of both types of spores. Ianovici (2016), reported in general, the daily concentrations of *Alteranaria, Cladisorium, Epicoccum* and *Pithomyces* spores are negatively correlated with wind speed. 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. Thus, study of root region microbial population is of great importance for understanding disease development and subsequently for controlling them.

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