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Population of *Trichoderma harzianum* strains in rice rhizospheres at different interval after seed sowing

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Abstract
When *Trichoderma harzianum* strains were applied to rice seed as seed biopriming and such seed were sown in the pots containing soil and population dynamics of each respective strain were measured in the rice rhizosphere, maximum recovery of CFUs were noticed in case of *T. harzianum* strain IRRI-1 followed by IRRI-6, IRRI-2 and IRRI-4 at 21 day, 32 days and 45 days after sowing. The population of *T. harzianum* could be only noticed up to 45 days after sowing, because due to drought exposure to these rice plants, they could not survived beyond 45 days, hence observation beyond 45 days was not possible. During present studies also, *T. harzianum* population in the rhizosphere of rice was at its highest at 45 days after sowing of *T. harzianum* treated seed.

Keywords: Population, rhizosphere, rice, *Trichoderma harzianum,* seed

1. Introduction
Rice (*Oryza sativa* L.) is the most important cereal crop plant for human consumption, providing staple food for more than half of the world's population. Rice is a member of family Gramineae and subfamily Oryzoidae. Total global area under rice is about 165.00 million ha with total output of 496.6 million tones of paddy at an average yield of 3.01 tons ha^{-1} (FAO, 2014) [2]. Out of 2.7 billion rice consuming peoples, more than 95% live in Asia. About 90.0% of world's total rice is grown in Asian countries alone (FAO, 2014) [2]. India is the second largest producer and consumer of rice in the world. Rice is one of the most important food crops of India in terms of area, production and consumer preference. In India, rice provides food calories for more than two third populations. Indian share in global rice production has been 21.34 percent. In India, area under rice cultivation is 43.97 m. ha with total output of 106.00 m tones with an average productivity of 2410 kg/ha (Department of Agriculture and cooperation, Govt. of India, 2014) [1]. One possibility to increase plant water acquisition or drought tolerance is to use beneficial microorganisms as inoculants. Recently, the positive interactions developed under drought conditions between *Pseudomonas putida* and AM fungi in stimulating plant growth and drought tolerance have been reported (Marulanda *et al*., 2009) [6]. However, incorporation of fungi like- *Trichoderma* in many cereals has been resulted in improved drought response. *Trichoderma* are free-living, soil borne, green-spores containing ascomycetes, that are ubiquitous in nature. These fungi are highly interactive in the rhizosphere and foliar environments. *Trichoderma* spp. is present in nearly all agricultural soils and in other environments such as decaying wood. *Trichoderma* spp. is characterized by rapid growth, mostly bright green conidia and a repetitively branched conidiophores structure. *Trichoderma* is imperfect fungi with its perfect stage *Hypocrea* are fast growing in culture and produce numerous green spores and chlamydospores. *Trichoderma* spp. are an integral component of eco-friendly, safe and non-chemical disease management system which have greater importance in organic agriculture. *Trichoderma* spp. is widely used as biocontrol agent against phytopathogenic fungi, and as a biofertilizer because of its ability to establish mycorrhizae like association with plants. The key factor to the ecological success of this *Genus* is the combination of very active mycoparasitic mechanisms with effective defense strategies induced in plants. There are reports of enhanced plant growth as a result of the association of *Trichoderma* strains with plants but the effects, as with other plant-growth-promoting microbes, are greater when plants are under suboptimal conditions or under biotic, abiotic, or physiological stresses (Shoresh *et al.* 2010; Mastouri and Harman, 2009) [8, 7].

Keepin in view the present investigation is undertaken with "Strains of *T. harzianum* population in rice rhizospheres at different interval after seed sowing".

2. Materials and Methods

The experiment was carried out on determination of population dynamics of different *Trichoderma harzianum* strains in rice rhizospheres, using cultivar IR-64 was sown in sandy loam soil on 10th July in both respective years and bio-control agent *Trichoderma harzianum* strains, with six replications and ten treatments in Completely Randomized Block Design (CRD) along with nine strains of *Trichoderma harzianum* during 2012-2014, under *in vitro* in the laboratory of Department of Plant Pathology and under the rain out shelter of the Department of Agriculture Biotechnology of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P.), India. Ten treatments were *viz*; IR-64 treated with IRRI-1 @ 10 g/kg (T_1), IR-64 treated with IRRI-2 @ 10 g/kg (T_2), IR-64 treated with IRRI-3 @ 10 g/kg (T_3), IR-64 treated with IRRI-4 @ 10 g/kg (T_4), IR-64 treated with IRRI-5 @ 10 g/kg (T_5), IR-64 treated with IRRI-6 @ 10 g/kg (T_6), IR-64 treated with TH-3 @ 10 g/kg (T_7), IR-64 treated with SVP-1 @ 10 g/kg (T_8), IR-64 treated with SVP-3 @ 10 g/kg (T_9) and T_{10} serve as untreated.

2.1 Determination of population dynamics: After each 7 days interval, *Trichoderma* (population) was determined by counting of colony forming units (CFUs).

2.2 Serial dilution: Monitoring of population dynamics as colony forming units (CFUs) of *T. harzianum* was done by serial dilution method. One gram of sample (substrate where *Trichoderma* was being grown) was suspended in 10 ml distilled water to make microbial suspension diluted 10 times, it was a 1: 10 conc. or 10^{-1} dilution of original sample, i.e. the original sample was diluted to 1/10th. Again, 1ml of suspension from first tube get into second tube contained 9 ml of sterile water; this suspension was used to make microbial concentration as 1:100 (10^{-2}). Similarly it was prepared as 1: 1000 (10^{-3}), 1: 10,000 (10^{-4}), 1: 100,000 (10^{-5}) up to 10^{-6} on dilution of the original sample. Finally 1ml of microbial suspension from last serial dilution i.e., 10^{-6} was added to sterile Petri dishes (triplicate in completely randomized manner) containing 30 ml of sterilized PDA medium, which was added with pinch of Streptomycin (to check bacterial contamination) when warm and molten. The Petri dishes were then incubated for 5 days at 28±2 ^{0}C.

2.3 CFUs counting: The density of cells, spores/conidia of *T. harzianum* (CFUs) was measured in laboratory by Plate Dilution technique (Johnson and Curl, 1972) [5]**.**

$$\frac{\text{Colony count (CFUs) on an agar plate}}{\text{Total dilution of tube (used to make plate for colony count)}} \text{ X Volume plated}$$

2.4 Population dynamics of *T. harzianum* strains: After 21 days of seed sowing, one gram soil sample from each replicate of every treatment was taken from rhizosphere with sterilized disk cutter into test tube. It was put into another test tube containing 10 ml sterilized distilled water and shaken well, and diluted up to 10^{-6}. 1.0 ml of the suspension was put into sterilized Petri plate poured with TSM and spread throughout surface with gentle shaking. All the procedure of serial dilution was done under aseptic condition in Laminar Air Flow. The Petri dishes were then incubated for 5 days at 28±2 ^{0}C and within this period colonies were formed which were counted. Population dynamics of *T. harzianum* were continuously determined on 21days, 32days and 45 days after rice seed sowing.

3. Results and Discussion

During rice crop season 2012, among all the strains of *T. harzianum* at every DAS from 21, 32 and 45 DASs it was found that highest CFUs were exhibited at 45 DAS that was significantly superior to the values at 32 and 21 DAS in IRRI-1, IRRI-6 and IRRI-2. The rest strains of *T. harzianum* resulted in low level of CFUs at 21 DAS and 32 DAS but there were higher CFUs found at 45 DAS while, strains of *T. harzianum* at every DAS starting from 21, 32 and 45 DASs were compared; it was found that highest CFUs were exhibited at 45 DAS that was significantly superior to the values at 32 and 21 DAS in IRRI-1, IRRI-6 and IRRI-2. The rest strains of *T. harzianum* resulted in low level of CFUs at 21 DAS and 32 DAS but they also resulted in higher CFUs at 45 DAS which were significantly lower than the IRRI-1, IRRI-6 and IRRI-2 in rice crop season 2013 [3].

Data given in Table 1 and 2 revealed that the maximum recovery of Colony Forming Units (CFUs) was noticed in case of *T. harzianum* strain IRRI-1 followed by IRRI-6, IRRI-2 and IRRI-4 at 45 days after sowing. Rest of the other strains also resulted in quite considerable level of population recovery but lower than earlier mentioned four strains. These findings suggested that when the rice seed treated with these strain of *T. harzianum* could have been sown in the soil, the CFUs of *T. harzianum* strain present in the seed coat must have been multiplied and colonized the rhizosphere after the root formation of seedling has been taken place. With increasing period after sowing, the activities of *T. harzianum* could have been further increased and with increasing rhizosphere area, the populations of *T. harzianum* also would have been increased, which is evident from the results where increasing in population have been noticed from 21 days, 32 days and up to 45 days. Since beyond 45 days population dynamics was not measured in the rhizospheric soil, what was the level of population dynamics beyond 45 days is not known.

However, during present investigation, population of *T. harzianum* could be only noticed up to 45 days after sowing, because due to drought exposure to these rice plants, they could not survived beyond 45 days, hence observation beyond 45 days was not possible. During present studies also, *T. harzianum* population in the rhizosphere of rice was at its highest at 45 days after sowing of *T. harzianum* treated seed. This result is supported by the findings of Tomer *et al*., (2016) [9] studied the suitability of de-oiled cakes of neem, jatropha, mahua and karanja along with cereals and millets substrates for mass multiplication of *Trichoderma harzianum*. Our results are also in accordance to those of Zhen and Zhao (2013) [10], Gangwar *et al*., (2013) [3] and, Jash and Pan, (2007) [4].

Table 1: Population of *T. harzianum* strains in rice rhizospheres at different interval after seed sowing in the rice crop season 2012

Treatments	Strains of *T. harzianum*	Colony forming units (CFUs x 10^6)			
		21 days	32 days	45 days	Average
T_1	*T. harzianum* (IRRI-1)	32.67	41.00	50.33	41.33
T_2	*T. harzianum* (IRRI-2)	10.67	12.33	38.00	20.33
T_3	*T. harzianum* (IRRI-3)	5.33	12.00	24.33	13.89
T_4	*T. harzianum* (IRRI-4)	9.67	14.00	27.33	17.00
T_5	*T. harzianum* (IRRI-5)	7.67	11.00	27.33	15.33
T_6	*T. harzianum* (IRRI-6)	29.00	32.00	46.67	35.89
T_7	*T. harzianum* (TH-3)	4.00	10.33	28.33	14.22
T_8	*T. harzianum* (SVP-1)	1.00	6.33	22.33	9.89
T_9	*T. harzianum* (SVP-3)	7.67	12.00	32.33	17.33
T_{10}	Control	0.00	00	00	00

Factors	Factor A (Interval)	Factor B (Strains)	Factors A X B
CD @5% level	0.663	1.21	2.09
SE(m)	0.234	0.428	0.741

Table 2: Population of *T. harzianum* strains in rice rhizospheres at different interval after seed sowing in the rice crop season 2013

Treatments	Strains of T. harzianum	Colony forming units (CFUs x 10^6)			
		21 days	32 days	45 days	Average
T_1	*T. harzianum* (IRRI-1)	20.67	25.00	28.33	24.67
T_2	*T. harzianum* (IRRI-2)	12.67	13.33	21.00	15.67
T_3	*T. harzianum* (IRRI-3)	6.33	15.00	18.33	13.22
T_4	*T. harzianum* (IRRI-4)	8.67	12.67	17.33	12.89
T_5	*T. harzianum* (IRRI-5)	5.67	7.00	18.33	10.33
T_6	*T. harzianum* (IRRI-6)	19.00	23.67	24.67	22.45
T_7	*T. har*zianum (TH-3)	4.00	12.00	16.33	10.78
T_8	*T. harzianum* (SVP-1)	4.00	6.33	16.33	8.89
T_9	*T. harzianum* (SVP-3)	4.00	6.00	20.33	10.11
T_{10}	Control	0.00	00	00	0.00

Factors	Factor A (Interval)	Factor B (Strains)	Factors A X B
CD @5% level	0.746	1.36	2.361
SE(m)	0.264	0.487	0.834

4. Conclusion

It can be concluded that the population of *T. harzianum* could be only noticed up to 45 days after sowing, because due to drought exposure to these rice plants, they could not survived beyond 45 days, hence observation beyond 45 days was not possible. During present studies also, *T. harzianum* population in the rhizosphere of rice was at its highest at 45 days after sowing of *T. harzianum* treated seed.

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