

Research Article

Variability in parasitic ability of *Trichoderma* isolates against sclerotia of *Sclerotium rolfsii* associated with chickpea

P. NAGAMANI¹*, SOMESHWAR BHAGAT², M. K. BISWAS³ and K. VISWANATH¹

¹Regional Agricultural Research Station, Tirupati - 517 502, Andhra Pradesh, India

²NRRI-Central Rainfed Upland Rice Research Station, Hazaribagh, Jarkhand

³Department of Plant Protection, Palli siksha Bavana, Viswava Bharati, Bolpur - 731235, West Bengal, India

*Corresponding author Email: manipath28@gmail.com

ABSTRACT: Parasitic ability of *Trichoderma* isolates was tested against *Sclerotium rolfsii* in two types of soils viz., natural and sterilized soils to assess the ecological adaptability of *Trichoderma* spp. to a soil. The isolate ATPU 1 (EID50 of 2.1 mycelia/gm of soil) was most effective in parasitization of sclerotia of *S. rolfsii* when used as live bait, under the natural soil of, when mycelial form of inoculum was used. This isolate was closely followed by KNO 2 and ATPP 6 which had the same EID50 of 2.2 mycelia/gm of soil, the next best isolates were KNP 3 and ATPPE 6 with EID50 of 2.3 mycelia/gm of soil. The isolate KT 6 (EID50 5.7) was rated as poorest competitive colonizer, requiring comparatively highest inoculum level to colonize 50% sclerotia of *S. rolfsii*. However the conidial form of inoculum from KNK1 (EID50 value of 2.2 conidia/gm of soil) was most aggressive isolate requiring lowest inoculum dose for 50% colonization of sclerotia of *S. rolfsii*.

KEY WORDS: Chickpea, Competitive parasitic ability, *Trichoderma* spp

(Article chronicle: Received: 13.04.2017; Revised: 28.08.2017; Accepted: 15.09.2017)

INTRODUCTION

Trichoderma possess great variability in their multifaceted mechanism of action against plant pathogens (Bhagat *et al.*, 2014). Some strains have a wide spectrum of activity (Harman, 1991) and few strains may control only specific pathogens (Smith *et al.*, 1990), while still others may have little or no bio-control efficacy. There is also a distinct variability in *Trichoderma* on ecological behaviour. One isolate, showing high hyperparasitic activity in a particular habitat does not grow profusely in another one. Hence, evaluation of the *Trichoderma* strains for their potential against the target pathogens is highly essential under both field and laboratory conditions. The success of hyperparasite depends on those conditions that allow its spores to germinate freely and

permit its survival through stress situations in soil. Two important modes of survival are (a) through operation of a phenomenon called fungistasis, and (b) free survival of dormant propagules in soils. Proliferation and subsequent establishment of *Trichoderma* and *Gliocladium* in rhizosphere largely depended upon age of inoculum with relation to food base, concentration of inoculum applied, soil properties and age of the plant. *Trichoderma* spp. are normally found to be dominant in both the rhizosphere and rhizoplane and are found associated with the roots throughout the life of plants. Rhizosphere competence of antagonistic fungi in root zone of many crops is a vital area of research that requires elaborate exploration for a successful management of plant diseases by biocontrol agents. Therefore, present investigation was aimed

to explore some isolates of *Trichoderma* spp. from chickpea growing Rayalaseema region of Andhra Pradesh.

MATERIALS AND METHODS

Antagonist isolates and culture conditions

Twenty isolates of *Trichoderma* were isolated from rhizosphere soil of chickpea growing areas of Raayalseema region and were maintained in potato dextrose agar media (PDA). The formulated product of

Trichoderma isolates in sorghum grains were prepared as per method followed by (Jeyarajan *et al.*, 1994).

Competitive parasitic ability

This experiment was conducted in two types of soils viz., natural and sterilized soil so as to assess the ecological adaptability of *Trichoderma* spp. to a soil which is geographically located at different places. 200g air dried (natural and sterilized soil) was mixed thoroughly with mycelia or conidial inocula of test isolates of *Trichoderma* separately, moisture holding

Table 1. Twenty different *Trichoderma* isolates and their NCBI accession numbers

Isolates	NCBI Accession number	ITS region analysis Species identified
ATPU-6	KY381941	<i>T. viride</i>
APTU-4	KY401450	<i>T. asperellum</i>
ATPP-6	KY358076	<i>T. asperellum</i>
ATPPE-6	KY401444	<i>T. asperellum</i>
APTU-1	KY401443	<i>T. asperellum</i>
KJ-12	KY381940	<i>T. longibrachiatum</i>
KNK-1	KY401447	<i>T. asperellum</i>
KNK-9	KY397967	<i>T. longibrachiatum</i>
KNN-2	KY381943	<i>T. asperellum</i>
KNN-4	KY397966	<i>T. viride</i>
KNO-2	KY401449	<i>T. asperellum</i>
KNO-9	KY401442	<i>T. asperellum</i>
KNP-1	KY231190	<i>T. asperellum</i>
KNP-3	KY401448	<i>T. longibrachiatum</i>
KNPG-3	KY381942	<i>T. asperellum</i>
KP 10	KY401445	<i>T. asperellum</i>
KR-4	KY401446	<i>T. asperellum</i>
KT-13	KY381939	<i>T. harzianum</i>
KT6	KY358077	<i>T. asperellum</i>
KT-7	KY401441	<i>T. asperellum</i>

capacity of each soil type was adjusted to 50% and fitted into the earthen cups (100 ml). 25 sclerotia of *S. rolfii* was buried 0.5 - 2.0 cm deep, covered with perforated aluminum foil and incubated at $28 \pm 1^\circ\text{C}$ for 7 days. The sclerotia of *S. rolfii* was harvested separately by sieving and plated on modified *Trichoderma* selective medium (TSM) after surface sterilization with 1.0% sodium hypochlorite solution for 2-3 min. and air dried. The Petridishes seeded with sclerotia of *S. rolfii*, were incubated at $28 \pm 1^\circ\text{C}$ for 7 days and the sclerotia with colonization by *Trichoderma* spp. in each treatment were recorded. The per cent age colonization of sclerotia of pathogens by *Trichoderma* spp. was calculated by dividing the number of sclerotia yielding *Trichoderma* by total number of sclerotia seeded in the Petri dishes.

Production of sclerotia of *Sclerotium rolfii*

Production of sclerotia of *Sclerotium rolfii*, mycelial plug (6 mm) of the fungus was inoculated into potato dextrose agar (PDA) medium and incubated at $28 \pm 1^\circ\text{C}$ for 25 days. The sclerotia were collected, washed, air dried and surfaced sterilized with 1.0% sodium hypochlorite solution, again air dried and used immediately. Required concentration of mycelia, conidial form of *Trichoderma* isolates prepared as per procedure given by Bhagat and Pan, 2011.

Antagonistic effects of *Trichoderma* isolates on germination and parasitization of sclerotia

The viability of mature sclerotia treated with different *Trichoderma* isolates was tested by on solidified potato dextrose agar media. Twenty five sclerotia from each *Trichoderma* treated were tested for its germination by placing sclerotia on PDA media incubated for 24h and examined under microscope. The number of germinated sclerotia and sclerotial parasitization % was calculated as per Vincent (1947). Effective Inoculums Densities (EID) was calculated by adopting probit analysis.

RESULTS AND DISCUSSION

All *Trichoderma* isolates prevented the sclerotia of *S. rolfii* to germinate within 2 days of incubation, green growth of *T. asperellum* (ATPU 1) more conidia was visible on the surface of sclerotia of pathogen. Fifth day, profuse growth of ATPU 1 and KNO-2 was

observed on the sclerotia of *S. rolfii*. The isolates *T. asperellum* (KNO-2, KNN-2), *T. longibrachiatum* (KNP-3) showed highest per cent parasitization of sclerotia. Whereas *T. asperellum* (KP-10, KR-4 and KT-13) recorded least per cent parasitization. The results of sclerotial antagonism of *Trichoderma* isolates is presented in Table 1, revealed that ATPU 1 isolate showed lowest sclerotial germination per cent (20.0%) and highest percentage of sclerotia parasitization followed by KNN-2 (22.67, 61.78) and ATPU6 (25.67, 59.77) germination per cent and percentage sclerotia parasitization respectively. Similarly, Mukherjee *et al.* (1995) observed that *G. virens* and *T. harzianum* prevented the germination sclerotia of *S. rolfii* and *R. solani* on soil plate and agar plate technique. Surface sterilized sclerotia of *S. rolfii* and *R. solani* from *T. harzianum* inoculated plates germinated on PDA but those colonized by *G. virens* failed to germinate.

Muthamilan and Jeyarajan (1992) reported that 67.4 % reduction of sclerotial production in *S. rolfii* in presence of *T. viride*. The variability in sclerotia production, inhibition of germination and susceptibility to degrade *in vitro* and infectivity of sclerotia of *R. solani* and *S. rolfii* suggests that there is considerable specificity in biocontrol. Mukherjee *et al.*, (1995) observed that, *T. harzianum* and *G. virens* readily parasitized the sclerotia of the *S. rolfii* and *R. solani* pathogens and was found to be more effective in destroying the sclerotia of pathogens. Parasitism of sclerotia is suggested as the principal mechanism of biological control of *S. rolfii* and *R. solani* by *G. virens*. However, Bunker and Mathur (2001) reported that *T. harzianum* was most effective causing significant suppression of both growth and sclerotial production of *R. solani in vitro* and they also reported that diffusible non-volatile antibiotic activity of *T. harzianum* was more potential than volatile antibiotics. Pan and Bhagat 2008 reported that ten *Trichoderma* spp. isolated from rhizosphere of different crops and were evaluated for their antagonistic potential against five soil borne plant pathogens. Among all, *T. 2* were most effective in inhibition of sclerotia formation, production and germination of sclerotia of *S. rolfii*.

The isolate ATPU 1 (*T. asperellum*, NCBI accession number KY401443) with EID50 2.1 mycelia/gm of soil was most effective in parasitization of sclerotia of *S. rolfii* used as live bait, under the natural soil of, when mycelial form of inoculum was used (Table 2).

Table 2. Antagonistic effect of *Trichoderma* isolates on germination and parasitization of sclerotia of *Sclerotium rolfsii*

Isolates	% germination of sclerotia ± SEM	% parasitization of sclerotia ± SEM
APTU-1	20.00±0.00	63.41±0.00
APTU-4	45.33±1.33	47.66±0.77
ATPP-6	25.67±1.2	59.77±0.87
ATPPE-6	30.33±2.85	57.26±2.21
ATPU-6	50.67±1.33	44.60±0.76
KJ-12	57.33±1.33	40.76±0.77
KNK-1	29.33±1.33	57.20±0.83
KNK-9	29.33±2.67	57.23±1.71
KNN-4	53.33±1.33	43.07±0.77
KNN-2	26.67±2.67	58.94±1.71
KNO-2	22.67±4.81	61.78±3.24
KNO-9	29.33±1.33	57.20±0.83
KNP-1	36.00±2.31	53.13±1.38
KNP-3	26.67±1.33	58.90±0.87
KNPG-3	29.33±3.53	57.26±2.21
KP 10	64.00±2.31	36.84±1.38
KR-4	66.67±3.53	35.20±2.14
KT-13	61.33±3.53	38.40±2.09
KT6	54.67±1.33	42.30±0.77
KT-7	48.00±4.00	46.14±2.30
Control	98.67±1.33	3.84±1.24
C.D	5.74	4.53
SE(m)	2.00	1.58

This isolate was closely followed by KNO 2 and ATPP 6 (*T. asperellum*, NCBI accession number KY358076) with same (EID50 2.2) next best isolates KNP 3(*T. longibrachiatum* NCBI accession number KY401448) and ATPPE6 (*T. asperellum* NCBI accession number KY401444) with (EID50 2.3). The isolate KT 6 (EID50 5.7) was rated as poorest competitive colonizer, requiring comparatively highest inoculum level to colonize 50% sclerotia of *S. rolfsii*. Whereas KNK1 (EID50 2.2 conidia/gm of soil) was most aggressive

isolate required lowest inoculum dose for 50% colonization of sclerotia of *S. rolfsii* when conidial inoculum was used. When conidial form of inoculum (antagonists) was used, the isolate ATPP 1 (EID50 1.3) (EID50, 2.2) was the most efficient colonizer of sclerotia of *S. rolfsii* and the isolate KT 6 and KT 7 (EID50 5.6,5.7) was rated as least competitive colonizer, requiring comparatively highest inoculum level to colonize 50% sclerotia of *S. rolfsii*.

Table 3. Effective inoculums potential of *Trichoderma* spp. required to colonize sclerotia of *Sclerotium rolfsii* in natural soil

<i>Trichoderma</i> spp.	Natural soils (Mycelia form)			EID	Natural soils (conidia form)			
	10 ³	10 ⁵	10 ⁷		10 ³	10 ⁵	10 ⁷	EID
APTU-1	23.67±1.45 ^{ab}	48.67±2.13 ^{ab}	70.33±2.15 ^{cdeb}	2.1±0.34 ^a	12.00±1.17 ^{cdeb}	20.3±1.74 ^{ef}	77.0±2.20 ^a	2.2±0.56 ^a
APTU-4	13.33±1.45 ^{ef}	35.33±2.13 ^{cdabe}	80.00±2.15 ^{ab}	4.6±0.34 ^{ab}	13.00±1.17 ^{cadbe}	25.7±1.74 ^{def}	65.0±2.20 ^{cdbe}	4.0±0.34 ^{cd}
ATPP-6	15.67±1.45 ^{def}	35.00±2.13 ^{cdafg}	60.33±2.15 ^e	2.2±0.34 ^a	9.67±1.17 ^{de}	25.7±1.74 ^{def}	48.3±2.20 ^g	2.3±0.34 ^a
ATPPE-6	27.33±1.45 ^a	48.00±2.13 ^{ab}	84.67±2.15 ^a	2.3±0.34 ^a	18.33±1.17 ^a	47.3±1.74 ^a	75.7±2.20 ^{ab}	2.4±0.34 ^{ab}
ATPU-6	21.67±1.45 ^{calbe}	44.00±2.13 ^{cab}	77.33±2.15 ^{cab}	4.7±0.34 ^{ab}	11.00±1.17 ^{cde}	25.3±1.74 ^{def}	69.0±2.20 ^{cadb}	4.0±0.34 ^{cd}
KJ-12	19.33±1.45 ^{cdbe}	36.00±2.13 ^{cdabe}	73.00±2.15 ^{cdlb}	5.8±0.34 ^{ef}	14.33±1.17 ^{cadb}	34.0±1.74 ^{deb}	77.0±2.20 ^a	5.7±0.34 ^{de}
KNK-1	10.00±1.45 ^{ef}	24.67±2.13 ^{hg}	68.00±2.15 ^{cde}	2.9±0.34 ^{ab}	7.33±1.17 ^e	19.3±1.74 ^{ef}	57.7±2.20 ^{fdge}	2.2±0.34 ^a
KNK-9	13.00±1.45 ^{ef}	39.67±2.13 ^{cadbe}	78.67±2.15 ^{cab}	2.9±0.34 ^{ab}	16.00±1.17 ^{cab}	33.7±1.74 ^{deb}	67.0±2.20 ^{cadbe}	2.6±0.34 ^{ab}
KNN-4	15.00±1.45 ^{ef}	28.00±2.13 ^{hige}	61.67±2.15 ^{de}	5.5±0.34 ^{cd}	9.33±1.17 ^{de}	24.0±1.74 ^{ef}	56.3±2.20 ^{ge}	3.8±0.34 ^{cde}
KNN-2	23.33±1.45 ^{cadb}	46.33±2.13 ^{cab}	74.33±2.15 ^{cab}	2.7±0.34 ^{ab}	14.00±1.17 ^{cadb}	46.0±1.74 ^a	65.0±2.20 ^{cadbe}	2.4±0.34 ^{ab}
KNO-2	11.00±1.45 ^g	25.33±2.13 ^{hg}	73.00±2.15 ^{cdlb}	2.2±0.34 ^a	9.00±1.17 ^{de}	24.7±1.74 ^{def}	56.3±2.20 ^{ge}	2.4±0.34 ^{bc}
KNO-9	16.67±1.45 ^{gcdbe}	49.33±2.13 ^{ab}	72.33±2.15 ^{cdlb}	2.7±0.34 ^{ab}	12.00±1.17 ^{cadbe}	35.7±1.74 ^{bc}	72.3±2.20 ^{cab}	2.8±0.34 ^{ab}
KNP-1	16.00±1.45 ^{gcdbe}	40.67±2.13 ^{cadb}	70.00±2.15 ^{cdeb}	2.9±0.34 ^{bc}	9.67±1.17 ^{de}	18.0±1.74 ^f	62.3±2.20 ^{cdle}	4.1±0.34 ^{cde}
KNP-3	10.33±1.45 ^g	39.33±2.13 ^{cdabe}	72.00±2.15 ^{cdlb}	2.3±0.34 ^a	10.00±1.17 ^{cde}	22.3±1.74 ^{ef}	56.7±2.20 ^{ge}	3.2±0.34 ^{cd}
KNPG-3	24.00±1.45 ^{ab}	29.33±2.13 ^{hidge}	68.67±2.15 ^{cdeb}	2.6±0.34 ^{ab}	13.67±1.17 ^{cadb}	41.3±1.74 ^{ab}	68.7±2.20 ^{cadb}	4.1±0.34 ^{de}
KP 10	12.00±1.45 ^{ef}	20.00±2.13 ^h	71.67±2.15 ^{cdeb}	5.4±0.34 ^{dce}	7.00±1.17 ^{ef}	22.7±1.74 ^{ef}	62.3±2.20 ^{cdle}	4.3±0.34 ^{def}
KR-4	15.33±1.45 ^{ef}	41.67±2.13 ^{cab}	77.67±2.15 ^{cab}	5.7±0.34 ^{ef}	12.00±1.17 ^{cadbe}	28.0±1.74 ^{dce}	69.3±2.20 ^{cadb}	5.5±0.34 ^{ef}
KT-13	19.33±1.45 ^{cdbe}	51.00±2.13 ^a	84.67±2.15 ^a	5.2±0.34 ^{de}	18.00±1.17 ^{ab}	46.3±1.74 ^a	70.7±2.20 ^{cab}	5.4±0.34 ^g
KT6	13.33±1.45 ^{ef}	29.33±2.13 ^{hidge}	72.33±2.15 ^{cdlb}	5.7±0.34 ^{ef}	9.33±1.17 ^{de}	19.7±1.74 ^{ef}	55.3±2.20 ^{ge}	5.6±0.34 ^{def}
KT-7	14.00±1.45 ^{ef}	28.67±2.13 ^{hg}	71.33±2.15 ^{cdeb}	4.9±0.34 ^{de}	10.67±1.17 ^{cde}	27.0±1.74 ^{dcef}	54.0±2.20 ^g	5.6±0.34 ^{def}
Control	1.00±1.45 ^h	1.00±2.13 ⁱ	1.00±2.15 ^f	-	1.00±1.17 ^f	1.0±1.74 ^g	1.0±2.20 ^h	-

*Mean of three replicates

*Means with different superscripts are significantly different with p<0.05 by Tukey's HSD test

Table 4. Effective inoculums potential of *Trichoderma* spp. required to colonize sclerotia of *Sclerotium rolfsii* in sterilized soil

<i>Trichoderma</i> spp.	Sterilized soils (Mycelia form)			EID	Sterilized soils (conidia form)			
	10 ³	10 ⁵	10 ⁷		10 ³	10 ⁵	10 ⁷	EID
KNP 3	35.7±2.46 ^{ab}	78.7±1.73 ^{bdac}	94.3±1.77 ^{bac}	2.84±0.22 ^a	28.0±1.18 ^{cdg}	79.3±2.33 ^{ab}	91.0±2.48 ^a	3.3±0.47 ^{bc}
ATPU 2	28.3±2.46 ^{cab}	72.7±1.73 ^{egdeh}	95.3±1.77 ^{ab}	2.45±0.22 ^a	23.7±1.18 ^{hij}	68.3±2.33 ^{def}	89.3±2.48 ^{dc}	4.9±0.47 ^{de}
KJ-12	21.7±2.46 ^c	59.0±1.73 ^{kij}	82.0±1.77 ^d	5.2±0.22 ^{de}	26.0±1.18 ^{hgef}	50.0±2.33 ^{gf}	86.0±2.48 ^{cab}	5.0±0.47 ^{dc}
KNK-9	30.7±2.46 ^{cab}	81.0±1.73 ^{bdac}	95.7±1.77 ^a	2.45±0.22 ^a	30.7±1.18 ^{cdg}	79.7±2.33 ^{def}	89.0±2.48 ^a	4.9±0.47 ^{dc}
KNO-9	22.3±2.46 ^c	68.7±1.73 ^{gh}	86.0±1.77 ^{bdac}	3.72±0.22 ^{ab}	25.3±1.18 ^{cab}	49.7±2.33 ^{def}	82.7±2.48 ^{ab}	4.1±0.47 ^{de}
KNO 2	38.7±2.46 ^a	86.3±1.73 ^a	96.7±1.77 ^a	2.10±0.22 ^a	33.3±1.18 ^a	68.7±2.33 ^a	98.3±2.48 ^a	2.50±0.47 ^{ab}
ATPP-6	34.3±2.46 ^{cab}	76.0±1.73 ^{ebdac}	95.3±1.77 ^{ab}	2.27±0.22 ^a	31.0±1.18 ^{hij}	79.3±2.33 ^{cab}	93.7±2.48 ^a	2.74±0.47 ^{ab}
ATPPE-6	25.7±2.46 ^{cab}	82.0±1.73 ^{abc}	93.7±1.77 ^{bac}	2.43±0.22 ^a	28.3±1.18 ^{gh}	67.0±2.33 ^{gdef}	89.7±2.48 ^a	2.5±0.47 ^{ab}
APTU-4	23.0±2.46 ^{bc}	59.0±1.73 ^{kij}	86.0±1.77 ^{bdac}	3.01±0.22 ^{ab}	25.0±1.18 ^{cab}	59.7±2.33 ^{cab}	82.3±2.48 ^a	2.76±0.47 ^{ab}
KNK-4	27.3±2.46 ^{cab}	65.7±1.73 ^{gh}	86.0±1.77 ^{bdac}	3.0±0.22 ^{ab}	25.7±1.18 ^{cdh}	46.0±2.33 ^{def}	90.0±2.48 ^{cd}	1.90±0.47 ^a
KR-4	27.0±2.46 ^{cab}	69.3±1.73 ^{egh}	95.7±1.77 ^a	2.9±0.22 ^{bc}	30.3±1.18 ^{hij}	79.3±2.33 ^{ab}	94.3±2.48 ^{ab}	5.8±0.47 ^{def}
KT 6	22.3±2.46 ^c	55.3±1.73 ^k	83.3±1.77 ^d	4.5±0.22 ^{bcd}	16.3±1.18 ^j	48.3±2.33 ^g	79.7±2.48 ^d	4.8±0.47 ^{de}
KNPG-3	33.7±2.46 ^{cab}	83.3±1.73 ^{ab}	96.7±1.77 ^a	4.3±0.22 ^{bcd}	27.0±1.18 ^{cdg}	85.0±2.33 ^a	92.0±2.48 ^a	3.8±0.47 ^{bcd}
KT 13	23.0±2.46 ^{bc}	64.0±1.73 ^{kljh}	93.0±1.77 ^{bac}	5.7±0.22 ^{de}	27.0±1.18 ^{ij}	48.7±2.33 ^{def}	90.7±2.48 ^a	5.5±0.47 ^{def}
KNK-2	27.7±2.46 ^{cab}	67.7±1.73 ^{gh}	87.3±1.77 ^{bdac}	2.7±0.22 ^{bc}	32.0±1.18 ^{cdh}	47.3±2.33 ^{gef}	88.3±2.48 ^{ab}	3.63±0.47 ^{bc}
KT-7	23.7±2.46 ^{bc}	66.3±1.73 ^{gh}	85.3±1.77 ^{dc}	4.7±0.22 ^{dec}	31.7±1.18 ^{hdef}	48.7±2.33 ^{cdeb}	84.3±2.48 ^a	5.7±0.47 ^{ef}
APTU-1	29.0±2.46 ^{cab}	66.7±1.73 ^{gh}	94.0±1.77 ^{bac}	2.36±0.22 ^a	23.3±1.18 ^{cab}	55.3±2.33 ^{cab}	83.7±2.48 ^a	2.65±0.47 ^{ab}
KNK-1	31.7±2.46 ^{cab}	73.7±1.73 ^{ebdac}	96.3±1.77 ^a	2.45±0.22 ^a	33.0±1.18 ^a	71.7±2.33 ^{def}	89.3±2.48 ^a	2.10±0.47 ^a
KNP-1	24.0±2.46 ^{bc}	58.0±1.73 ^{klj}	89.7±1.77 ^{bdac}	2.40±0.22 ^a	20.0±1.18 ^{cdh}	52.0±2.33 ^{def}	86.7±2.48 ^d	3.3±0.47 ^{ab}
KP 10	33.0±2.46 ^{cab}	78.3±1.73 ^{ebdac}	96.3±1.77 ^a	2.7±0.22 ^{ab}	25.0±1.18 ^{cab}	78.0±2.33 ^{bde}	91.7±2.48 ^a	5.9±0.47 ^{ef}
Control	0.0±2.46 ^d	0.0±1.73 ^l	0.0±1.77 ^e	-	0.0±1.18 ^k	0.0±2.33 ⁱ	0.0±2.48 ^e	-

*Mean of three replicates

*Means with different superscripts are significantly different with p<0.05 by Tukey's HSD test

Under steam sterilized (Table 3), lowest EID50 values of *Trichoderma* were recorded against sclerotia of *S. rolfsii*. The isolate KNO 2 required least amount of mycelial inoculum. This isolate followed by ATPP 6 (EID50 2.26), ATPU 1 (EID50 2.36) and the isolate KT 13 (EID50 5.7) was rated as poorest performer in colonizing 50% sclerotia of *S. rolfsii* in same soils. Similarly, when conidial form of inocula was used, KNK 4 continued to perform well requiring lowest EID50 value (1.9) whereas the isolates KNK 1 (EID50 2.1), ATPPE (EID50 2.50) and ATPU 1 (EID50 2.65) were the next best isolates in sterilized soil respectively.

The parasitic or saprophytic ability of antagonists, *Trichoderma* spp. are two major attributes which make them a potential antagonist against many soil borne plant pathogens. The *in situ* competitive parasitic potential of the *Trichoderma* can be studied using the sclerotia as live baits, produced by several phytopathogenic fungi. The penetration and subsequent colonization by antagonists on the sclerotia of many pathogens obviously reflect much upon their parasitic ability rather than saprophytic attributes in the intensely competitive microbiotic environment in soil. *Sclerotium rolfsii* is well known to produce sclerotia, the principal structure as the means of survival propagules under adverse environmental condition, sclerotia are known to survive for several years in soil (Coley Smith and Cook, 1971) and how they behave so has been a subject of research to find new methods of biological control. They are commonly thought to be resistant to desiccation and this resistance is usually attributed to the presence of rind (Cook and Al-Hamdani, 1986). Benhamou and Chet (1996) investigations revealed that under light microscope the hyphae of the *T. harzianum* multiplied on the sclerotial surface and displayed the ability to penetrate the rind, growth of the antagonist in the rind layer was mainly intracellular, and host wall penetration was achieved by means of constricted hyphae.

Present findings suggested that the test isolates of *Trichoderma* varied in their ability to colonize the sclerotia of *S. rolfsii* in different soils with two forms of inoculum. The possible explanation of this result may be due to the phenomenon of soil fungistasis in natural and sundried soil (Roy and Pan, 2005). The fungistatic nature of soil and the nature of annulment of soil fungistasis may have considerable impact on the survival and population of dynamics of natural and

introduced species of *Trichoderma* and *Gliocladium* in soil. Natural soil often ecologically does not allow introduced biocontrol agents to perform well due to some abiotic and biotic factors (Roy and Pan 2005, Bae and Knudsen, 2005). Soil solarisation and sterilization upsets the ecosystem to the extent that it may allow proliferation of *Trichoderma* spp.

The present results were in agreement with Bhagat and Pan 2011, isolates ThrWB-1 and ThrWB-2 were showed most effective in sclerotial parasitization of *S. rolfsii* under Mohanpur (WB) soils, followed by isolates ThrWB-1, ThrAN-5, ThrAN-7, TvAN-3 and TvAN-5 were most promising isolates with high competitive colonization of sclerotia of *S. rolfsii* under natural and sterilized soils. Monika *et al.*, (2016) conducted research on competitive parasitic ability against sclerotia of *S. sclerotiorum*. With mycelial form of inoculums, the isolate UP:Bam 003 and MP:Kha030 appeared most efficient in their competitive parasitic ability against sclerotia of *S. sclerotiorum*, whereas the isolate MS:Mar016 was with intermediate effect and the isolate UP:Kus008 were poor competitive colonizer on sclerotia of *S. sclerotiorum*. Considering the conidial form of inoculum, the isolate UP: Bam003 appeared most efficient colonizer of sclerotia of *S. sclerotiorum*, whereas UP:Kus008 exhibited as least colonizer and isolate MP:Kha030 and MS:Mar016 showed intermediate colonizing ability of sclerotia.

REFERENCES

- Bae YS, Knudsen GR. 2005. Soil microbial biomass on growth and biocontrol efficacy of *Trichoderma harzianum*. *Biological Contro.* **32**: 236–242. Crossref.
- Benhamou N, Chet I. 1996. Parasitism of sclerotia of *S. rolfsii* by *Trichoderma harzianum*: Ultrastructural and Cytochemical aspects of the interaction. *Phytopathology* **86**: 405–416. Crossref.
- Bhagat S, Pan S. 2011. Parasitic ability of *Trichoderma* isolates against sclerotia of *Sclerotium rolfsii* and management of collar rot of brinjal. *Biopesticide Int.* **7**(1): 52–59.
- Bhagat S, Birah A, Kumar R, Yadav MS, Chattopadhyay C. 2014. Plant disease management: prospects of pesticides of plant origin. *Advances in Plant Biopesticides* 119–129.

- Bhagat S, Pan S. 2011. Competitive parasitic ability of *Trichoderma* spp. and biocontrol of damping off of solanaceous vegetables. *J Mycopathol Res.* **49**(1): 65–72.
- Bunker RN, Mathur K. 2001. Antagonism of local biocontrol agents to *Rhizoctonia solani* inciting dry root rot of chilli. *Indian J Mycol Pl Pathol.* **31**: 50–53.
- Cole-Smith JR, Cook RC. 1971. Survival and germination of fungal sclerotia *Ann Rev Phytopathol.* **9**: 65–92. Crossref.
- Cook RC, Al-Hamdani AM. 1986. Water relations of sclerotia and other infective structure. In: Ayes PG, Body L (Eds.). *Water, Fungi and Plants*. Cambridge: Canbridge University Press. 49–63. PMID:3957782
- Harman GE. 1991. Seed treatments for biological control of plant disease. *Crop Prot.* **10**: 166–171. Crossref.
- Monika S. Sharma OP, Bhagat S, Neetu P. 2016. *In-Vivo* Evaluation of Competitive parasitic Ability and Rhizosphere Colonisation of Different *Trichoderma* isolates. *Int J Curr Microbiol Appl Sci.* **5** (4): 32–38. Crossref.
- Mukherjee PK, Mukhopadhyay AN, Sarmah DK, Shrestha SM. 1995. Comparative Antagonistic Properties of *Gliodadium virens* and *Trichoderma harzianum* on *Sclerotium rolfsii* and *Rhizoctonia solani* —its Relevance to Understanding the Mechanisms of Biocontrol. *J Phytopathol.* **143**: 275–279. Crossref.
- Muthamilan M, Jeyarajan R. 1992. Effect of antagonistic fungi on *Sclerotium rolfsii* causing root rot of groundnut. *J Biol Control* **6**: 88–92.
- Pan S, Bhagat S. 2008. Characterization of antagonistic potential of *Trichoderma* spp. against some soil borne plant pathogens. *J Bioi Control* **22**(1): 43–49
- Roy A, Pan S. 2005. Competitive parasitic ability and biocontrol potential of mutants of *Trichoderma harzianum* and *Gliocladium virens* against *Sclerotium rolfsii* induced root rot of groundnut. *Indian J Mycol Pl Pathol.* **35** 326–330.
- Smith VL, Wilcox WF, Harman GE. 1990. Biological potential for biological control of phytophthora root rot and crown rots of apple by *Trichoderma* and *Gliocladium* species. *Phytopathology* **80**: 880–885. Crossref.
- Vincent JH. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* **15**: 850. Crossref.