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In vitro Compatibility Study between the *Rhizobium* and Native *Trichoderma* Isolates from Lentil Rhizospheric Soil

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A B S T R A C T

Combination of *Rhizobium* and *Trichoderma* species were studied to document their compatibility their synergism, antagonism under *in vitro* condition and their effects on the growth of lentil *in vivo* condition. In respect this, few strains of *Trichoderma* were isolated from the rhizosphere soil of Pulse crop from different locations of West Bengal and they were tested *in vitro* and *in vivo* along with *Rhizobium* to know the interaction between them and the growth behaviour on lentil. The results indicated that *Rhizobium* and *Trichoderma* isolates showed compatibility in between them in their growth. The cultural filtrate of different isolates of *Trichoderma* were also used to find out the antagonistic potentiality or compatibility against *Rhizobium* and found that some strains showed compatibility for their growth and some cause inhibition on the growth of *Rhizobium*. The pot culture experiment was done through seed treatment of different *Trichoderma* isolates and *Rhizobium* individually and in combination, showed differential growth characteristics of Lentil. The numbers of root nodules, weight of fresh and dry nodule were significantly increased when *Trichoderma* isolate of Shekhampur and Market sample were mixed with *Rhizobium* in comparison to sole application of these two organisms. Whereas, shoot length, fresh shoot weight, root length and dry root weight were increased though their differences were statistically insignificant. The result therefore suggested that combined application of native *Trichoderma* isolate and *Rhizobium* can increase the growth and total biomass of lentil which play an important role in organic agriculture.

Introduction

Lentil (*Lens culinaris*) is an edible pulse, commonly used for human nutrition, animal feed and soil fertility (Sarker and Kumar, 2011). Its cultivation enriches soil nutrient status by adding nitrogen, carbon and organic matter which promotes sustainable cereal-based systems of crop production (Frederick *et al.*, 2006; Badarneh, 1995; O' Hara *et al.*, 2002; Schulze *et al.*, 2006). Combined or simulation application of rhizobacteria with other bio-inoculants and pesticides are not always compatible and synergistic or additive (Medina-Martinez *et al.*, 2002). The inoculation of seeds with *Rhizobium* is known to increase nodulation, nitrogen uptake, and growth and yield response of crop plants (Dorosinsky and Kadyrov, 1975; Patil and Shinde, 1980; Herandez and Hill, 1983; Rudresh *et al.*, 2005). The beneficial effect of *Rhizobium* sp. has been a main focus in terms of biological nitrogen fixation in the recent past (Deshwal *et al.*, 2003). *Trichoderma* have long been known as effective antagonists against soil borne plant

pathogenic fungi, nematodes (Rifai, 1969, Papavizas, 1985; Mukhopadhay *et al.*,1986; Chet, 1987; Bennett and Lane, 1992; Kumar and Mukherji, 1996, Rudresh *et al.*, Sivan *et al.*, 1984; Coley- Smith *et al.*, 1991) and consider as a plant growth enhancer and plant defence inducer (Verma *et al.*, 2007). It is encouraging that the application of *Trichoderma* spp. with other biofertilizer is now also getting importance for enhancing nutrition and suppressing soil borne plant pathogens (Rudresh *et al.*, 2005; Whipps and Lumsden, 2001). There is no doubt that both this two are highly potential genus with several of its species having antagonistic potential. So, attention needs to be given towards this bio-control agent. So, the study on the interaction of rhizobacteria with other bio-inoculants and their effects on PGPR both *in vitro* and *in vivo* need to be done to judge their compatibility with other bio-inoculants. The study of combining these two organisms is of great potential value to organic agriculture in order to avoid fertilizers and pesticides. In the present investigation two groups of microorganisms viz., *Rhizobium* and *Trichoderma* spp. were studied to document both their compatibility and their combined effects on the growth and yield of lentil both i*n vitro* and *in vivo* conditions. In respect this, few strains of *Trichoderma* were isolated from different lentil rhizosphere of West Bengal and they were then tested *in vitro* and *in vivo* along with *Rhizobium* to know the interaction between them and their on the enhancement the seedling vigour of Lentil.

Materials and Methods

Collection of soil samples

Soils were collected from different locations of Nadia district, West Bengal. Samples were collected from lentil rhizosphere. The location from where the samples were collected was far away from one another so as to represent an eco-distribution pattern of *Trichoderma* habitation of West Bengal. Sl. No. Isolation code Village/Sample

MSTh1 Marketed Sample
HTh2 Hoogly
ShTh3 Shekhampur
RaTh4 Ranaghat
ChTh5 Chakdah
KaTh6 Kakdwip

The *Rhizobium* was collected from the Survey selection and Mass production, BCKV.

Isolation technique

Trichoderma were isolated from the soil of the Lentil rhizosphere (Harris and Sommers, 1968) using TSM (Elad and Chet, 1983). The soils were collected from rhizosphere of different locations of WB and dried under shade and ground to powder with a mortar and pestle and passed through 2mm mesh sieve, 10 gm of powdered soil was mixed with 90 ml of sterile distilled water to prepare mother suspension. This suspension was used for serial dilution continued up to 10-2 to 10-7.1ml of the suspension from each of 10-6 and 10-7 dilution was placed on 20 ml of TSM in each of the sterilized petriplates by giving a gentle whirling motion to the plate and allowed them to incubate in 28 (±1°C) temperature (Islam *et al.*, 2005) for seven days. 3 replications were maintained for each dilution for a particular soil.

The green colonies of the bio antagonists usually appeared at the 4th and 5th day of incubation. Each colony was studied carefully under microscope using 0.1% lactophenol cotton blue stain (0.1gm cotton blue mixed in 100ml of lactophenol solution) and compared to the monographs of *Trichoderma* (Rifai, 1969) for identification at genus and species level. The separated colonies were then

transferred to PDA slants by using single hyphal tip method (Rangaswami, 1958). The slants were maintained at 5°C for further use.

Maintenance of rhizobacterial isolates

The individual bacterial colony was picked up and streaked on Yeast extract manitol agar (YEMA) and after incubation at 28ºC (±1°C), kept at 4°C for short period maintenance. Periodic (at 15 days intervals) sub culturing was done on the same medium.

Compatibility study between the *Trichoderma* isolates and *Rhizobium* (*In vitro*)

Dual culture method

The *Tricoderma* isolates were plated with *Rhizobium* to evaluate their interaction by dual culture technique as described by Morton and Strouble (1955). A 5mm diameter mycelia disc from the margin of the 7 days old culture of *Trichoderma* isolates was placed at the one end of the plate one cm away from the periphery and 108 cfu/ml suspension of *Rhizobium* was streaked at the other periphery.

The experiment was setup in completely randomised design with 3 replications for each treatment. In control only *Trichoderma* discs were placed. Inoculate plates were incubated at 28o C (±1°C) for 7 days. 2-3 days after the incubation period, radial growth of *Trichoderma* isolates was measured and per cent inhibition of average radial growth was calculated in relation to mycelia growth of the control (Vincent, 1947).

I= x100

I= % Inhibition of growth
C= mycelial growth in control plate
T= mycelial growth in test plate

Culture filtrate method

Trichoderma harzianum was inoculated to flasks containing sterilized Czapeck's broth with 0.1 per cent yeast extract and incubated for 12 days and the contents were filtered and passed through membrane filter. The filtrate was incorporated into Yeast extract manitol broth at 20 % (VN) and inoculated with 0.5ml of *Rhizobium* cell suspension (3x 10^7 CFU/ml) and incubated on a rotary shaker for 72 at 28°C (±1°C). At the end of incubation the population of *Rhizobium* was estimated (Jayaraj and Ramabadran, 1999).

Evaluation of growth interaction of *Rhizobium* and *Trichoderma in vitro* (by colorimeter)

Four strains of *Trichoderma* were grown with *Rhizobium* species in YEMB to study the growth pattern of both. 50 ml of Yeast Extract Manitol broth was sterilized and inoculated with 0.5 ml of spore suspension *(*3x10^7 spores/ml) and 0.5 ml of the cell suspension of *Rhizobium* (3x10^7 CFU/ml). Both *Trichoderma* and *Rhizobium* was grown individually in YEMB in different flasks for comparison. The flasks were continuously shaken in an orbital shaker for 72 h at 28°C (±1°C) in 100 rpm after which the intensity of growth was assessed by observing the optical density of the medium in a colorimeter at 530 nm (Jayaraj and Ramabadran, 1999). To count the population of *Trichoderma*, *Rhizobium* individually and in combination serial dilution and plating method using specific medium were followed.

Evaluation of interaction between selected *Trichoderma* isolates with *Rhizobium* (*in vivo*)

Lentil seeds (Var. B-177) were tested for germination percentage by wet blotter technique to ascertain that it is free from any

diseases (De Tempe and Binnerts, 1979) fifty seeds were arranged at equal distance on a wet blotter placed in a petriplate and incubated at room temperature for three days. The numbers of germinated seeds out of total number of seeds observed were recorded along with pathogen infestations, if any. After testing the lot for germination percentage and seed health, Lentil seeds (B- 177) were soaked in water for 24 hours (one day before the experiment). The seeds were surface sterilized with $HgCl_2$ and treated with the following organisms: (a) *Trichoderma spp.* spore suspension (1-3 x $l0^8$ spores/ml) @ 3ml/10gm of seed; (b) *Rhizobium* cell suspension (1-3 x $l0^8$ CFU/ml) @ 3 ml/ 20g of seed; (c) *Trichoderma* + *Rhizobium* at the above level and (d) control seeds surface sterilized 0.2% $HgCI_2$ with treating them with *Rhizobium* and *Trichoderma* individually.

The seeds (6 Nos.) were sown in earthen pots (10 x15 cm) filled with unsterilized soil. Regular watering and observations were made during the development of the plants. The plants were removed after 30 days from each pot and the root zone was washed under running tap. The pinkish nodules were counted and the other observations were recorded (Jayaraj and Ramabadran, 1999).

Results and Discussion

Evaluation of antagonistic potential of selected *Trichoderma* isolate against *Rhizobium*

Different *Trichoderma spp.* were isolated, one from Market sample (MSTh1) and rest 5 from different places of West Bengal like Hoogly (HTh2), Shekhampur (ShTh3), Ranaghat (RaTh4), Chakdah (ChTh5), Kakdwip (KaTh6) from the root rhizosphere of different pulse crops. These *Trichoderma* isolates were grown with the *Rhizobium* collected from the surface selection, mass production, BCKV, to know the antagonistic potential or compatibility against the *Rhizobium in vitro* condition. The dual culture plating was done and observations were recorded at 2 days interval after the date of inoculation. It was observed that in every date of observation the growth of the *Trichoderma* was different and increased significantly with the increase age of the culture. Among the different isolates of *Trichoderma* Market sample (MSTh1) produced profused growth along with *Rhizobium* and significantly at par with individual growth of this Market sample of *Trichoderma* (untreated control) (7.60 cm) which cover the full plate after 9 days of inoculation (Table 1 and Fig. 1).

It was also observed that, the Shekhampur isolate of *Trichoderma* (ShTh3), and Ranaghat isolate of *Trichoderma* (RaTh4) were also significantly at par with each other and also grow profusely (7.53 cm and 7.50 cm respectively), whereas minimum growth were observed from Hoogly of *Trichoderma* (HTh2) at 9 days after inoculation which is 5.93 cm. According to their growth over *Rhizobium*, Market sample of *Trichoderma* (MSTh1) produced maximum followed by *Trichoderma* isolated from Shekhampur (ShTh3) *i.e.* 5.23cm and minimum in Hoogly (HTh2) isolate 4.33cm irrespective of their age of growth. According to their growth against *Rhizobium*, (Plate: 3) Ranaghat (RaTh4), Chakdah (ChTh5) and Kakdwip (KaTh6) were similar (4.68, 4.46, and 4.47cm respectively) (Table 4).

The *in vitro* study of different *Trichoderma* isolates and *Rhizobium* in dual culture plate technique showed Market sample of *Trichoderma* produced profuse growth along with *Rhizobium* and minimum by Hoogly isolate. It indicated that *Rhizobium* and *Trichoderma* isolates showed a compatible reaction in growth and it was suggested by Jayaraj and Ramanadran (1999).

Influence of cultural filtrate of *Trichoderma spp*. on the growth of *Rhizobium* and vice versa

Influence of *Rhizobium* on the mycelia growth of *Trichoderma*

Different cultural filtrate of different isolates of *Trichoderma* also used to find out the antagonistic potentiality or compatibility against *Rhizobium* up to 240 hours after inoculation. Every 48 hours after inoculation the result showed that the growth of *Trichoderma* were increased significantly and maximum was observed after 240 hours of inoculation (6.905 cm). Though, the interaction between the *Trichoderma* isolates and the age of the colony was not statistically significant. Here, Shekhampur (ShTh3), Ranaghat (RaTh4), and Chakdah (ChTh5) isolate of *Trichoderma* showed no significant difference among themselves in increase the growth of colony diameter (4.87, 4.93. and 4.72 cm respectively) whereas maximum as noticed on Market sample isolate (MSTh1) 5.267cm and minimum is Kakdwip isolate (KaTh6) 3.517cm (Tables 2 and 5).

Influence of *Trichoderma filtrate on Rhizobium* population

Rhizobium was inoculated on 6 different culture filtrate of *Trichoderma* and it was observed that increase in the age if inoculation there is significant increase in *Rhizobium* population irrespective of different *Trichoderma* isolates.

Though within the cultural filtrate of different *Trichoderma* isolates, the growth of *Rhizobium* were reduced significantly in comparison to untreated control (only *Rhizobium* isolate), no *Trichoderma* inoculation, except Chakdah isolate of *Trichoderma* (ChTh5) where the *Rhizobium* colony was highest (119.67) statistically at par with untreated control *Rhizobium* without *Trichoderma* filtrate (124.60) irrespective of date of observations (Fig. 1). It was also observed that the *Rhizobium* growth was statistically insignificant when they are inoculated on cultural filtrate of *Trichoderma* Hoogly isolate (163 nos.), Ranaghat isolate (161.67), and Kakdwip isolate (168.33) on last date of observation. In every date of observation the colony growth of *Rhizobium* on cultural filtrate of Chakdah isolate of *Trichoderma* (ChTh5) was statistically at par with each other, whereas, minimum *Rhizobium* growth (colony count) was noticed when the *Rhizobium* grow on the cultural filtrate of Market sample of *Trichoderma* in every date of observation statistically at par with Hoogly isolate of *Trichoderma* (Table 3). The results therefore indicate that, except Chakdah isolate of *Trichoderma*, other cultural filtrates of *Trichoderma* showed some inhibitory effect on the growth of *Rhizobium* (Table 5).

When the cultural filtrate of *Trichoderma* was in corporate into the YEMA and inoculated with *Rhizobium* cell suspension, it was observed that all the cultural filtrate showed some inhibitory effect on the growth of *Rhizobium* except Chakdah isolate of *Trichoderma* which was similar to that of the result Sethi and Subha Rao (1968) that growth of *Rhizobium* showed slight inhibitory effect on the certain strains of *Trichoderma.* Similarly, the growth of different strain was increased significantly without the effect of *Rhizobium*. It indicated that growth of *Trichoderma* showed no inhibition when they incorporated with *Rhizobium*.

Intensity of growth of different isolates of *Trichoderma* with *Rhizobium* and its OD Value

The results showed that OD value of different *Trichoderma* isolates over *Rhizobium* was

different and their differences were statistically significant. Maximum OD value was observed on Market sample of *Trichoderma* over *Rhizobium* (0.590) followed by Ranaghat isolate over *Rhizobium* (0.513) and Hooghly isolate of *Trichoderma* (0.510). Though, their differences were statistically significant except later two treatments and minimum in Shekhampur isolate of *Trichoderma* over *Rhizobium* (0.280).

Growth of *Rhizobium* and their OD value within the different *Trichoderma* isolates were statistically significant. Maximum *Rhizobium* growth was increased over Shekhampur isolate (51.28%) followed by the growth of *Rhizobium* on Ranaghat and Hoogly isolates of *Trichoderma* (21.02% and 21.53% respectively) and minimum within the market sample of *Trichoderma* (9.23). though the OD value of *Rhizobium* was statistically at par with Ranaghat and Hoogly isolates of *Trichoderma*.

Growth of different isolates of *Trichoderma* over *Rhizobium* were decreased significantly and maximum growth reduction of OD value was observed on Ranaghat isolate (-49.60) and minimum in market sample of *Trichoderma* (-21.19) followed by Shekhampur isolate (-22.18) and Hoogly isolate (-26.95) though later 3 *Trichoderma* isolates showed no significant differences in reduction of OD value within the *Rhizobium* over control growth of *Trichoderma.* The population of *Rhizobium* in control condition and their OD value showed no significant differences within themselves.

Similarly the growth of *Trichoderma*, the growth of *Rhizobium* within different *Trichoderma* isolates showed no significant differences among themselves over the untreated control of *Rhizobium.* The population of different isolates of *Trichoderma* was statistically significant and maximum population was observed in Market sample of *Trichoderma* (5.66) statistically at par with Shekhampur isolates of *Trichoderma* (5.33) and minimum in Ranaghat isolate of *Trichoderma* (2.33) statistically at par with Hoogly isolate (2.66).

Table.1 *In vitro* antagonistic potential of *Trichoderma* isolates against *Rhizobium*

Interval of Observations (D) / **Location (L)**	**72hrs**	**120hrs**	**168hrs**	**216hrs**	**Mean**
T(MS) (MSTh1)	4.30	5.40	5.83	7.60	**5.78**
Hooghly (HTh2),	3.10	3.97	4.30	5.93	**4.33**
Shekhampur (ShTh3)	3.70	4.53	5.17	7.53	**5.23**
Ranaghat (RaTh4)	1.93	4.00	5.30	7.50	**4.68**
Chakdah (ChTh5)	2.43	3.73	5.00	6.67	**4.46**
Kakdwip (KaTh6)	2.40	3.73	5.37	6.37	**4.47**
Control	3.40	5.10	6.07	7.60	**5.54**
Mean	**3.04**	**4.35**	**5.29**	**7.03**	
	D	**L**	**D × L**		
SEm (±)	**0.195**	**0.257**	**0.515**		
CD (P=0.05)	**0.552**	**0.728**	**NS**		

Table.2 Effect of *Rhizobium* on mycelia growth of *Trichoderma (in cm)*

Interval of Observations (D) / Location (L)	96hrs	144hrs	192hrs	240hrs	Mean
T(MS) (MSTh1)	3.133	4.033	6.433	7.467	**5.267**
Hooghly (HTh2),	2.600	4.000	5.567	7.333	**4.875**
Shekhampur (ShTh3)	2.867	3.800	5.567	7.500	**4.933**
Ranaghat (RaTh4)	2.933	3.833	5.333	6.800	**4.725**
Chakdah (ChTh5)	2.633	3.233	4.833	5.433	**4.033**
Kakdwip (KaTh6)	1.600	2.300	3.967	6.200	**3.517**
T(MS) (MSTh1)	3.333	4.967	6.900	7.600	**5.700**
Mean	**2.729**	**3.738**	**5.514**	**6.905**	
	D	**L**	**D × L**		
SEm (±)	**0.118**	**0.156**	**0.313**		
CD (P=0.05)	**0.334**	**0.442**	**NS**		

Table.3 *In vitro* colony count of *Rhizobium* in nos. over different isolates *Trichoderma filtrate*

Date of Observation (D) / Isolates (I)	96hrs	144hrs	192hrs	240hrs	Mean
I_1 = T (MS)	51.33	122.33	137.33	151.00	**92.60**
I_2 = Hooghly	59.67	127.67	145.33	163.00	**99.53**
I_3 = Shekhampur	66.00	132.67	148.67	156.00	**101.27**
I_4 = Ranaghat	74.33	139.33	150.00	161.67	**105.87**
I_5 = Chakdah	78.33	147.00	180.00	188.00	**119.67**
I_6 = Kakdwip	66.33	124.00	152.00	168.33	**103.33**
I_7 = Control (*Rhizobium*)	88.00	157.00	180.67	190.33	**124.60**
Mean	**62.40**	**121.03**	**139.28**	**150.07**	
	Date of Observation (D)	**Isolates (I)**	**D × I**		
SEm (±)	**2.740**	**3.625**	**7.250**		
CD (P=0.05)	**7.762**	**10.270**	**NS**		

Table.4 Effect of interaction between *Trichoderma* and *Rhizobium* in their OD value as well as population

Doses	OD VALUE	A	B	C	D	E	F
R+T(MS)	0.590	9.23	-21.19	95.33	43.18	5.66	46.11
		(17.68)	(27.24)		(41.07)		(42.76)
R+T(S)	0.280	51.28	-22.18	101.66	42.27	5.33	35.64
		(45.73)	(27.83)		(40.53)		(36.56)
R+T(H)	0.510	21.53	-26.95	99.33	41.34	2.66	57.93
		(27.64)	(31.10)		(40.00)		(49.60)
R+T(R)	0.513	21.02	-49.60	95.66	42.93	2.33	67.85
		(27.24)	(44.77)		(40.91)		(55.56)
SEm (±)	**0.005**	**1.279**	**2.390**	**4.038**	**1.604**	**0.236**	**2.328**
CD (P=0.05)	**0.015**	**3.941**	**7.364**	**NS**	**NS**	**0.727**	**7.173**

A=OD value over control *Rhizobium* (increase),B=OD value over control *Trichoderma* (decrease),C=Population of *Rhizobium* as $1x10^8$ cfu/ml, D=Population of *Rhizobium* decrease over control, E=Population of *Trichoderma* as $1x10^8$ spore/ml, F=Population of *Trichoderma* decrease over control, R+T(MS)= *Rhizobium+Trichoderma* (Market sample), R+T(S)= *Rhizobium+Trichoderma* (Shekhampur), R+T(H)= *Rhizobium+Trichoderma* (Hoogly), R+T(R)= *Rhizobium+Trichoderma* (Ranaghat)

Table.5 Effect of *Rhizobium-Trichoderma* interaction on the nodulation and root

Treatments	Nodule		Shoot			Root		
	No. of Nodule (nos.)	Fresh Nodule wt. (gm)	Shoot length (cm)	Fresh shoot wt. (gm)	Dry shoot wt. (gm)	Root length (cm)	Fresh root wt.	Dry root wt.
Rhizobium	57.67	0.048	87.50	0.853	0.336	37.83	0.103	0.064
T(MS)	44.67	0.039	81.37	0.699	0.315	41.70	0.114	0.039
T(S)	43.67	0.038	83.33	0.812	0.302	43.83	0.110	0.051
T(H)	42.00	0.037	84.07	0.817	0.284	40.49	0.102	0.017
R+T(MS)	76.33	0.068	79.67	0.864	0.333	41.13	0.081	0.042
R+T(S)	67.67	0.058	83.33	0.838	0.342	38.90	0.089	0.179
R+T(H)	61.00	0.049	78.20	0.829	0.289	40.00	0.101	0.034
Control	34.00	0.035	79.87	0.825	0.253	39.93	0.090	0.038
SEm (±)	**3.219**	**0.003**	**3.54**	**0.032**	**0.017**	**2.490**	**0.007**	**0.048**
CD (P=0.05)	**9.651**	**0.009**	**NS**	**NS**	**0.051**	**NS**	**0.021**	**NS**

R+T(MS)= *Rhizobium+Trichoderma* (Market sample), R+T(S)= *Rhizobium+Trichoderma* (Shekhampur), R+T(H)= *Rhizobium+Trichoderma* (Hoogly), R+T(R)= *Rhizobium+Trichoderma* (Ranaghat)

Fig.1 Dual culture technique showing interaction between 6 *Trichoderma* isolates and one *Rhizobium*

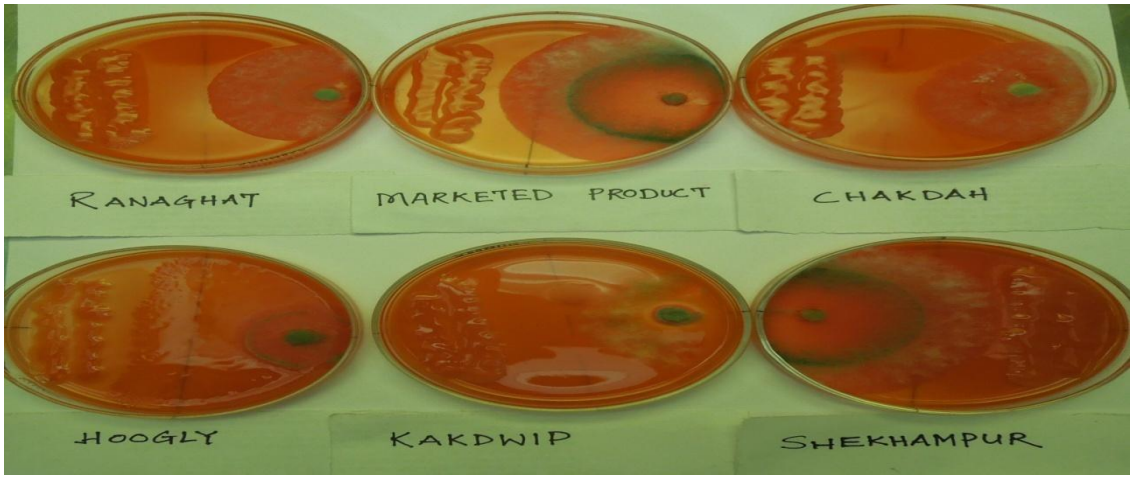

Fig.2 Effect of cultural filtrate of different *Trichoderma* isolates on the population of *Rhizobium*

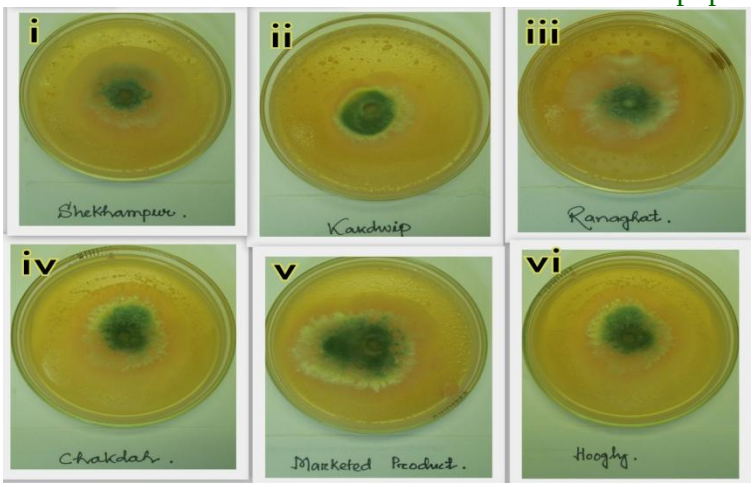

Fig.3 1(a) & 1(b) are 30 days old lentil seedlings in pot in control and treated with Market Product respectively. 2(a) & 2(b) are 30 days old lentil shoot in control and treatment condition (Market product) respectively. 3(a) & 3(b) are root of 30 days old lentil in control and treatment Condition (Market product) respectively

Population of different isolates *Trichoderma* were also decreased significantly over the *Rhizobium* and the maximum reduction was observed by Ranaghat isolate of *Trichoderma* (67.85%) followed by Hoogly isolate (57.93%) and minimum in Shekhampur isolate (35.64%) followed by market sample of *Trichoderma* (46.11%) and their differences were statistically significant. (Table 13)It was also observed that the individual growth of *Trichoderma* and *Rhizobium* was affected significantly when they were dual cultured in broth. The OD value as well as the population of *Rhizobium* showed no significant effect when cultured along with different isolated of *Trichoderma* though Sethi and Subha Rao (1968) observed a slight inhibitory effect of *Rhizobium* in the presence of certain strains of *Trichoderma*. But Jayaraj and Ramabadran (1999) indicated that mycelia mat of certain isolates of *Trichoderma* spp. strongly inhibit the growth of the *Rhizobium*. This inhibition may be due to the competition by the individual organism on the nutrient utilization and growth, antibiosis and lysis. Similarly, Jayaraj *et al.*, (1994), observed a significant but slight inhibition of growth of 2 strains of *Rhizobium* by *T. viride* and *T. harzianum in vitro.*

Rhizobium – *Trichoderma* interaction and their effect on Lentil *in vivo*

A pot culture experiment were carried out using 3 isolates of *Trichoderma* i.e. Market sample (MSTh1), Shekhampur (ShTh3), Hoogly (HTh2) along with *Rhizobium* culture obtained from BCKV mass culture production were applied individually or in combination showed 100% germination of seeds in every treatment. Other growth factors were also collected the shoot length, root length, fresh and dry shoot weight, fresh and dry root weight, number of nodules and their fresh and dry weight. It was observed that shoot length, root length, fresh shoot weight and dry root weight showed no significant difference among themselves in increase their growth pattern. Whereas, number of nodules produced in the root, fresh nodule weight, dry nodule weight, dry shoot weight, fresh root weight were significantly differ in their growth behavior.

Number of nodules

Maximum number of nodules were produces on Market sample (MSTh1) of *Trichoderma* along with *Rhizobium* inoculated seed (76.33 nos.) followed by *Trichoderma* isolated from Shekhampur isolate (ShTh3) along with *Rhizobium* (67.67 nos.) though their difference were not statistically significant. The minimum number of nodules was observed in untreated control where no *Rhizobium* or no *Trichoderma* were added (34 numbers). It was also observed that combined application of *Trichoderma* and *Rhizobium* increase the number of nodule/6 plants in comparison to individual application of *Trichoderma* and *Rhizobium* (Table5).

Fresh weight of nodules

Similarly fresh weight of nodules was also increased in different treatment combination and their differences were statically significant. Maximum weight was observed in *Rhizobium* along with Market sample (MSTh1) combination (0.068gm) followed by combination with *Rhizobium* along with *Trichoderma* Shekhampur isolate (ShTh3) (0.058gm). *Rhizobium* along with *Trichoderma* Hoogly (0.049gm) and only *Rhizobium* (0.048gm), their difference were statistically significant except later two treatments. Minimum fresh nodule weight was observed only *Trichoderma* of Shekhampur (ShTh3), Market sample (MSTh1), Hoogly and untreated control though their differences were statically in significant.

Shoot length

In case of shoot length, it was observed that, all the treatments combinations increase their shoot length in comparison to untreated control though their differences were not statistically significant. Here, individual application of *Rhizobium* and *Trichoderma* and their combined application showed no significant difference increase the shoot length. Similarly, fresh shoot weight was maximum in combined application of *Rhizobium* along with Market sample (MSTh1) (0.864) followed by single application of *Rhizobium* (0.853gm), combined application of *Rhizobium* along with Shekhampur isolate (ShTh3) (0.838gm) though their difference were not statistically significant were as minimum was observed Market sample (MSTh1) of *Trichoderma* only (0.699gm). Individual application of *Rhizobium*, *Trichoderma* and its combination showed differential dry shoot weight and their difference were statistically significant. Maximum dry shoot weight was found on *Rhizobium* and *Trichoderma* Shekhampur (ShTh3) isolate combination (0.342) followed by sole application of *Rhizobium* 0.33643gm combined application of *Rhizobium* and *Trichoderma* Market sample (MSTh1) isolate (0.333gm) and sole application of *Trichoderma* (Market sample (MSTh1) (0.315gm) and differences were not statistically significant (Table 5 and Fig. 3).

Root length

Root length was also different in different treatment sand maximum was observed sole application of *Trichoderma* Shekhampur (ShTh3) isolate 43.83cm followed by combined application of *Rhizobium* and *Trichoderma* Market sample (MSTh1) isolate (41.13). Sole application of *Trichoderma* Market sample (MSTh1) 41.70 and they were statistically at par among themselves. It was observed that root length of different treatments showed no significant among themselves. Fresh root weights in different treatments were different and their differences were statistically significant. Among the treatments maximum weight was observed on sole application of *Trichoderma* Market sample (MSTh1) 0.114gm followed by *Trichoderma,* Shekhampur (ShTh3), *Trichoderma* Hoogly (HTh2), *Rhizobium* and their differences were not statistically significant Dry root weight was maximum on combined application of *Rhizobium, Trichoderma* and *Trichoderma* Shekhampur (ShTh3) isolate 0.17947gm followed by sole application of *Rhizobium* 0.06487gm sole application of *Trichoderma* Shekampur (ShTh3) 0.05180gm though their differences were not statistically significant (Table 5 and Fig. 3).

The pot culture experiment on Lentil seed (Var.- B-177) treated with different *Trichoderma* isolates ($1x10^8$ spore suspension) and *Rhizobium* cell suspension ($1x10^8$ cfu/ml) were sown on earthen pot under greenhouse condition to find out the different growth behavior of lentil plant due to combined application of *Rhizobium* and *Trichoderma* and its was observed that different growth behavior like no. of nodule, fresh nodule weight, dry root weight, dry shoot weight, fresh root weight are significantly different among themselves, in increase or decrease of growth behaviors' when they were applied individually or in combination. Whereas shoot length, fresh shoot weight, root length and dry root weight showed no significant difference among the different treatment combination. Here, it was observed that the combination of *Rhizobium* and Shekhampur and Market sample isolate of *Trichoderma* (MSTh1) increased the number of root nodules, fresh nodules, dry nodule weight, dry nodule weight, dry shoot weight significantly in comparison to control

and sole application of *Rhizobium.* Whereas, the result contradict with the result of Jayaraj and Ramabadran (1999) that *Rhizobium* alone and *Rhizobium Trichoderma* mixture were statistically at par on increase the number of nodules. Though, biomass yield increased slightly when *Rhizobium* and *Trichoderma* were applied in combination. Similar type of result was also observed by Sridhar *et al.*, (1993) that slight increase in nodulation of Urd bean following combined inoculation of *Rhizobium* with *Bacillus subtilis.*

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