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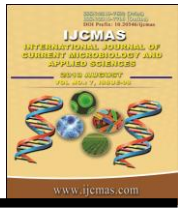

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Physicochemical Characterization and Papulation Dynamics of Mycoflora in Infected Rhizosphere Soil of Onion White Rot caused by *Sclerotium cepivorum*

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A B S T R A C T

The present study describes physicochemical characterization and rhizosphere soil mycoflora in the field of onion white rot caused by *Sclerotium cepivorum*. Soil fungi in onion infected field need to improve knowledge of diversity of fungi associated with white rot of onion. Infected rhizosphere soil, sixteen physicochemical parameters were analysed. It founds alkaline pH but EC, N, Ca, Na, B, S and Mo contents were found least whereas OC, P, K, Zn and Cu high in infected soil as compared to standard range. Due to infected soil, chemical analysis is also hanged. Fifteen samples of soil were carried out during June- Sept and Dec-Feb 2017. In all the 09 genera and 10 species were observed from infected soil. *Mucor*, *Rhizopus* and *Aspergillus* species were found dominant. Total number of fungal species colony was found dominant in Osmanabad (OD) site. Parentage of frequency and % of abundance was found more in *Rhizopus stolonifer* (80% and 15.39%) respectively.

Introduction

Onion (*Allium cepa* L.) is an important vegetable for potential foreign exchange earners for a country like India, as it is second largest producer of onions after China, producing 1.6 million MT annually FAO (2012). It is also known as "queen of kitchen". Productivity of onion is affected by many biotic and abiotic stresses especially diseases. The onion producing states in India includes mainly, Maharashtra, Karnataka, Gujrat, Bihar and Madhya Pradesh, among these state Maharashtra is contributed 32.6% of the total production (Anonymous, 2009). The soil microbes decompose the plant and animal residues entering the soil and convert them into soil organic matter, which influences on soil physical, chemical and biological properties and on creating a complimentary medium for biological reactions and life support in the soil environment. Never the less enhanced site-specific diversity typically results in higher levels of below ground

microbial diversity and production. Large quantities of readily decomposable organic matter are added to agricultural soils every year as crop residues or animal wastes and have a significant outcome on soil microbial population. The physicochemical study of parameters is important to agriculturist for plants growth and soil management. The plant species growing on the soil also equally influence the population and species composition of the soil fungi along with infected pathogen. Some studies dealt with the influence of plant community and others attempted to examine seasonal trends (Kennedy *et al*., 2005). Soil mycoflora plays a pivotal role in evaluation of soil conditions and in stimulating plant growth (Singh *et al*., 1999) by biochemical transformation and mineralization activities in soils.The soil is a complex organization being made up of inorganic matter, organic matter, soil organisms, soil moisture, soil solution and soil air and soil contains 50-60% mineral matter, 25-35% water, 15-25% air and little percentage of organic matter (Chatwal *et al.*, 2005). Other non-point sources of contamination affecting agricultural soils include inputs such as, fertilizers, pesticides, sewage sludge and organic (Singh, 2001). Shamir and Steinberger (2007) reported that the topsoil contains high organic matter, which in the presence of adequate moisture supply, acted upon by the microorganisms to decompose the complex organic residues into simpler forms; hence, microbial populations are generally higher in the surface soil layer as compared to the lower depths. Higher fungal population during rainy and autumn season supported the findings of other workers (Arunachalam *et al*., 1997), due to prevailing favorable moisture and temperature condition. Therefore the objective of the present study was to find the physiological changes and population of mycoflora due to white rot of onion caused by *Sclerotium cepivorum.*

Materials and Methods

Physico-chemical characterization

Physico-chemical analysis of infected rhizosphere soil were collected from study area and used for physicochemical characterization. Soil were spread out on a tray for air drying and sieved over a 150 mm and used for characterization. Each sample is weighed using digital balance. The samples were then oven-dried at a temperature of 110^0C for 24 hours and reweighed. Electrical conductivity and pH of compost were measured (Subbiah and Asija, 1956). Nitrogen content was determined by the Kjeldahl method (Sahilemedhin and Bekele, 2000). Organic Carbon was evaluated (Walkely and Black, 1934) method by oxidizing organic carbon with potassium dichromate and sulphuric acid.

Phosphorus in soil was determined by Olsens method by using spectrophotometer (Olsen *et al.,* 1954; Bray and Kurtz, 1945). Water soluble and exchangeable Potassium was calculated by Ammonium acetate method (Hanway and Heidel, 1952) using Flame photometer. Sodium, Calcium and Magnesium cations were estimated by EDTA titration (GOI, 2011). Analysis of Ferrous, Manganese, Copper, Boron, Sulphur, Zinc and Molybdenum were done by acid digestion of soil (Jackson, 1967).

Population dynamics of mycoflora

Infected rhizosphere soil collection sites

Isolation of fungi from infected rhizosphere soil of onion white rot caused by *Sclerotium cepivorum* from different localities viz. ND-Nanded, LT-Latur, OD-Osmanabad, SR-Solapur, BD-Beed, AD-Aurangabad, TR-Tuljapur, LA-Lohara, MM-Murum, OA-Omerga, UR- Udgir, PR-Pandharpur. SA-

Sangola, MA-Mangalweda and NG-Naldurg was carried out.

Isolation of fungi by dilution plating method

For the isolation of mycoflora, dilution plate method was employed (Apinis, 1963; Warcup (1950). Ten grams of sample were transferred to a flask containing 100 ml sterile water. The contents were crushed and shaken on a mechanical centrifuge for 15 min and then serially diluted to obtain 10^{-3}andof 0.5 ml of each was transferred to sterile petri plates containing potato dextrose agar (PDA) medium.

The pH of medium was adjusted by adding 0.1N HCl or 0.1N NaOH. Petri plates were incubated in an inverted position at 27 $\pm 2^0$C in dark.

Identification of fungi

Fungal morphology were studied macroscopically by observing colony features (Colour and Texture) and microscopically by staining with lacto phenol cotton blue and observe under compound microscope for the conidia, conidiophores and arrangement of spores (Aneja, 2001). The fungi were identified with the help of literature (Nagamani *et al.*, 2006; Ellis, 1976; Ainsworth *et al*., 1973). The percentage of incidence, frequency and abundance were calculated by employing the following formulae (Girisham *et al*., 1986).

$$\% \text{ of incidence} = \frac{\text{No of colonies of species in all plates}}{\text{Total no of colony of the all the species in all plates}} \times 100$$

$$\% \text{ of frequency} = \frac{\text{No of observation in which species appeared}}{\text{Total no of observations}} \times 100$$

$$\% \text{ of abundance} = \frac{\text{No of colonies of species in all observations}}{\text{Total no of colonies in all observations}} \times 100$$

Statistical analysis

The number of colonies per plate in 1 g of soil was calculated and the percent contribution of each isolated fungi were determined. Data were statistically analysed and the significance of differences was determined by using book (Mungikar, 1997).

Results and Discussion

Physico-chemical characterization

Infected rhizosphere soil was collected from white rot of onion and sixteen physicochemical parameters were analysed. It founds alkaline pH but EC, N, Ca, Na, B, S and Mo contents were found least whereas OC, P, K, Zn and Cu high in infected soil as compared to standard range. Due to infected soil, chemical analysis is also changed. Among 16 characterization, Phosphorus (467±12.11kg/ha) and Potassium (526.8±11.22kg/ha) contents was found very high as compared to standards. In case of Nitrogen (94.05±3.22kg/ha), Calcium (4.68±1.33mg/kg) and Sodium (1.69±3.33mg/kg) was found very poor support in infected soil (Table 1).

Population dynamics of mycoflora

Fifteen samples of infected rhizosphere soil (surface of 0-5cm deep) from different localities were collected during pre and post-harvest infection of onion and carried out for isolation, quantification and identification of microflora by dilution plate technique. In all the 09 genera and 10 species viz. *Mucor muudo, Aspergillus niger, Aspergillus flavus, Rhizopus stolonifera, Fusarium oxysporum, Pythium* sp, *Trichoderma harzianum, Rhizoctonia solani, Cladosporium* spand *Penicillium chrysogenum* were observed. *Mucor*, *Rhizopus* and *Aspergillus* species were found dominant. Total number of fungal species colony was found dominant in Osmanabad (OD) site. Parentage of frequency and % of abundance was found more in *Rhizopus stolonifer* (80% and 15.39%) respectively (Table 2; Fig. 1 and 2). It observed from finding that when more population dynamics of mycoflora shows less white rot of onion infection by *Sclerotium cepivorum.* Graphical representation of percent contribution of fungal species in infected onion fields was showed in figure 3.

Organic matters acts as glue for binding soil components and improve water infiltration and water holding capacity and organic carbon or organic matter is the indicator of soil quality and productivity (Fawcett and Caruana, 2001). Chaudhari (2013) studied that the physicochemical study of soil is based on various parameters like total Organic Carbon, Nitrogen (N), Phosphorus (P_2O_5), Potassium (K_2O), pH and Conductivity and results showed that all the eight selected places of Bhusawal have medium or high mineral content.

Table.1 Physico-chemical characters of infected soil of onion white rot caused by *Sclerotium cepivorum*

Sr. No	Parameters	Standard Range	Infected soil (±SE)
1	pH	6.5 to 7.5	7.6±0.99
2	Ele. Conductivity³ mS	Less than1.0	0.15±0.11
3	Organic carbon %	0.41 to 0.60	1.86±0.11
4	Nitrogen ³kg/ ha	161 to 320	94.05±3.22
5	Phosphorus ³ kg/ha	31 to 50	467±12.11
6	Potassium ³ kg/ha	181 to 240	526.8±11.22
7	Calcium (mg/kg)	65 to 80	4.68±1.33
8	Magnesium (mg/kg.)	10 to 15	12.32±2.66
9	Sodium (mg/kg)	5 to 15	1.69±3.33
10	Zinc (ppm)	1.0 to 5.0	4.99±1.11
11	Ferrous (ppm)	2.5 to 5.0	3.35±1.01
12	Manganese (ppm)	2.0 to 5.0	3.74±1.13
13	Copper (ppm)	0.2 to 0.5	2.83±0.10
14	Boron (mg/gm)	30 to 100	23±2.55
15	Sulphur (mg/kg)	10 to 20	09±2.32
16	Molybdenum(mg/kg)	0.8to3.3	0.43±0.34

Values are Mean ± Standard Error

Table.2 Population dynamics of mycoflora in infected rhizosphere soil of onion white rot caused by *Sclerotium cepivorum*

Sr No	Fungi / Locations	*Sclerotium cepivorum* infected soil samples sites																	
		ND	**LT**	**OD**	**SR**	**BD**	**AD**	**TR**	**LA**	**MM**	**OA**	**UR**	**PR**	**SA**	**MA**	**NG**	**TS**	**PF**	**PA**
1	***Mucor muudo***	+	-	+	+	+	+	-	+	+	-	+	+	+	+	-	11	73.33	14.11
2	***Aspergillus niger***	+	+	+	+	-	-	+	+	-	+	+	+	+	-	-	10	66.67	12.83
3	***Aspergillus flavus***	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	11	73.33	14.11
4	***Rhizopus stolonifer***	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	12	80.00	15.39
5	***Fusarium oxysporum***	-	+	-	+	-	+	-	+	-	+	+	+	+	+	-	9	60.00	11.54
6	***Pythium sp***	+	-	+	-	-	-	+	-	+	-	+	-	-	-	+	6	40.00	7.70
7	***Trichoderma harzianum***	-	-	+	-	-	-	+	-	-	+	+	-	-	-	+	5	33.34	6.42
8	***Rhizoctonia solani***	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	3	20.00	3.85
9	***Cladosporium sp***	-	-	+	-	-	+	+	-	-	+	-	-	-	-	+	5	33.34	6.42
10	***Penicillium chrysogenum***	-	+	-	+	+	-	-	-	-	-	-	+	+	-	+	6	40.00	7.70
Total No. of colonies		**4**	**5**	**8**	**5**	**3**	**5**	**5**	**5**	**4**	**6**	**6**	**6**	**6**	**4**	**6**	**78**		
% Incidence		**5.13**	**5.13**	**10.26**	**5.13**	**3.85**	**5.13**	**5.13**	**5.13**	**5.13**	**7.70**	**7.70**	**7.70**	**7.70**	**5.13**	**7.70**			

Legands:ND-Nanded,LT-Latur,OD-Osmanabad,SR-Solapur,BD-Beed,AD-Aurangabad.TR-Tuljapur, LA-Lohara, MM-Murum, OA-Omerga, UR- Udgir,PR-Pandharpur.SA-Sangola,MA-Mangalweda,NG-Naldurg,TS-Total Species, PI-Percentage Incidence, PF- Percentage Frequency, PA- Percentage Abundance

Fig.1

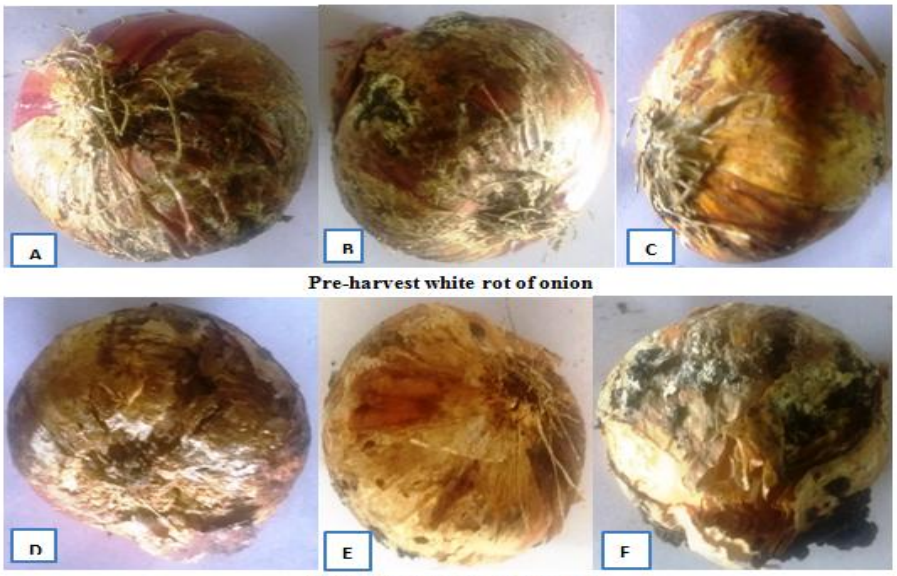


Fig.1. Pre and post-harvest white rot infection of onion bulb caused by *Sclerotium cepivorum*.

Fig.2

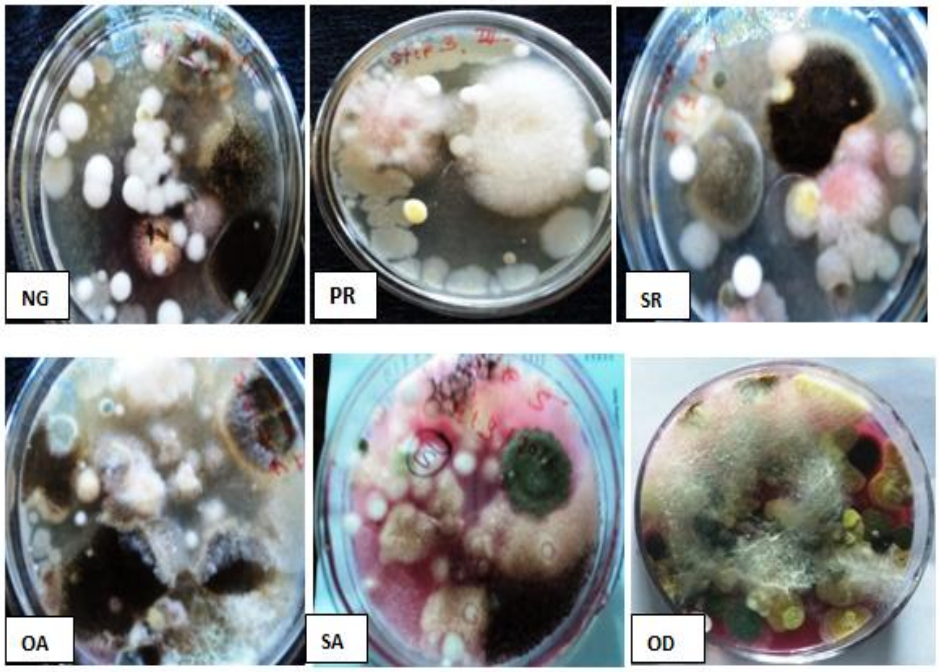
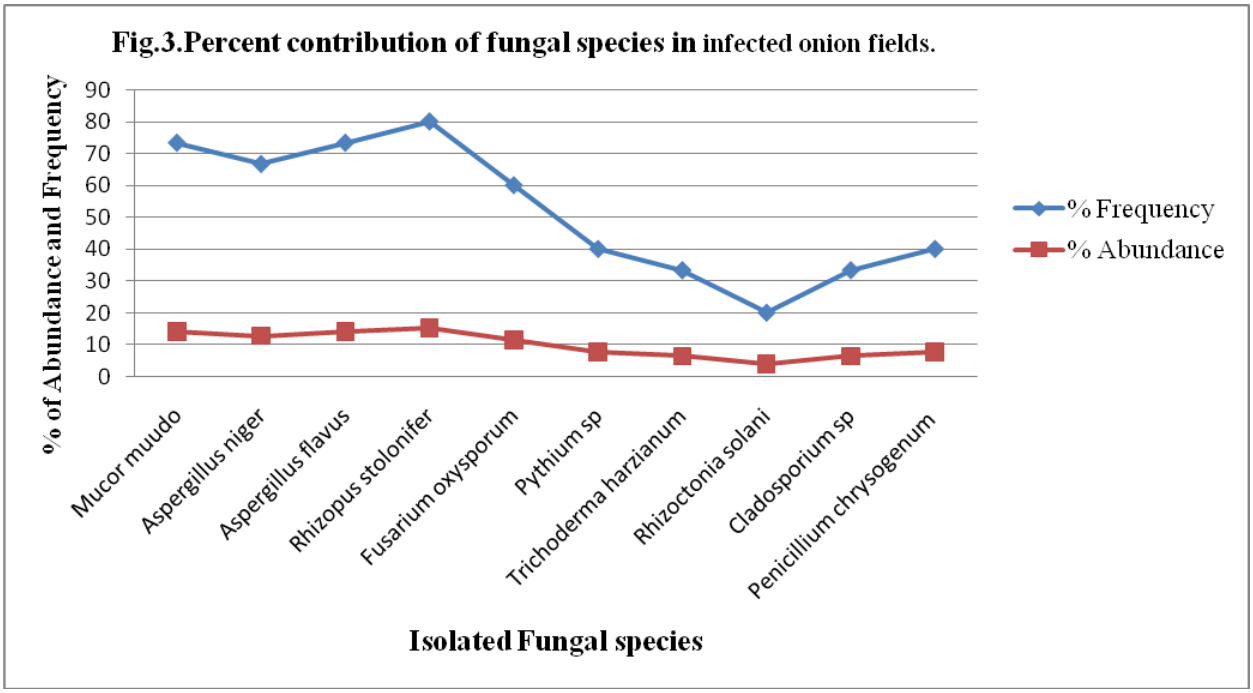


Fig.2. Dominant fungal colony in infected rhizosphere soil of onion white rot caused by *Sclerotium cepivorum* from different localities (NG- Naldurg, PR-Pandharpur, SR-Solapur, OA-Omerga, SA-Sangola , OD-Osmanabad).

Fig.3



Ganorkarand Chinchmalatpure (2013) studied on soils with physical properties, chemical properties and micronutrients of soils have been done and the values of pH indicated that all samples of the soils are alkaline and all samples were containing moderate amounts of available micronutrients. Joel and Amajuoyi (2009) studied some selected physicochemical parameters and heavy metals in a drilling cutting dump site and test results indicated that some of the heavy metals like copper, iron and calcium showed a high level of contamination in most of the plots under the study area. Mahajan and Billore (2014) studied on the physicochemical parameters like pH, specific conductivity, chloride, total alkalinity, calcium, magnesium nitrate, sulphate, phosphate sodium and potassium from July 2008 to June 2009 and fluctuation were observed in several parameters.(Vernon Paren (2010) reported that the salinity values above 2 dS/m begin to cause problems with salt sensitive plants, and values above 4 dS/m are problems for many garden and landscape plants.

The conservation of diversity of mycoflora in agricultural fields becomes very essential for the development of sustainable agriculture. The soil pH, organic content and water are the main factors affecting the fungal population and diversity (Yu *et al*., 2007; Zhang et.al, 2001; Jha *et al*., 1992). Hackl *et al* (2000) reported the plant species growing on the soil also equally influence the population and species composition of the soil fungi. Soil fungi have significant impact on the several activities of soil ecosystem. Some studies on soil fungi of agricultural fields of Tamilnadu, Andhra Pradesh, Odisha and other remaining states of India enlightened the importance of soil mycoflora in agricultural fields (Prince and Prabakaran, 2012; Gaddeyya *et al*., 2012; Behera *et al*., 2012). It was reported that the density of fungal population occurred during the monsoon season when the soil moisture was significantly high (Deka and Mishra, 1984) and environmental factors such as pH, moisture, temperature, organic carbon, organic, nitrogen play an important role in the distribution of mycoflora. Fungal diversity of

any soil depends on a large number of factors of the soil such as pH, organic content and moisture (Rangaswami and Bagyaraj, 1998; Alexander, 1977).

In conclusion, the physicochemical parameters are important to plant growth and status of microbiota, therefore the study concluded that the soil quality can be carried out by different parameters. Most of the parameters are quite higher or lower than acceptable limits. The saprobic fungi represent the largest proportion of fungal species in soil and they perform a crucial role in the decomposition. In conclusion, in the present study fifteen different onion field soil samples of four districts were studied for screening and detected of fungal diversity. *Aspergillus, Penicillium* and *Mucor* species were found dominant. It observed from finding that when more population of fungi shows less onion infection by *Sclerotium cepivorum.* Our finding determines the differences in fungal species composition of onion infected soils and management practices have greater potential to influence the soil fungal community in future.

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