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Studies on Quantitative Analysis of Rhizosphere and Non-Rhizosphere Mycoflora at Different Stages of Plant Growth in Different Varieties of Pigeon Pea [*Cajanus cajan* (L.) Millsp.]

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ABSTRACT

The rhizosphere and non-rhizosphere mycoflora of ten different varieties of pigeon pea [Cajanus cajan (L.) Millsp.] were studied in relation to different stages of plant growth (non-flowering, flowering and fruiting stages). From the results obtained it was found that always the fungal population was higher in rhizosphere than the non-rhizosphere in all the varieties studied. Species of Aspergillus, Fusarium and Penicillium were very commonly isolated from the rhizosphere. The rhizosphere mycoflora was very high at flowering stage of plant growth i.e. the microbial population was increased with age of plant up to flowering stage then it was decreased. In the present study the quantitative analysis of rhizosphere soil mycoflora of ten varieties of pigeon pea, at different stages of plant growth was also studied. The number of fungi per gram of dry soil, R:S ratio and the number of fungal species was also higher at flowering stage of plant growth. The variety BDN-708 exerted maximum rhizosphere effect at all the different stages while ICPL-2376 exerted minimum rhizosphere effect.

Key words: *Rhizosphere, Mycoflora, Pigeon pea varieties.*

INTRODUCTION

Pigeon pea (*Cajanus cajan* (L.) Millsp.) is an important legume crop of rain fed agriculture in the semiarid tropics. It is second most important food legume of India. The Indian subcontinent, eastern Africa and Central America are the world's three main pigeon pea producing areas. Pigeon pea crop is cultivated in more than twenty five tropical and subtropical countries, either as a sole crop or mixed crop with cereals, such as sorghum, pearl millet or maize or with other legumes, such as peanuts, soybean, black gram and cotton. Being a legume capable of symbiosis with *Rhizobia*, the pigeon pea enriches soil through symbiotic nitrogen fixation.Rhizosphere is a metabolically active region with conspicuous variations in its surrounding mycoflora depending upon the root exudates, genus, species, variety, age and phase of growth, soil, environmental conditions and foliar sprays etc.

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It is well known that the rhizosphere, apart from different form the rest of the soil in its mycoflora, exhibits a pattern of fluctuation depending upon factors such as plant age and environment. Earlier workers have visualized the rhizosphere effect as something closely connected with the active growth of the plant till the peak period of vegetative growth i.e. the flowering stage and then decreased[1,2,3,4,5]. Some workers have been reported that decrease in the fungal population of the rhizosphere with plant age [6,7]. The rhizosphere effect may primarily due to the influx of mineral nutrients to the plant roots through mass flow diffusion and accumulation of chemicals and organic compound secreted in to the soil by the roots[8]. The exact composition of the root exudates determine by many factors including species and nutritional status of the plant, soil structure and micronutrient status[9]. The main aim of the present work is to study quantitative analysis of rhizosphere mycoflora in relation to different stages of plant growth in different varieties of pigeon pea

MATERIAL AND METHODS

Collection of soil samples: Rhizosphere soil samples were collected from different varieties of pigeon pea such as PUSA-992, BDN-2, BDN-708, BSMR-853, BSMR-736, BSMR-175, ICP- 8863, ICPL-87119, ICPL-2376 and AKT-9913 by shaking up-rooted plants (at non-flowering, flowering and fruiting stages of plant growth) in sterile paper bags from Pulses research center, Badnapur, Dist., Jalna (M.S). Non-rhizosphere soil was sampled from trenches away from root zone effect and nearly at the same depth travelled by pigeon pea plants and brought to the laboratory. Soil samples were shade dried.

Chemical analysis of soil samples: At the same time of isolation simultaneously soil analysis experiments was carried out. In this pH of the soil, water holding capacity was calculated by the methods described by Subramanyam[10]. Organic carbon content, organic matter[11]. Total nitrogen by micro Kjeldhal distillation method[12]. Potassium by flame photometry method, phosphorus was also analyzed[13].

Isolation of rhizosphere and non-rhizosphere mycoflora: Isolation of rhizosphere and non-rhizosphere mycoflora at different stages (at non-flowering, flowering and fruiting stages of plant growth) of plant growth was done on peptone dextrose agar medium containing Rose Bengal (1:30,000) and streptomycin[14] by dilution plate technique[15]. After inoculation plates were incubated at room temperature for 7 days, on incubation developing colonies were identified[16,17,18] and some unidentified cultures were sent to Agarkar Research Institute, Pune (M.H.). Number of colonies of each species as well as total number of colonies in each plate was recorded. Number of fungi per gram of moisture free soil in rhizosphere and non-rhizosphere were also recorded[19].

RESULTS AND DISCUSSION

It is well known that in the rhizosphere, the microorganisms are more abundant than in the soil free from the influence of roots that is non-rhizosphere[20]. The present studies also supported the above view as the numbers of fungi in the rhizosphere were found to be greater than in the soil away from it. In this present investigation total 32 fungal species were isolated from rhizosphere and non-rhizosphere soil of pigeon pea varieties at different stages of plant growth (Table 3). Species of *Aspergillus*, *Fusarium* and *Penicillium* were always dominant[21,22] were studied fungal and actinomycete flora of the rhizosphere of citrus plants and observed that 4 to 8 times more fungi in the rhizosphere than in soil. Relatively they were more numerous in the rhizosphere of non-growing roots than in the growing roots. In general, *Aspergilus* and *Penicillium* species were more numerous in the rhizospheres of both growing and non-growing roots than in soil.It has been observed from the quantitative analysis of rhizosphere soil of ten varieties of pigeon pea at different stages of plant growth that, the number of fungi/gram of dry soil, R:S ratio and number of fungal species were high at flowering stage. The positive rhizosphere effect was noted in all the varieties at various stages (Table 4).

Regarding the effect of different stages of plant growth on rhizosphere mycoflora of ten varieties of pigeon pea, it has been observed that, the fungal population increases from non-flowering to flowering stage. At fruiting stage, a decline in fungi/gram of dry soil, R:S ratio and number of species was observed in all the varieties with slight variation.It is evident from Table 3 that, variety BDN-708 exerted maximum rhizosphere effect at all the three stages and the ICPL-2376 showed minimum. Quantitatively there was marked difference in rhizosphere mycoflora of ten varieties of pigeon pea at different stages of plant growth, number of fungi/gram of dry soil, R:S ratio and number of species were also higher than the non-flowering and fruiting stages. Stimulation of fungal population in the rhizosphere of all the verities was observed. Soil fungi play an important role in biogeochemical cycles, decomposition of organic matter, growth of the plant and disease development and control[23]. There is a close relationship between rhizosphere fungi are plant health and growth, due to their roles in antagonizing pathogens, decomposition of plant debris, and supplying of nutrients[24]. Variation in the fungal population of the rhizosphere is plant-dependent because roots release different type of organic compounds that make a unique rhizosphere nutrient pool, which is available to soil microorganisms[25,26,27]. Physical and chemical properties of soil are also known to be significantly correlated with changes in the rhizosphere fungal population[28]. Texture of soil also affects the organic carbon content and indirectly it shows the effect on rhizosphere microbial population[29,30]. The rhizosphere effect of ten varieties of pigeon pea was increased from non- flowering to flowering stage. Minimum number was observed at non-flowering (Vegetative) stage and maximum at flowering stage [1,2,3,4,5,31,32,33,34]. It seems that increase in rhizosphere mycoflora at flowering stage than the non-flowering (vegetative) & fruiting stages was probably stimulated by various factors like increased root exudation, decomposition of moribund root hairs, epidermal cells and cortex, accumulation of cell materials[35,36,37].

Table 1: Phenotypic characters of pigeon pea varieties

Name of Variety	Year of release	Plant Height (cm)	Flower colour	Grain colour	Growth habit	Days to maturity	100 seeds wt. (g)	Protein (%)	Avr. yield (kg/ha)	Special features
PUSA -992	2003	140-145	Yellow	Red	Interme -diate	130-160	9-10	19.0	1300-1400	Moderately resistant to wilt
BDN -2	1976	140-145	Yellow	White	Interme -diate	160-165	9-10	20.5	1000-1200	Sun red stem colour, maroon pod colour
BDN -708	2004	140-145	Yellow	Red with shining	Interme -diate	160-165	11-12	20.5	1300-1400	Maroon colour pods, suitable for low rainfall area (550-560 cm), tolerant to wilt & SM
BSMR -853	2001	155-160	Red	White	Interme -diate	175-180	10-11	21.5	1400-1450	Dorsal side of standard is red & ventral side yellow. Resist -ant to wilt & sterility mosaic.
BSMR -736	1994	155-160	Yellow	Red	Interme -diate	175-180	10-11	19.0	1350-1450	Stem colour green, pods at maturity are green turning to maroon colour towards maturity. Resistant to wilt & sterility mosaic.
BSMR -175	1991	135-140	Yellow	White	Interme -diate	165-170	10-11	19.0	1100-1200	Resistant to wilt and sterility mosaic.
ICP -8863	1993	150-180	Yellow	Orange to brown	Interme -diate	150-180	9-10	19.0	1400-1450	Resistant to wilt, susceptible to sterility mosaic. Green stem, pods are four seeded.
ICPL -87119	1993	140-227	Yellow	Brown	Interme -diate	160-202	10-11	21.2	1510-1540	Resistant to wilt and sterility mosaic, large seeded.
ICP -2376	-	140-145	Yellow	White	Interme -diate	160-165	9-10	19.0	-	Resistant to sterility mosaic, suscepti -ble to wilt (100%).
AKT -9913	-	140-150	Yellow	White	Interme -diate	160-165	10-11	19.0	1100-1200	Moderately resistant to wilt

(Source: Krishi Dainandini (2011) Marathwada Agricultural University, Parbhani & Panjabrao Krishi Vidyapeeth, Akola)

Table 2: Chemical analysis of rhizosphere and non-rhizosphere soil from different varieties of pigeon pea

Name of Var.	Diff. stages	pH	Water holding Capacity (%)	Organic Carbon Cont. (%)	Organic matter (%)	Total Nitrogen (%)	Phos. in mg/100g of soil	Pott. (%)
PUSA-992	NFS	7.3	52.66	0.24	0.413	0.11	12	0.02
	FLS	7.0	54.33	0.42	0.724	0.15	16	0.05
	FRS	7.4	52.33	0.36	0.620	0.13	14	0.04
BDN -2	NFS	7.4	52.00	0.50	0.862	0.10	13	0.04
	FLS	7.0	52.66	0.58	0.999	0.15	14	0.06
	FRS	7.3	52.33	0.52	0.896	0.11	12	0.02
BDN -708	NFS	7.4	52.00	0.42	0.724	0.13	15	0.03
	FLS	6.9	52.66	0.52	0.896	0.15	17	0.05
	FRS	7.1	52.33	0.48	0.827	0.11	14	0.04
BSMR-853	NFS	7.0	52.66	0.54	0.930	0.12	12	0.02
	FLS	6.8	54.33	0.54	0.930	0.16	14	0.03
	FRS	7.0	52.00	0.50	0.862	0.13	13	0.02
BSMR -736	NFS	7.4	53.00	0.42	0.724	0.11	14	0.04
	FLS	6.7	55.66	0.54	0.930	0.14	14	0.06
	FRS	7.0	54.00	0.50	0.862	0.12	12	0.03
BSMR-175	NFS	7.2	54.00	0.52	0.896	0.14	14	0.04
	FLS	6.6	53.66	0.56	0.965	0.17	15	0.06
	FRS	6.9	53.00	0.50	0.862	0.15	12	0.02
ICPL -87119	NFS	6.6	52.66	0.26	0.448	0.11	15	0.02
	FLS	6.4	54.33	0.36	0.620	0.13	16	0.05
	FRS	6.8	52.00	0.32	0.551	0.11	12	0.04
ICP -8863	NFS	6.9	53.00	0.28	0.428	0.13	13	0.02
	FLS	6.6	53.66	0.46	0.793	0.16	16	0.03
	FRS	7.0	52.00	0.36	0.620	0.12	14	0.02
ICP -2376	NFS	7.6	53.00	0.50	0.862	0.10	13	0.04
	FLS	7.0	52.00	0.60	1.034	0.14	16	0.05
	FRS	7.7	53.66	0.54	0.930	0.11	14	0.02
AKT -9913	NFS	7.4	52.00	0.56	0.965	0.13	14	0.03
	FLS	6.7	53.00	0.68	1.172	0.16	15	0.04
	FRS	7.2	52.66	0.56	0.965	0.14	15	0.02
Non Rhiz. soil	NFS	7.2	50.00	0.30	0.517	0.10	12	0.02
	FLS	6.6	51.33	0.36	0.620	0.11	13	0.03
	FRS	7.0	51.00	0.34	0.586	0.11	11	0.02

Note: NFS= at non-flowering stage, FLS= at flowering stage and FRS= at fruiting stage.

Table 3: Isolation of rhizosphere and non-rhizosphere fungi from different varieties of pigeon pea at different stages [Non-flowering (1), Flowering (2) and Fruiting (3)] of plant growth

Name of fungus	PUSA -992			BDN -2			BDN -708			BSMR -853			BSMR -736			BSMR -175			ICP -8863			ICPL -87119			ICP -2376			AKT -9913			Non-rhizo.		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Alternaria alternata	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
A. solani	+	+	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	+	-	+	-	+	-	+	-	-	-	-	+	-	-	+	-
Aspergillus candidus	-	+	-	-	-	-	-	+	-	-	+	-	-	+	-	+	-	+	-	+	-	-	+	-	-	-	-	-	-	+	-	-	-
A. flavus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
A. fumigatus	-	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-
A. niduans	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
A. niger	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
A. terrus	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
Aspergillus sp.1	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-
Aspergillus sp.2	-	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-
Chaetomium sp.	-	+	-	+	+	-	-	-	+	-	+	-	+	-	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-
Cladosporium herbarum	+	+	-	-	+	-	+	+	+	-	-	+	-	+	-	+	+	-	-	-	+	-	+	+	-	+	-	-	-	-	-	+	+
Curvularia lunata	-	+	-	-	-	+	-	+	-	+	-	+	-	-	-	-	+	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-
C. paliscence	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Drechslera tetramera	-	+	-	-	+	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+
Fusarium oxysporum	+	+	-	+	+	+	-	+	-	+	+	-	+	-	-	+	+	-	-	-	+	-	+	+	-	+	-	-	+	-	+	+	-
Fusarium sp.	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
Helminthosporium sp.	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Isaria felina	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Mucor sp.	-	-	-	+	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	+
Nigrospora sp.	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
Penicillium adametzi	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. citrinum	-	+	-	+	+	-	+	+	-	-	+	-	-	+	-	-	+	-	+	+	-	+	+	-	+	-	+	-	+	+	+	+	-
P. islandicum	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Pythium sp.	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-
Rhizoctonia solani	-	+	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	+	-

Rhizopus stolonifer	-	-	+	-	+	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	-	+	+	-	-
Talaromyces sp.	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Trichoderma viride	-	+	+	-	-	+	-	+	-	+	-	-	-	-	+	+	-	-	+	+	-	-	+	-	+	-	+	-	+	+	-	-	+
Trichoderma sp.	-	-	-	-	+	-		-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Talaromyces sp.	-	+	-	-	+	-	+	-	+	-	+	+	+	-	-	+	+	-	+	-	-	+	-	+	-	-	-	-	-	-	-	+	-
Brown sterile mycelium 1	-	-	-	+	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-
Total	6	23	7	11	20	9	11	14	10	7	15	11	7	12	8	10	16	9	8	15	7	10	16	10	7	11	8	8	13	10	8	11	7

Table 4: Quantitative analysis of rhizosphere fungi from different varieties of pigeon pea at different stages of plant growth

Sr. No.	Pigeon pea Varieties	Diff. Stages	No. of fungi/g of dry soil in Rh.	No. of fungi/ g of dry soil in NRh.	R:S Ratio	No. of sp. in	
						Rh.	NRh.
1	PUSA -992	NFS	43,500	18,166	2.39	06	06
		FLS	72,000	22,666	3.17	23	11
		FRS	32,833	19,533	1.68	07	07
2	BDN-2	NFS	39,500	18,166	2.17	11	06
		FLS	54,166	22,666	2.38	20	11
		FRS	29,833	19,533	1.52	09	07
3	BDN-708	NFS	61,166	18,166	3.36	11	06
		FLS	**80,000**	22,666	**3.52**	14	11
		FRS	41,500	19,533	2.12	10	07
4	BSMR-853	NFS	23,500	18,166	1.29	07	06
		FLS	38,500	22,666	1.69	15	11
		FRS	26,666	19,533	1.36	11	07
5	BSMR-736	NFS	23,000	18,166	1.26	07	06
		FLS	32,333	22,666	1.42	12	11
		FRS	29,000	19,533	1.48	08	07
6	BSMR-175	NFS	25,166	18,166	1.34	10	06
		FLS	50,500	22,666	2.22	16	11
		FRS	34,166	19,533	1.74	09	07
7	ICP-8863	NFS	22,500	18,166	1.23	08	06
		FLS	33,166	22,666	1.46	15	11
		FRS	24,166	19,533	1.23	07	07
8	ICPL-87119	NFS	37,000	18,166	2.03	10	06
		FLS	41,000	22,666	1.80	16	11
		FRS	22,666	19,533	1.16	10	07
9	ICPL-2376	NFS	23,833	18,166	1.31	06	06
		FLS	33,500	22,666	1.47	10	11
		FRS	**22,333**	19,533	**1.14**	08	07
10	AKT -9913	NFS	25,833	19,166	1.42	08	06
		FLS	35,500	23,666	1.56	13	11
		FRS	23,333	19,533	1.19	10	07

Note: NFS= Non-flowering stage, FLS=Flowering stage and FRS=Fruiting stage,
NR= Non-rhizosphere and R= Rhizosphere.

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