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POPULATION DYNAMICS OF RHIZOSPHERE MYCOFLORA ON SOME IMPORTANT PULSES

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ABSTRACT: The present study deals with the isolation & identification and population dynamics of rhizosphere mycoflora on traditional pulses. The rhizosphere mycoflora of four pulses i.e. green gram, black gram, pigeon pea *and* cowpea was studied. There are about total 19 species and 6 genera of fungi were isolated from these soil samples. Physiochemical parameters such as water holding capacity, pH, moisture content and texture were analysed. The mycoflora were isolated by using soil dilution and soil plate techniques on three different media. Among isolated fungi, *Asper gillus nige, A. flavus, A. oryzea, Cladosporium herbarum, Penicillium adametzi, P. aurantiogriseu m, P. brevicompactum, P. funiculosum, P. glandicola, P. implicatum P. islandicum, rugulosum, P. si mplicissimum, Fusarium oxysporum* and *Trichoderma harizanum* were found dominant. Out of these, nine *Penicillium* species was observed. The highest number of colonies i.e *P. adametzi* was observed on RBA medium (38) and % contribution (38.8) as compared to others. The dominant % contribution of *C. harbarum* (47.6%) was reported on PDA media. The diversity of fungi on different media and % contribution of mycoflora were statistically analysed.

Keywords: Rhizosphere, Mycoflora, Population dynamics, Pulses. Media.

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1. INTRODUCTION

Soil is normally considered as the fine earth which covers land surfaces and accumulation of mineral matter transported by water, wind or ice. A simple definition of soil is the material that plants grow

in and which provides them with physical support, water and nutrients [10]. The rhizosphere is a "zone of soil surrounding the root which is affected by it" and compound released from roots into the soil change its chemical and physical properties and stimulate the growth of various organisms [2]. It was reported the rhizodeposits of various exudates, sloughed cells and decaying roots provide an important substrate for the soil and there is a complex interplay between mycoflora and the quantity and type of compounds [3]. It was reviewed that fungi are fundamental for soil ecosystem functioning especially in forest and agricultural soils, they play key role in many essential processes such as decomposition and elemental release by mineralization [4]. Generally, in India popular pulses i.e chickpea, pigeon pea, green gram, black gram and Lentil are mostly grown in two seasons' kharif and rabi [5]. It was reported the pusles are healthy, nutritious and easy to cook, growing pulses also promotes sustainable agriculture as pulse crop help decrease greenhouse gases, increase soil health and use less water than other crops [6]. It was reported that environmental factor such as pH, moisture, temperature, organic carbon, organic nitrogen play an important role in the distribution of mycoflora of green gram and black gram [7]. It was reported the physicochemical analysis of soil from pulses and 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 fields of Salur mandal [8]. It was reported the fungal diversity of any soil depends on number of physichochemical characters of the soil such as pH, organic content and moisture [9].

2. MATERIALS AND METHODS

Collection of Rhizosphere sample from Crop Field

Soil samples were collected from each crop by digging out soil around the rhizosphere area upto 5-10 cm from plant. Rhizosphere soil samples where usually collected after each 30 days of the plant growth from each selected crop plants i.e. green gram (*Vigna radiata* (L). R.Willczek), black gram (*Vigna munga* (L), pigeon pea (*Cajanus cajan* (L) Millsp) and cowpea (*Vigna ungiculata* (L). walp.) field. The soil samples were collected from the surface of underground roots and their surroundings. Surface sterilized scalpel was used to transfer rhizosphere soil to clean sterilized ziplock polythene bags separately.

Soil Analysis

Soil is a set of various chemical processes that determine the amount of available plant nutrients but also the chemical, physical and biological soil properties important for plant nutrition or soil health. There are analyse the soil water holding capacity, pH of soil, texture of the soil, moisture content of the soil [8].

Isolation method

Soil dilution plate method: (serial dilution)

1gm of soil sample was suspended into 10ml of double distilled water to make microbial suspensions (10^{-1} to 10^{-5}). Dilution of 10^{-1}, $10^{-,2}$ 10^{-3}, were used to isolate fungi. One ml of microbial suspension

of each concentration were added to sterile petri dishes (triplicate of each dilution) containing 15ml of sterile Potato Dextrose Agar, Czapek's Dox Agar and Rose Bengal Agar [10].

Soil plate method: About 0.05g of soil was scattered on the bottom of a sterile petri dish and molten cooled (40-45^{0}c) agar medium (PDA) and (CZA) (RBA) was added and rotated gently to disperse the soil particles in the medium. The petri dishes were then incubated at 28±2^{0}C in dark f or three day [11].

Identification of fungi from the rhizosphere sample

The soil mycoflora enumerated by two method, namely soil dilution and soil plate method on different media such as Potato Dextrose Agar, Czapek's Dox agar and Rose Bengal Agar by The fungi were identified with help of literature[12,13].

Statistical analysis

The number of colonies per plate in one gram of soil was calculated and the % contribution of each isolated fungi were determined. Data were statistically analysed by using book [14].

$$\%\ \text{contribution} = \frac{\text{Total no. of } * \text{CFU of an individual species}}{\text{Total no. of CFU of all species}} \text{X100}$$

*CFU-Colony Forming Unit

3. RESULTS AND DISCUSSION

Rhizospere soil analysis of pulses crop plants.

The collected soil was characterized its different four physic-chemical properties and illustrated in table 1. Water holding capacity of the studied crop plant highest water holding capacity shows cowpea i.e 85%, and lowest water holding capacity shows black gram 28.5%, after cowpea , green gram shows 70% and pigeon pea 60%.The pH of crop plant was check by using the handy –pH meter. pH of the all pulses ranges from 7.6 to 8.2, alkaline pH the highest pH is 8.2 shown by green gram and lowest pH is 7.6 both pigeon pea and cowpea Soil texture is clay loamy. Green gram, pigeon pea and cowpea fields shows the clay texture of soil. Black gram field soil shows the loamy texture of soil. The moisture content of soil ranges from 51.5% to 66.6%. There are highest moisture content is present in green gram field soil and lowest moisture content is present in cowpea and black gram soil field. Pigeon pea shows 56.25% moisture content.

Isolation and identification of rhizosphere fungi

Study revealed that, total 402 colonies and 19 fungal species from 6 genera were isolated from different agricultural pulses i.e. *Vigna radiata*, *V. munga*, *Cajanus cajan*, *V. unguiculata* field. The isolated fungal species are 19 species viz. *Aspergilus niger*, *A. flavus*, *A. parasiticus*, *A. oryzea* , *Caldosporium herbarum, Penicillium adametzi*, *P. aurantiogriseum*, *P. brevicompactum*, *P. funiculosum*, *P. glandicola*, *P. implicatum*, *P. islandicum*, *P. rugulosum*, *P. simplicissimum*, *Fusarium oxysporum, F. solani*, *Trichoderma harizanum* , *Trichoderma viride* . *Helminthosporium sp* etc (fig.1).

Green gram-In rhizosphere mycoflora of green gram, 19 species were observed on PDA, RBA,(19) and CZA (18) medium. Among 19 species, *Penicillium adametzi* was observed maximum colonies on RBA medium (38) followed by PDA & CZA. Average number of colonies was formed maximum in PDA (31), then on CZA average number of colonies was (15) *P. adametzi* .The average number of percent contribution was maximum in *C.herbarum (47.6%)* on PDA medium followed by *P. adametzi* (38.8%) on RBA medium. In case of CZA medium the highest % contribution was found in P. *isanadicum* (9.2) (Table 2)

Black gram- In rhizosphere mycoflora of black gram, 18 species were observed on PDA CZA(18) and RBA(17) medium. Among 18 species, *P. rugulosm* (16) was observed maximum colonies on CZA medium followed by RBA & PDA. Average number of colonies was formed maximum in RBA (15), then on PDA average number of colonies was (8).The average number of percent contribution was maximum in *P. rugulosm* (23.8%) on CZA medium followed by *P*. *rugulosm* (19.5%) on PDA medium. In case of RBA medium the highest % contribution was found in *C. harbarum (8) on* RBA (Table 3).

Pigeon pea- In rhizosphere mycoflora of pigeon pea, 19 species were observed on PDA, RBA (17) and CZA (14) medium. Among 19 species, *P. rugulosm* and *P.implicatum* was observed maximum colonies on RBA medium (36) followed by CZA & RBA. Average number of colonies was formed maximum in CZA (21) of *P. implicatum* , on PDA (12) of *A. parasiticum* and *P. implicatum*(12) . The average number of percent contribution was maximum in *P. implicatum* (46.6%) on CZA medium. In case of RBA medium, the highest % contribution was found *in P. funiculosum* (3.3%) (Table 4)

Cowpea- In rhizosphere mycoflora of cowpea, 18 species were observed on PDA, RBA (17), and CZA (14) medium. Among 19 species, *P. aurantiogrisum* was observed maximum colonies on CZA medium (30) followed by PDA & RBA. Average number of colonies was formed and found maximum in PDA (28*)* of *P. herbarium*. Average number of colonies on RBA was (5) of *P. herbarium* .The average number of percent contribution was maximum in *A. parasiticus* (41.4%) on PDA medium followed by *Helminthosporium* sp. (20.1%) on RBA medium. In case of CZA medium the highest % contribution was found in *P. herbarium* (18.6) (Table 5) The present study gives the information about diversity of rhizosphere mycoflora of different pulses from different media. Physical and chemical properties of soil are also known to be significantly correlated with changes in the rhizosphere fungal population. The present study was supported the above view as the numbers of fungi in the rhizosphere were found to be greater than in the soil away from it and total 32 fungal species were isolated from rhizosphere and non- rhizosphere soil of pigeon pea varieties at different stages of plant growth and among it, *Aspergillus, Fusarium* and *Penicillium* were found dominant [15]. It was detected the total 24 fungal species were isolated from the rhizosphere and non-rhizosphere soil of three leguminous crop plants viz. *Cicer arietinum, V.*

radiata and V. mungo at three different stages of plant growth and it was observed that the most dominant fungal species were *Aspergillus, Penicillium*, *Rhizoctonia* and *Cladosporium* [16]. It was reported the occurrence of fungal species from black gram cultivation fields. *Aspergillus niger* (14.2), *Aspergillus nidulans* (17.8), *Aspergillus terreus* (17.8) and *Trichoderma viride* (14.2) were dominant in the soil of black gram field [7]. It was reported the rhizospheric mycoflora of pigeon pea fields and mostly found *Penicilium citrinum*, *P. decumbens, A. niger, A.terrus, A.flavus, Trichoderma viride* [17]. It was reported fungi include *Alternaria spp., Aspergillus niger, Botrytis cineria, Chaetomium spp., Curvularia spp., Fusarium udam, Mucor racenosus., Penicillium spp., Rhizoctonia solani, Rhizopus arrhizus, Sclerotinia rolfsii, Trichoderma* spp. and *Verticillium spp.* from pigeon pea [18]. It was reported 33 species and two varieties belonging to 144 genera were isolated *Aspergillus niger*, *A. flavus* , *A. tamarii*, *A. oryzae*, *A. ochraceus*, *Penicillium chrysogenum*, *P. viridicatum*, *P. lanosum* like more fungi found in rhizosphere of Cowpea field [19]. It was reported mycoflora from rhizosphere of cowpea field *Fusarium* sp*., Macrophomina* sp*., Alternaria* sp*, Curvularia* sp*., Cladosporium* sp*., Aspergillus sp., Penicillium* sp*. Pythium.* sp*., Fusarium* s*p. Rhizoctonia* sp*., Macrophomina* sp*. Alternaria* sp*. Curvularia* sp*. Cladosporium* sp [20]. It was isolated mycoflora possessed 8 genera and 14 species and results clearly indicated that *Trichoderma, Aspergillus, Fusarium* and *Penicillium* were of high occurrence in all fields and some species of *Curvularia*, and *Rhizopus* were least encountered and *Trichoderma* and *Aspergillus* were found dominant in all pulse fields [21].It was reported total of 15 species belonging to 6 genera of fungi were isolated from agricultural fields at Salur Mandal by using soil dilution and soil plate technique on Potato Dextrose Agar and Czapek's Dox Agar medium and 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 stol*anifer were isolated and characterized [8].

Table 1: Rhizospere soil analysis of pulses crop plants.

Sr.No	Soil sample	Water Holding Capacity (%)	pH	Texture	Moisture content (%)
1	Green Gram (*Vigna radiata)*	70	8.2	Clay	66.6
2	Black Gram (*Vigna munga)*	28.5	8.0	Loamy clay	51.5
3	Pigeon Pea (C*ajanus cajan)*	60	7.6	Clay	56.25
4	Cowpea (Vigna ungiculata)	85	7.6	Clay	51.5

Table 2. Rhizosphere mycoflora of green gram on different media and its % contribution.

Sr.no.	Name of the fungi	Number of colonies			% Contribution		
		PDA	CZA	RBA	PDA	CZA	RBA
1	*Aspergillus flavus*	01	02	01	1.5	3.7	1.8
2	*A. niger*	01	02	01	3.0	3.7	1.8
3	*A. oryzae*	02	01	01	1.5	3.7	1.8
4	*A. parasiticus*	01	00	02	1.23	1.8	1.3
5	*Cladosporium herbarum*	08	08	18	47.6	00	5.7
6	*Penicillium adametzi*	31	15	38	1.5	1.8	38.8
7	*P. aurantiogriseum*	01	01	07	1.5	1.8	4.1
8	*P.brevicompactum*	01	01	02	1.5	1.8	1.8
9	*P. funiculosum*	01	02	01	1.5	1.8	1.8
10	*P.glandicola*	01	03	04	3.6	3.7	3.7
11	*P.implicatum*	03	04	02	3.0	5.5	4.1
12	*p. isandicum*	02	02	01	1.5	9.2	2.3
13	*P.rugulosm*	01	01	07	1.5	3.7	3.7
14	*P. simplicissimum*	01	01	01	1.5	1.8	1.3
15	*Fusarium oxysporum*	05	05	05	1.6	1.8	1.9
16	*F. solani*	03	02	01	4.6	00	2.7
17	*Trichoderma harzianum*	01	02	01	1.5	3.7	1.3
18	*Trichoderma viride*	01	01	01	1.5	1.8	1.3
19	*Helminthosporium sp.*	01	03	01	1.5	5.5	2.3
Average number of total colonies		65	54	97			

Table 3. Rhizospere mycoflora of black gram on different media and its % contribution.

Sr.no	Name of the fungi	Number of colonies			% Contribution		
		PDA	CZA	RBA	PDA	CZA	RBA
1	*Aspergillus flavus*	00	06	03	00	9.6	03
2	*A. niger*	01	02	03	2.4	3.2	03
3	*A. oryzae*	05	02	02	12.1	3.2	00
4	*A. parasiticus*	03	01	01	7.3	1.6	01
5	*Cladosporium herbarum*	01	02	01	2.4	3.2	08
6	*P. adametzi*	06	08	15	14.6	12.9	00
7	*P. aurantiogrisum*	01	00	01	2.4	00	01
8	*P.brevicompactum*	01	01	02	2.4	1.6	03
9	*P. funiculosum*	02	01	03	4.8	16.1	01
10	*P.glandicola*	01	02	01	2.4	3.2	00
11	*P.implicatum*	01	01	01	2.4	1.6	01
12	*P. isandicum*	01	02	00	2.4	3.2	01
13	*P.rugulosm*	08	16	06	19.5	23.8	01
14	*P. simplicissimum*	01	02	00	2.4	3.2	08
15	*Fusarium oxysporum*	02	02	01	4.8	3.2	00
16	*F. solani*	02	01	00	4.8	2.8	01
17	*Trichoderma harzianum*	01	01	03	2.4	3.2	00
18	*Trichoderma viride*	03	02	01	7.3	1.6	01
19	*Helminthosporium sp.*	01	01	01	2.4	1.6	00
Average number of total colonies		41	62	42			

Table 4. Rhizosphere mycoflora of pigeon pea on different media and its % contribution.

Sr.no.	Name of the fungi	Number of colonies			% Contribution		
		PDA	CZA	RBA	PDA	CZA	RBA
1	*Aspergillus flavus*	01	00	00	2.0	2.2	00
2	*A. niger*	02	01	00	4.1	4.4	00
3	*A. oryzae*	02	02	02	4.1	00	2.2
4	*A. parasiticus*	12	00	01	4.1	2.2	1.1
5	*Cladosporium herbarum*	02	01	01	25	2.2	1.1
6	*Penicillium adametzi*	03	01	01	4.1	00	1.1
7	*P. aurantiogrisum*	01	00	01	6.25	4.4	1.1
8	*P. brevicompactum*	04	01	01	2.0	11.1	1.1
9	*P. funiculosum*	01	02	03	8.3	8.2	3.3
10	*P .glandicola*	01	05	01	2.0	2.2	1.1
11	*P. implicatum*	12	21	36	8.3	46.6	1.1
12	*P. isandicum*	01	01	01	2.0	00	4.0
13	*P. rugulosm*	01	20	36	2.0	11.1	4.0
14	*P. simplicissimum*	03	00	01	2.0	4.4	1.1
15	*Fusarium oxysporum*	01	05	01	2.0	4.4	1.1
16	*F. solani*	01	02	01	6.25	00	1.1
17	*Trichoderma harzianum*	01	02	01	2.2	00	1.1
18	*T. viride*	01	00	01	2.0	00	1.1
19	*Helminthosporium sp.*	01	00	01	00	4.5	1.1
Average number of total colonies		48	45	90			

Table 5. Rhizosphere mycoflora of cowpea on different media and its % contribution.

Sr.no.	Name of the fungi	Number of colonies			% Contribution		
		PAD	CZA	RBA	PDA	CZA	RBA
1	*Aspergillus flavus*	01	01	03	1.4	1.1	8.8
2	*A. niger*	01	02	02	1.4	1.1	5.8
3	*A. oryzea*	01	00	01	2.9	00	2.9
4	*A. parasiticus*	02	01	03	41.4	1.1	8.8
5	*Cladosporium herbarum*	28	16	05	2.0	18.6	14.7
6	*Penicillium adametzi*	05	03	01	7.3	3.4	5.8
7	*P. aurantiogrisum*	01	30	00	1.4	00	00
8	*P. brevicompactum*	05	00	01	7.3	34.8	5.8
9	*P. funiculosum*	01	05	00	1.4	1.1	2.9
10	*P .glandicola*	01	02	01	7.3	00	2.9
11	*P. implicatum*	03	02	01	1.4	9.3	2.9
12	*P. isandicum*	05	03	01	1.4	5.8	2.9
13	*P. rugulosm*	02	02	01	4.4	2.3	2.9
14	*P. simplicissimum*	02	04	01	7.3	2.3	2.9
15	*Fusarium oxysporum*	01	06	01	2.9	3.4	2.9
16	*F. solani*	03	01	03	2.9	2.3	2.9
17	*Trichoderma harzianum*	01	01	07	1.4	4.6	2.9
18	*T. viride*	05	00	01	7.3	00	8.8
19	*Helminthosporium sp.*	00	00	02	00	6.9	20.1
Average number of total colonies		44	34	34			

4. CONCLUSION

In conclusion, the physicochemical parameters are important to plant growth and status of rhizosphere microbiota, therefore the study concluded that the soil quality can be carried out by different parameters. The saprophytic fungi represent the largest proportion of fungal species in soil and they perform a crucial role in the decomposition. In the present study soil samples of 4 pulses crop fields viz Green gram, Black gram, pigeon pea and cowpea were studied for screening and detection of fungal diversity.Among isolated fungi, *Aspergillus nige, A. flavus, A. oryzea, Cl adosporium herbarum, Penicillium adametzi, P. aurantiogriseum, P. brevicompactum, P. funicul osum, P. glandicola, P. implicatum P. islandicum , rugulosum, P. simplicissimum, Fusarium oxysporum* and *Trichoderma harizanum* were found dominantand out of which *Penicillium* species was observed frequently. Our finding determines the differences in fungal species composition of

with pulses. The frequencies of mycoflora in agricultural fields were found to be regulated by many abiotic factors.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

The authors of this paper do not have any financial relation with any company, agricultural sectors that might lead to a conflict of interest for any of the authors

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