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STUDIES THE EFFECT OF *TRICHODERMA HARZIANUM* FOR BIOCONTROL OF CUCUMBER (*CUCUMIS SATIVUS L.*) AND SUPPRESSION OF *RHIZOCTONIA SOLANI*

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ABSTRACT

Incidence and severity of the disease and resultant damage vary considerably among planting from one growing season to another. The genus *Trichoderma* are alternatives to chemical pesticides in agriculture as a biocontrol agent. The present study was undertaken to investigate the biocontrol potential of *Trichoderma harzianum* against the phytopathogenic fungus *Rhizoctonia solani*. Inoculum was applied as wheat bran preparation. Control of disease by *Trichoderma* were measured after 7,14,21 and 28 days. 60-40% reduction in disease were recorded after 14 and 28 days. Decrease in *Rhizoctonia solani* was observed when the *Trichoderma harzianum* rate was increased from 10^6 to 10^8 log cfu g^{-1}soil.

***Keywords*:** *Trichoderma harzianum, Rhizoctonia solani*, Cucumber, Biocontrol

Introduction

Disease management strategies, utilizing biological control agents are suitable alternative to replace the excessive use of pesticide as well as fungicide. Biocontrol is a promising tool to maintain current level of agriculture production while reducing the release of polluting chemical pesticide to the environment(Marian and Shimizu, 2019). Useful biocontrol system comes in part and as a result a group of beneficial microorganism have been evolved (Han Jigang *et al.*, 2005; Julien and Manker 2005; Perez-Piqueres *et al.,* 2006; Roberts *et al.,* 2005; Bhattacharjee and Dey, 2014; Raza *et al.,* 2017).

Among all the biocontrol agent *Trichoderma* is one of the most commonly used biocontrol agent (Ommati *et al.*, 2012; Kamala and Devi 2012; Lopez *et al.*, 2019). *Trichoderma harzianum* is a common inhabitant of rhizosphere and contribute to control many soil borne plant disease caused by fungi. *Trichoderma harzianum* interacts with rhizosphere microflora in favour of plant health (Harman *et al.*, 1989). The increased growth response was explained mainly by influence of *Trichoderma* on control of many soil borne pathogens, rapid colonization of roots (Singh *et al.*, 2018) and triggering of induced systemic resistance (Meera *et al.*, 1995; Shivanna *et al*, 1996; Yedidia *et al*, 1999). Solubilization, increased uptake and translocation of physiologically less available mineral (Moharam *et al.*, 2012). Production of growth hormones and vitamin are also suggested as part of the mechanism of growth promotion (Baker, 1989).

Rhizoctonia solani is one of the most important soil borne fungal pathogen. *Rhizoctonia solani* is ubiquitous soil borne pathogen that inflict damage on a wide range of economically important crop. The pathogen variously caused pre and post emergence damping off root, crown rots and foliar and flower blight (Jambulkar *et al.*, 2016).

Materials and Methods

The biocontrol ability of *Trichoderma harzianum* isolates was evaluated in green house experiment. *Rhizoctonia solani* was cultured on sand maize meal (9; 1w/w) at 28 ±2℃. After 7 days of incubation, the inoculum was mixed with the soil at rates 0.1%,0.5%and 1% to have the final inoculum densities approximately 10^3,$5x10^3$ and 10^4Cfu g^{-1}soils. Conidial suspension of *Trichoderma harzianum* harvested from exponential phase SM culture, were mixed at different inoculum levels (ca 10^6-10^8 cells g^{-1} soil) in pathogen infested soil. The inoculated soil mixture were transferred into pots (400g pot^{-1}). Surface sterilized cucumber seeds (6 pot^{-1}) were sown and the pots were maintained in a greenhouse under complete randomized block pattern. Growing plant were samples for disease incidence and root colonization by *Trichoderma harzianum* at regular interval of 7 days upto 28 days after emergence. Disease was grading using the following scale.

1. Healthy plant, no symptom
2. Browning of collar region
3. Dark brown to black spot on collar as well as primary roots
4. Weak and stunted plants with rooting of roots
5. Plant dead

Lesion on entire root system and the disease severity index were calculated as describe by Bull *et al.* (1991). To determine the root colonizing population of *Trichoderma harzianum* the roots were cut into segment, 2 gm. of root segments (1cm long) were macerated and vortexes for 2-3 min in 10 ml of sterilized PBS. Aliquotes (0-2 ml) of diluted suspensions ($10^{-4}$and 10^{-5}) were plated on SM. After 72-96 hours colonies of *Trichoderma harzianum* were counted and expressed as cfug^{-1}root. The frequencies of *Trichoderma harzianum* isolates and *Rhizoctonia solani* in the soil at different inoculum density of pathogen were recorded by dilution plating of the soil sample collected at the termination (28th days)of the experiment.

Results

Biocontrol efficacy of *Trichoderma harzianum* was evaluated in the greenhouse in sand infested with *Rhizoctonia solani* and inoculated with *Trichoderma harzianum* (10^6-10^8cfug^{-1}soil). No disease was noticed in 7 days old plants whereas 60-40% reduction in disease was recorded after 14 and 28 days, for example after 28 days 45% disease was recorded in the plant inoculated with *Trichoderma harzianum* (10^7cfu g^{-1}) as compared to control (69%). In general the severity of disease was increased from 0.1 to 1 %. Marked decrease in disease was observed when the *Trichoderma harzianum* amendment rate was increased from 10^6-10^8 log cfu g^{-1}soil. A significant negative correlation (P = 0.05, R ^{2}= 0.97) was observed in disease and population of *Trichoderma harzianum*.

Table 1 : Effect of *Trichoderma harzianum* on root rot disease at various concentration in Green house

Days	*Rhizoctonia solani* concentration (%w/w)	Control		*Trichoderma harzianum* (Disease)		*Trichoderma harzianum* (Disease)		*Trichoderma harzianum* (Disease)	
				10^6		10^7		10^8	
		A	B	A	B	A	B	A	B
7	0.1	–	–	–	–	–	–	–	–
	0.5	–	–	–	–	–	–	–	–
	1.0	5	1	4	1	4	1	–	–
14	0.1	27	1.9	22	1.1	18	1	12	1
	0.5	29	2	25	1.1	21	1.1	14	1.1
	1.0	33	2.3	26	1.3	22	1.1	15	1.1
21	0.1	45	2.8	35	1.5	26	1.2	24	1.2
	0.5	52	3.1	40	2.0	33	1.5	28	1.3
	1.0	58	3.6	41	2.1	35	1.6	32	1.4
28	0.1	56	3.2	38	1.9	37	1.5	33	1.4
	0.5	65	3.7	48	2.4	46	2.2	39	2.2
	1.0	69	4.1	47	2.5	45	2.3	43	2.3

Note: x = root rot disease severity was recordedfor A= disease % and B = disease severity index on scale 1-5.The plant was considered diseased with first localized symptom on collar region = no disease. No disease was observed in 7 days old plants. Data are means of 20 replicates.

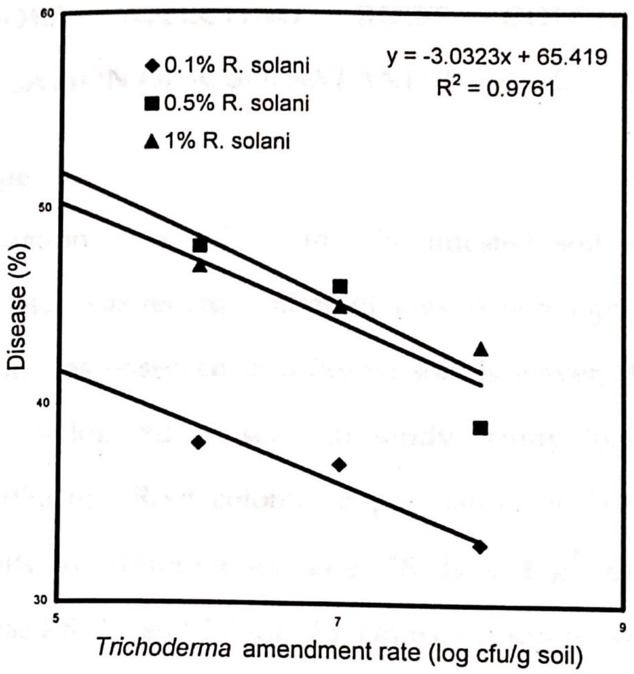

Fig. 1 : Relationship between amendment rate of *Trichoderma harzianum* and root rot disease in cucumber. Data are mean of 20 replicate.

The reduction in disease was more pronounced at higher inoculum concentration of *Trichoderma harzianum* (10^6-10^8cfu g^{-1}). However the disease severity was greater when the pathogen was mixed at a rate of 1% of the soil as compared to 0.5 or 0.1in all the treatments. Though a significant reduction in disease severity was observed in all the treatments with increase in time gradual increase in disease severity was noticed in treated plants, for example the disease indexes in *Trichoderma harzianum* (10^6cfu g^{-1}) inoculated cucumber was 1.3,2.1,2.5 after 14, 21 and 28 days. Corresponding to the 26,41 and 47 % disease in the pots infested with *Rhizoctonia solani* at 1% inoculum density.

Discussion

Increased growth response in cucumber has been demonstrated by application of *Trichoderma harzianum* under greenhouse condition (Kleifeld and Chet 1992; Ousley *et al*, 1994; Inbar *et al.*, 1994; Yedidia *et al.*, 2001; Paudel *et al.*, 2017; Raza *et al.*, 2017, Ban *et al.*, 2018; Lopez *et al.*, 2019). The result indicate that the significant increase in growth of cucumber in all parameter. *Trichoderma harzianum* effect plant growth as a result of their ability to influence plant hormones and vitamins (Baker, 1989; Kleifeld and Chet, 1992). Such substances could influence the early stage of plant growth with better development of plant roots (Hermosa *et al.*, 2013). The enhancement in root total area and growth rate enable the plant to explore a greater volume of soil due to an increase in number of active site of uptake per unit area. Thus they might be able to sequenster more phosphate and other mineral ions liberated as a result of solubilization by microorganism. Although management of *Rhizoctonia solani* is difficult because of its wide host range and its ability to survive under adverse environmental condition through scleotia (Ajayi *et al.*, 2018; Jambulkar *et al.,* 2016).

Trichoderma harzianum colonize plant root they invade the superficial layer of the root but do not penetrate further, at least in part because they elicite plant defense reaction. *Trichoderma harzianum* probably have an intrinsic ability to attack *Rhizoctonia solani* (Chao and Wan-Ying 2019)). The plant defense reaction can become systemic and protect the entire plant from range of *Rhizoctonia solani* and disease even when *Trichoderma* species grow only on the roots (Yedidia *et al.*, 2000). This root colonization also increase the growth of roots and of entire plant thereby increasing plant productivity and the yield of reproductive organ. They also help plant to overcome abiotic stress and improve nutrient uptake. Production of hydrolytic enzyme has frequently been emphasized as one of the major factor (Cherkupally *et al.*, 2017). Seedling disease symptom on cucumber range from root rot, pre emergence damping off under high inoculum density to root. So *Trichoderma* could prove better option to minimize the incidence of root rot and other disease (Makal *et al.*, 2020). So the genus *Trichoderma* is highly effective against *Rhizoctonia solani* causing seed and soil borne disease (Dubey *et al.*, 2011; Huang *et al.*, 2012; Jambhulkar *et al.*, 2015). These finding indicate that *Trichoderma harzianum* have developed a symbiotic rather than parasitic relationship with plants.

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