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Study of Bio-efficacy of Alkali tolerant *Trichoderma* against damping off and rotting diseases of Tomato and Cauliflower caused by *Pythium* spp. and *Sclerotina* spp.

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Abstract: The main aim of the study is to analyze the bioefficacy of alkali tolerant *Trichoderma viride* IPL/VP/T2 under field conditions for the management of damping off and rotting disease on tomato crop and cauliflower crop caused by *Pythium spp* and *Sclerotina spp* respectively.Alkali tolerant *Trichoderma viride* isolated from alkaline agricultural soil and were tested *in-vitro* and *in-vivo* for disease control. The pathogen was isolated from diseased tomato and cabbage plant by direct standard plating method. The assessment of Alkali tolerant *Trichoderma viride* against *Pythium* and *Sclerotina* was conducted by dual culture techniques and in field condition.Under field conditions results showed significant control of pre-emergence and post-emergence damping off disease on tomato and cauliflower to the minimum when compared to control under alkaline soil condition.The study showed the effective biological control of *Pythium* and *Scelrotina* in the presence of Alkali tolerant *Trichoderma in* Tomato and cauliflower respectively.
Keywords: Alkali tolerant *Trichoderma*, Biocontrol, Damping off, *Pythium* spp, Rotting, *Sclerotina.*

Introduction:

Biological control is a disease control method with low environmental impact and less contamination risk for humans, domestic animals and the environment.For this to occur biological control systems that are effective, reliable and economical must be developed(1).Biological control of a plant disease has always been thought to be the best remedial measure for disease management. Biological control offers an environmentally friendly approach to the management of plant disease and can be incorporated into cultural and physical controls and limited chemical usage for an effective integrated pest management (IPM) system (2).It is an eco-friendly strategy of disease control and if the organism is self-perpetuating its repetitive applications are not required, thus reducing the cost of raising plantations considerably. There is an extensive data in the biological control literature which refers to genus *Trichoderma.* The activity of this useful species has been recognized from 1930 and today there are modern technologies for including them in biological control of various diseases (3).

Trichoderma strains are of great importance as biocontrol agents, and should have better stress tolerance levels than the plant pathogens against which they can be applied for biological control under field condition(4).The abiotic factors deteriorate the antagonistic properties of *Trichoderma*, against the phytopathogenic fungi(5).Damping-off is a common problem in almost all field and greenhouse crops. A number of soil-borne pathogenic fungi have been associated with pre- or post-emergence damping off like *Pythium, Phytophthora, Fusarium, Aphanomyces and Rhizoctonia solani,* etc. (6) Controlling damping-off with antagonistic micro-organisms can be relatively easy, compared with other soil-borne diseases. Several species of micro-organisms have been tested as potential bio control agents of damping-off, including bacteria, *Pseudomonas spp. and Bacillus spp., and fungi, Gliocladium spp., P. oligandrum* and others. Very few, however, have been developed into commercial products. Currently, most available commercial products for damping-off biocontrol are based on fungal antagonists(7).Antagonistic bacteria are common soil inhabitants with potential to be developed into biofungicides for the management of seedling damping-off, root rot, and other soil-borne diseases of various crops(8).Soil borne pathogen were also isolated from cabbage and were identified as *Scelrotina spp.* In cauliflower it is responsible for causing stalk rot disease as a result of which seed production is greatly affected. The disease was first observed in 1973 in a few isolated fields in Saproon valley of Himachal Pradesh but has since spread rapidly (17).In the present study, Alkali tolerant *Trichoderma* spp. were isolated from different alkaline agricultural soils and were screened for maximum biocontrol activity. *Trichoderma* isolates were studied for *in vitro* biocontrol assay and experiments were conducted on field to study the bio efficacy of the isolate under natural condition.

Material & Methods:

Isolation and Screening of Alkali tolerant *Trichoderma* from agricultural and non-agricultural soil.

Twenty *Trichoderma spp* isolates were obtained from soil sample. The isolates were allowed to grow *in-vitro* at high alkaline medium of pH 10.5 (13). From the twenty isolates obtained in the previous study, five isolates were further screened and confirmed as most alkali tolerant isolates. The isolates were further studied for bio control of plant pathogen.

In-vitro study of bio control activity of alkali tolerant *Trichoderma* spp. isolates

Rotting diseases causing phytopathogen *Sclerotina sclerotiorum* and damping off disease causing phytpathogen *Pythium* spp. were isolated from infected stem of Cauliflower and diseased tomato plant respectively. Dual cultures were performed for determination of diffusible metabolites by five *Trichoderma spp* isolate and isolated phyto pathogenic test fungus were grown on PDA plates. 5 mm disc of both the fungi (*Trichoderma spp*. and phytopathogenic test fungus) were placed on PDA plate about 2.0 – 2.5 cm away from each other (9).The plates were incubated at 25^{o}C for one week. Inhibition of the pathogenic fungal growth was measured. Control was taken without *Trichoderma spp*. Observation was recorded after one week and percentage inhibition was calculated. All the experiments were conducted in five replications.

To study the bio efficacy of *Trichoderma viride* for management of damping off disease in tomato caused by *Pythium* spp. and rotting diseases in cauliflower caused by *Sclerotina* spp. in field condition.

From the five alkali tolerant isolates of *Trichoderma spp* IPL/VP/02 was further selected as the best biocontrol agent and were further studied under field condition. The field experiment were conducted at Alipur, Delhi to study the effectiveness of the formulation of *Trichoderma viride* IPL/VP/02 against the Pathogen *Pythium* spp. and *Scletotina* spp. The experiment were conducted in Randomized Block Design with five treatments and five replications. The cauliflower variety Pusa synthetic (Susceptible to Sclerotinia rot) and the tomato seeds PKM 1 variety treated with liquid formulation of *Trichoderma viride* IPL/VP/02 at 5, 10, 20 ml/kg of seeds by soaking overnight in 1 % (w/v) Sodium Carboxy Methyl cellulose (Na-CMC) and were sown in line The treated seeds were kept for 24 hrs in shade and then sown. Seeds treated with Chemical Fungicide (Carbendazim 50%) at 2g/kg of seed were used for comparison and a suitable control was also maintained. The crop was irrigated at proper intervals and all the agronomic practices and fertilizer schedule were followed as per standard protocols. The treatments were designated as CT1, CT2, CT3, CT4, CT5 and TT1, TT2, TT3, TT4, TT5 respectively(10).The nursery beds prepared for the experiment were raised bed of 10 cm height and plot

size were 3.0 m in length and 1.0 m in width. The method of sowing was manual and in line with 2 cm of depth of sowing.

Assessment of Disease percentage:

The pre emergence disease was recorded at 7 days after sowing and post-emergence disease was recorded at 30th day after sowing and percent disease incidence was calculated using the standard procedure (11)

$$\text{Percentage of disease incidence (pre-emergence)} = \frac{\text{Number of seeds not germinated}}{\text{Total number of seeds sown.}} \times 100$$

$$\text{Percentage of disease incidence (post-emergence)} = \frac{\text{Number of seedling infected}}{\text{Total number of seeds geeminated.}} \times 100$$

Tomato and cauliflower were also observed for phytotoxic symptoms such as chlorosis, necrosis, scorching, epinasty and hyponasty on 1,3,5,7 and 10 days after treatment *Trichoderma* isolate IPL/VP/02 and grading was done as per guidelines (12).

Results

***In-vitro* study of alkali tolerant *Trichoderma* isolates against *Phythium* and *Sclerotina* causing damping off and rotting respectively on tomato and cauliflow qwsgxber.**

The results of the *in-vitro* dual culture study revealed that alkali tolerant *Trichoderma viride* IPL/VP/02 reduced mycelia growth of *Pythium* and *Sclerotina* to the minimum. All the five isolates were able to effectively bio control the growth of the pathogen to different percentage summarized in the table 1. The highest percentage of antagonistic activity was by the isolate *Trichoderma viride* IPL/VP/02.The isolates showed profuse sporulation and mycoparasitism the *Phythium* and *Sclerotina* upto 79.5 and 79.1 per cent respectively (Table1&2)..The isolate *Trichoderma viride* IPL/VP/02 were further considered for study of bio efficacy to control the disease under field condition.

Table 1: Percentage inhibition of growth of mycelia of *Sclerotina* by *Trichoderma* isolates in dual culture techniques.

Tr. No.	Isolate no.	Organisms	Radial Growth of *Pythium*spp. (mm)*	Percent growth reduction	Growth characters of *Trichoderma* isolates
T1	IPL/VP/02	*T.viride*	21.22 ± 1.20	79.5 ± 2.12	Profuse Sporulation and over growth on pathogen
T2	IPL/VP/04	*T. harzianum*	26.33 ± 1.44	70.2 ± 1.22	Profuse Sporulation and over growth on pathogen
T3	IPL/VP/09	*T.asperellum*	28.78 ± 2.10	65.1 ± 2.01	Profuse Sporulation and over growth on pathogen
T4	IPL/VP/16	*T. viride*	27.44 ± 1.45	67 ± 1.66	Profuse Sporulation and over growth on pathogen
T5	IPL/VP/20	*T. harzianum*	26.11 ± 1.23	69.6 ± 1.23	Profuse Sporulation and over growth on pathogen
T6	Carbendazim	----	26.22 ± 1.56	70.64 ± 1.67	---
T7	Control	----	88.00 ± 0.55	--	---
	SE CD (p=0.05)		0.56 1.45		

*The results are mean of 5 replicates.

Table 2: Percentage inhibition of growth of mycelia of *Sclerotina* by *Trichoderma* isolates in dual culture techniques

Tr. No.	Isolate no.	Organisms	Radial Growth of *Scelotina* spp. (mm)*	Percent growth reduction	Growth characters of *Trichoderma* isolates
T1	IPL/VP/02	*T. viride*	20.22 ± 1.20	79.1 ± 1.65	Profuse Sporulation and over growth on pathogen
T2	IPL/VP/04	*T. harzianum*	24.4 ± 1.22	70.4 ± 1.44	Profuse Sporulation and over growth on pathogen
T3	IPL/VP/09	*T.asperellum*	25.2 ± 1.44	67.1 ± 1.5	Profuse Sporulation and over growth on pathogen
T4	IPL/VP/16	*T. viride*	24.3 ± 1.53	69.1 ± 1.34	Profuse Sporulation and over growth on pathogen
T5	IPL/VP/20	*T. harzianum*	24. 3 ± 1.6	69.3 ± 1.51	Profuse Sporulation and over growth on pathogen
T6	Carbendazin		24.22 ± 1.56	68.64 ± 1.45	---
T7	Control		80.00 ± 0.55	--	---
	SE CD (p=0.05)		0.47 1.62		

*The results are the mean of 5 replicates.

Bioefficacy of isolates of *Trichoderma viride* IPL/VP/02 liquid formulation against *Phythium* and *Sclerotina* causing damping off tomato and rotting in cauliflower under field condition.

Table 3: Bioefficacy of *Trichoderma viride* IPL/VP/02 liquid formulation for the management of *Pythium* spp causing Damping off of tomato under field condition.

Tr. No.	Treatment details	Damping off disease (%)		Disease Control (%)	
		Pre-emergence	Post-emergence	Pre-emergence	Post-emergence
TT1	Seed Treatment with *T.viride* A.S. @ 5 ml/kg of seed	10.21 (20.18)	14.70 (22.45)	51.23	56.21
TT2	Seed Treatment with *T.viride* A.S. @ 10 ml/kg of seed	8.23 (16.24)	10.22 (18.92)	70.34	71.33
TT3	Seed Treatment with *T.viride* A.S. @ 20 ml/kg of seed	5.21 (12.11)	7.76 (12.65)	79.33	80.11
TT4	Seed Treatment of Carbendazim 50 % WP @ 2 g/kg seed	11.25 (21.22)	17.22 (24.69)	32.11	37.65
TT5	Untreated control	19.22 (26.12)	30.11 (34.12)	----	----

*The results are the mean of 5 replicates.

Table 4: Bioefficacy of *Trichoderma viride* IPL/VP/02 liquid formulation for the management of *Sclerotina spp.* causing rotting of cauliflower under field condition.

Tr. No.	Treatment details	Rotting disease (%)		Disease Control (%)	
		Pre-emergence	Post-emergence	Pre-emergence	Post-emergence
CT1	Seed Treatment with *T.viride* A.S. @ 5 ml/kg of seed	9.11 (18.16)	12.70 (20.95)	49.93	52.71
CT2	Seed Treatment with *T.viride* A.S.	8.03	9.72	67.14	63.47

	@ 10 ml/kg of seed	(15.24)	(17.92)		
CT3	Seed Treatment with *T.viride* A.S. @ 20 ml/kg of seed	6.21 (11.91)	7.19 (11.65)	70.33	73.21
CT4	Seed Treatment of Carbendazim 50 % WP @ 2 g/kg seed	10.20 (19.22)	15.22 (22.39)	34.84	37.60
CT5	Untreated control	18.22 (24.22)	29.15 (33.02)	----	----

*The results are the mean of 5 replicates.

The results of the experiments conducted under field conditions revealed the supremacy of the bio-formulation of alkaline tolerant *Trichoderma viride* IPL/VP/02. Among the various dosage levels of bio formulation tested as seed treatments, T3 treatment (Seed treated with 20ml/kg of seed) significantly reduced the pre emergence and post emergence damping off tomato and rotting of cauliflower respectively when compared to control (Table 3 &4) Seeds treated with *Trichoderma viride* IPL/VP/02 10 ml/kg also recorded statistically (p<0.05) as per results with that of T3 whereas, untreated control showed the maximum of 19.22 and 30.11 per cent pre and post emergence damping off and 18.22 and 29.15 per cent pre and post emergence rotting disease respectively.

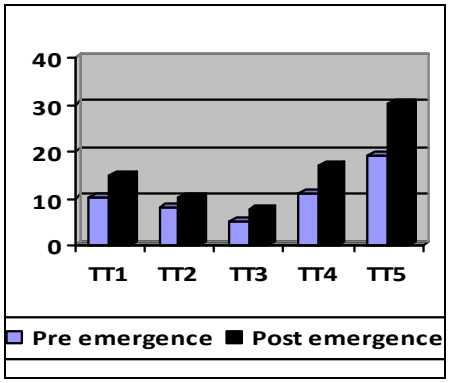

Fig 1: Effect of different treatments of *Trichoderma viride* IPL/VP/02 liquid formulation on pre and post emergence of damping off in tomato.

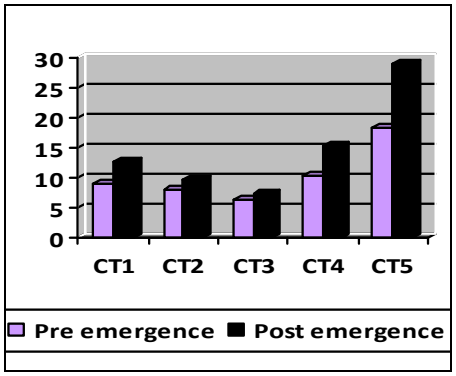

Fig 2: Effect of different treatments of *Trichoderma viride* IPL/VP/02 liquid formulation on pre and post emergence of Rotting of Cauliflower

Phytotoxicity and effect:

No phytotoxicity symptoms such as chlorosis, necrosis, scorching, epinasty and hyponasty on 1,3,5,7 and 10 days were observed throughout the period of investigation in field trial on the seedlings of Tomato Variety PKM 1 and cauliflower variety Pusa when treated with *Trichoderma viride* isolate IPL/R&D/VP/TV/02 liquid formulation at various levels viz, @ 5 ml/kg, 10 ml/kg and 20 ml/kg of seeds.

Discussion:

Trichoderma viride isolates IPL/VP/02 were isolated from alkaline agricultural soil.The strain were screened initially on the basis of its alkaline tolerance and effective biocontrol of several fungal phytopathogen(13).In the present study the alkaline tolerant *Trichoderma* strains were studied *in-vitro* and *in-vivo* for its bio efficacy as biocontrol agent against *Phythium* and *Sclerotina* spp. a common pathogen in tomato and cauliflower respectively. In lab study the *Trichoderma viride* isolate were able to control the pathogen effectively from 65 % to 79 %. Isolates of *Trichoderma viride* IPL/VP/02 were found to be the most effective biocontrol agents in dual culture study controlling both the pathogens upto 79.5 % and 79.1 % respectively. This proves the bioefficacy nature of *Trichoderma* isolates (14). *Trichoderma viride* IPL/VP/02 were studied for its bio-efficacy under field condition against *Pythium* and *Scelotina.* Field study conducted in alkaline soil pH above 8.5 and tomato and cauliflower variety susceptible to the pathogen were sown together with the 2% liquid formulation of *Trichoderma viride* IPL/VP/02.The results of the experiments conducted under field conditions revealed the supremacy of the bio-formulation of alkaline tolerant *Trichoderma viride* IPL/VP/02.*Trichoderma viride* treated seeds were able to inhibit the growth of the pathogen *Phythium aphanidermatum* which causes damping off diseases in tomato(15) and *Scelotina sclerotiorum* which casuse stem rot in cauliflower (17).Among the various dosage levels of bioformulation tested as seed treatments, the treatment T3 (Seed treated with 20ml/kg of seed) significantly reduced the pre emergence and post emergence damping off tomato and rotting of cauliflower respectively (Table 3 & 4) when compared to control.

Seed treated with the *Trichoderma viride* IPL/VP/02 10 ml/kg also recorded statistically as per results with that of T3 (TT3 & CT3 respectively) whereas, untreated control recorded the maximum of 19.22 and 30.11 per cent pre and post emergence damping off and 18.22 and 29.15 per cent pre and post emergence rotting disease respectively.

Conclusion:

Crop Cultivation in Alkali soils where pH is high is been a serious concern for Indian agriculture. Biological disease management is a cost effective way of disease management. To facilitate the Biological disease management in alkaline soils, the biocontrol agent should be able to multiply and perform under high pH(16).This can be achieved only by Alkali tolerant biocontrol agent *Trichoderma* which was able to perform both under *in-vitro* and under natural field condition. The study showed the effective biological control of *Pythium* and *Scelrotina* in the presence of Alkali tolerant *Trichoderma in* Tomato and cauliflower respectively. It confirmed that the alkali tolerant strain can be studied further to look into the prospects of commercialisation of these strains as potent Biocontrol agents.

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