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RESEARCH ARTICLE

STUDY OF SOME ANTAGONISTIC SOIL FUNGI FOR PROTECTION OF FRUIT ROT (*PHOMOPSIS VEXANS*) AND GROWTH PROMOTION OF BRINJAL.

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Abstract

Application of chemical fungicides and fertilizer in agrifield for protection and growth enhancement is now serious concerns for sustainable agriculture and environmental monitoring as these two items are creating health hazards and environmental pollution. There are many soil fungi and they play an important role in soil formulation, soil fertility, soil structure, soil improvement and crop protection. In this experiment, three soil fungi namely *Trichoderma viride*, *T. harzianum* and *Beauveria bassiana* were applied in brinjal (*Solanum melongena*) for combating dangerous brinjal disease *Phomopsis* fruit rot of brinjal and growth enhancement of this vegetable. Their efficacy was compared with blitox 50 fungicide. In lab. condition by incised method, *Phomopsis vexans* along with *T. viride* inoculated fruits showed 20 PDI while *Phomopsis vexans* inoculated fruits 100 PDI. *Phomopsis vexans* with *T. harzianum* and *Phomopsis vexans* with *B. bassiana* inoculated fruits show 30 and 50 PDI respectively. *T. viride* gave maximum crop protection followed by *T. harzianum* and *B. bassiana* respectively. The three years field trial indicated that continuous three years (2014-2016), PDI is lesser (30.25, 35.00 and 28.75 respectively) in the treatment of *T. viride* in comparison with other treatments (*T. harzianum* and Blitox 50) and untreated. It also showed that the height, the dry weight, no. of leaves and no. of leaf area of the brinjal plant, treated with *T. viride* (spore suspension 1×10^7 per ml) gave better result in comparison to control (untreated) brinjal plant. It reflects the potentiality of plant growth promotion. The recommendation is to avoid fungicides and chemical fertilizer; farmers may alternatively apply *T. viride* spore suspension (10^7 spore / ml) during seedling treatment and four consecutive spraying of this suspension at the interval of fifteen days after initiation of fruits.

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Introduction:-

Recently modern science has paid much attention to soil fungi for disease management and environmental monitoring. The vital function played by soil fungi together with other microbes is the decomposition of organic matter and the release of the nutrients locked up in plants and animals, bringing about the recycling of nutrients.

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There are about 75,000 species of soil fungi in the world (Finlay, 2007), and the soil formulation, soil fertility, and crop protection largely depend on microbial community (Schimel *et al.*, 2005). Once a suitable isolate of antagonistic soil fungi has been identified from the bioassay screening procedure, the isolate then be applied and tested on a larger scale, in the field. Plant disease and lack of soil nutrients such as NPK continue to threaten crop production in modern agriculture and play a direct role in net loss of crop productivity in agriculture. With the advent of chemical fertilizers and pesticides /fungicides, it was thought that a permanent and reliable solution of soil fertility and crop pathogens have been achieved but it has been proved that chemical fertilizers and pesticide application are not safe to the environment as the toxicants cause environmental pollution and have harmful effects on human beings (PAN, 2007). Recently, US Dept of Health and Human Services (2008) reported that the chemicals benomyl, carbamates and mancozeb are carcinogenic. The triazoles fungicides are causing reproductive defect of male (Goetz *et al.*, 2007) and female mice (Rockett *et al.*, 2006). All humans now carry a body burden of persistent pesticides, many of which are linked to chronic health effects (Schafer *et al.*, 2004; PAN, 2007). Some pesticides are even carcinogenic and causing some human cancer such as colorectal cancer (Lee *et al.*, 2007), breast cancer (Abdalla *et al.*, 2003), leukemia and non-Hodgkin's lymphoma in childhood (Meinert *et al.*, 2000). However, the potential impact on environment as well as health largely limits their application (Eckert *et al.*, 1994). Chemical fertilizers may have harmful effects on the soil and its life, especially when they are very concentrated and water soluble (Smith *et al.*, 2008). Ammonium sulphate is a very strong biocide, hindering nitrogen fixation and killing nematodes and earthworms. Superphosphate has a negative effect on free-living nitrogen-fixing bacteria (Primavesi and Primavesi, 1990). Nitrate levels above 10 mg/L (10 ppm) in groundwater can cause 'blue baby syndrome' (acquired methemoglobinemia), leading to hypoxia (which can lead to coma and death if not treated) (Ward *et al.*, 2005; Powler, 2006). Unfortunately to gain a target crop production with chemical fertilizers and pesticides, over 100 species of non target organisms are adversely affected (Alabouvette and Couteadier, 1992). Despite realization of adverse effects of chemical fertilizers and pesticides on plants, animals and environment, they are being applied indiscriminately (Eckert and Ogawa, 1988; PAN, 2007). Current problems include the continued development of fungicide resistance among pathogens (Spotts and Cervantes, 1986; Holmes and Eckert, 1999; Kanetis *et al.*, 2008; Smilanick, 2011). Hence, to reduce the use or dose of chemicals, one possibility is to utilize the disease suppressing activity and plant growth promoting capacity of certain microorganisms in agrifields which should be highly ecofriendly. Such microorganisms are commonly referred to as biological control (biocontrol) agents (BCA) and plant growth promoters (PGP) and their commercial formulations are as biopesticides and biofertilizer. *Trichoderma* spp. are active rhizosphere colonisers (Tronsmo and Harman, 1992) and act as BCA and PGP. The use of PGP/BCA offers an attractive way to replace chemical fertilizer, pesticides, and supplements; most of the isolates result in a significant increase in plant height, root length, and dry matter production of shoot and root of plants. *Aspergillus* sp., *Penicillium* sp., *Trichoderma* sp., *Beauveria bassiana* amongst fungi offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to the plants (Sharma, 2003; Chen *et al.*, 2006). It has also been established that biocontrol agents enhance growth by producing growth stimulating factors (Windham *et al.*, 1986, Ponmurugan and Baby, 2006b). *Trichoderma* has a superior capacity to mobilize and take up soil nutrients compared to other organisms (Chet *et al.*, 1997). Egg plant (*Solanum melongena* L.; family- Solanaceae) being native to India, is one of the most common vegetable crops of the country. Based upon its highest production potential and availability of the products to consumers, it is also termed as poor man's vegetable. Brinjal is grown for its unripe, immature fruits, which are used as cooked vegetable and in curries (Bhat and Vasanthi 2008). The area under brinjal cultivation is estimated at 680,000 Ha with a total production of 11,896,000 Mt at the rate of 17.5 Mt/Ha (FAO data, 2012). In most of the tropical and sub tropical areas *Phomopsis* blight and fruit rot of eggplant incited by *Phomopsis vexans* (Sacc. & Syd.) Harter is considered to be the most serious and wide spread disease. *Phomopsis* blight of eggplant occurred almost every year in the months of March- April and October- November in severe forms. Near about throughout the year the people of W. Bengal consume the fruits as vegetable as farmers generally treat the pesticides which are carcinogenic; so, we are constantly in taking these. Keeping environment pollution and human health hazards in mind, we have tried to cultivate brinjal in agrifield without chemical fertilizer and fungicide but by using three ecofriendly soil fungi which protect the crop and enhance the crop growth.

Materials and methods:-

Antagonistic fungi:-

Trichoderma viride (Genebank Accession no. KY966032), *Trichoderma harzianum* (Genebank Accession no. KY966020), and *Beauveria bassiana* (Genebank Accession no. KM604668.1) antagonistic fungi were previously isolated from soil and phenotypically and molecularly (PCR based ITS1-5.8S -ITS2) identified in our laboratory.

Isolation and purification of pathogen from disease rotted fruit (Brinjal):-

Five gram of rotten fruit was aseptically cut out and smashed and mixed in 50 ml sterile distilled water under Laminar air flow. The solution and diluents were streaked on to PDA petri dishes containing penicillin (1.6ug /ml) and incubated at 28 °C temperature for 3 days. After three days, each colony of the fungus was transferred to PDA slant by inoculating needle and incubated at 28 °C for one week and stored at 4°C in refrigerator (Dingra & Sinclair 1985).

Purification of pathogen:-

It was done by hyphal tip method in PDA medium

Characterization and identification of isolated pathogen:-

The isolated pathogen was grown separately in Petri plates (9.00cm) containing PDA medium at 28 ±2⁰C in B.O.D. incubator for 7 days. Different cultural (colony texture, growth pattern, linear growth, colour of reverse plate) were recorded. The Mycelium was taken on slides. It was stained by cotton blue, mounted by lactophenol and covered by covered slip. Finally observed under compound microscope for character of hypha, conidiophore, phialide and conidia. All characters were recorded. The measurement of breadth, hyphae, conidiophore, phialide and breadth and length of conidia were done with the help of ocular and stage micrometer. The fungal pathogen was identified with the help of literature (Barnett *et. al.*, 1998; Gilman 2001; Subramanian 2007, Subramanian 1971, Domsch *et. al.*, 1980, Nagamani *et. al.*, 2006).

Establishment of pathogenicity of isolated pathogen:-

Phomopsis vexans - The pure culture of *Phomopsis vexans* isolated were raised in 9 mm petridishes containing 20 ml sterilized PDA medium at 28 ±2⁰C in B.O.D. incubator. Fresh & disease free brinjal fruits were taken & washed under tap water. After washing, water was soaked by blotting papers. The washed brinjals were surface sterilized by alcohol soaked cotton. With the help of sterilized cork borer (5 mm diameter) a bore was made in each fruit near the flame under Laminar Air flow chamber. A bit of injured inoculum from the growing colony of *P. vexans* was aseptically transferred to each hole. The inoculated hole was sealed by sterilized cotton & the cotton was attached to fruit with the help of cello tape. After 3 days the rotted portion was recorded.

The Study of protection of brinjal by antagonists (artificial inoculation method):-

Fresh and disease free more or less equal size of brinjal fruit (variety :muktakeshi) were harvested from field and washed by tap and distilled water simultaneously. Then their surfaces were sterilized by alcohol soaked cotton under Laminar air flow chamber. 5 mm. injury was done by sterilized incised needle and equal amount of inoculum of *Phomopsis vexans* and antagonist was inoculated into the injured hole. One piece of sterilized cotton was covered over the inoculated zone with the help of cello tape. The inoculated brinjals were incubated at 28 ± 2⁰ C. temperature in BOD incubator for 7 days. For each antagonist, 10 brinjal fruits were taken. One set inoculated with only *P.vexans* was also made. Similarly one control (without any inoculation by placing only a piece of agar medium) set was also run. Similar experiment was repeated by 3 times. After 7 days, number of brinjal fruit infected, rotted and also uninfected were recorded. PDI (Percent of disease index) was calculated by the formula:

$$PDI = (\text{Total number of fruit} - \text{total number of fruit rotted}) \div (\text{Total number of fruit inoculated}) \times 100$$

Comparative study of efficacy of different antagonists for controlling fruit rot of brinjal (*P. vexans*):-

Experimental set was similar to earlier. Here radius of infected or rotted tissue (Inoculated by both pathogen and antagonist) was measured by 12 hr interval. The progress of rotted tissue was calculated.

Effect of antagonists on seed germination, root length & shoot length of brinjal (var muktakeshi):-

A suspension of spore (10⁷spores/ml) fungal antagonist was made in sterile distilled water. 10 gms of fresh & disease free brinjal seeds (var. muktakeshi) were dipped in 100ml suspension of spore of antagonists for over night, then they were placed in the wetted blotting papers placed in petri dishes. In each petri dish 20 seeds were taken. For each antagonist, 100 seeds were taken. The sets were placed in room temperature. One control (untreated set) was run. The number of germinated and non-germinated seeds were recorded and percent of seed germination was calculated,

The length of shoot and root of germinated seeds were measured by mm scale or mm graph paper by 24 hr. intervals up to 15 days.

Effect of antagonists on the root and shoot length, number of leaf and area of leaf:-

Seeds of brinjal (var.muktakeshi) were treated with antagonist by dip method. The treated and untreated seeds were sown in minipot (plastic tea cup) filled with fertile soil. Regular watering and watching were conducted and seedlings of brinjal were raised. In each pot one seedling was raised. For each antagonist 10 seedling (pot) were considered. After 30 days the treated & non-treated seedling of brinjal were uprooted and the soils adherence on roots were washed. The water of washed seedling were blotted by blotting paper. The root length, & shoot length were measured by mm scale. The number of leaf per seedlings were recorded. The area of leaves of both treated and untreated were calculated. It was done by placing the leaves of mm graph paper and area of leaf was drawn and by counting the square cells of graph paper the area was calculated.

Miniplot Design and cultivation for percentage of disease analysis in three years:-

Total twelve plots were prepared. Each plot was 6/6ft size. Each miniplot was differentiated by 1ft border Three of them were untreated (control) plots. Three plots were treated by *T.viride*, another three were treated by *T.harzianum*. The rest three plots were treated by blitox 50 fungicide. The plant sapling (20 day old) of muktakeshi variety were dipped in spore suspension of antagonistic soil fungi for one hour and planted within 6/6 ft plots in two rows; each row contained three plants with distance of 1.5 ft; rows were distance by 2 ft. In Blitox treated plot saplings were dipped in 3% blitox solution for one hour. The spore suspension of fungi (1×10^7 spore/ml) in respective tagged plot were sprayed, 3% of blitox 50 solution was sprayed in three plots and distilled water was sprayed by knapsack sprayer @ 500L/hectare in three plots of untreated plots. 1% Tween was mixed with all solutions before sprayings. The first spray was on the initiation of fruits and more three sprayings at the interval of 15 days. The plots were designed by Randomized Block Design (RBD). All plants were maintained with regular irrigation, fertilizer and weeding as per cultivation needs. The percentage of fruit rot disease was calculated by the formula as given earlier.

Results and discussion:-

Study of protection of fruit rot of brinjal by selected antagonists (in lab condition by incised method):-

Phomopsis along with *T. viride* inoculated fruits showed 20 PDI while *Phomopsis* inoculated fruits 100 PDI. *Phomopsis* with *T. harzianum* (Fig 1) and *Phomopsis* with *B. bassiana* inoculated fruits show 30 and 50 PDI. *T.viride* gave maximum crop protection followed by *T. harzianum* and *B. bassiana* respectively (Table 1).



Fig. 1 Study of antagonists effect of *T.viride* by inoculating *Phomopsis* along with *T. viride*.

Table 1:-PDI of treated fruits in Lab condition (incised method).

Sl No.	Treatment	PDI
1.	<i>T. viride</i> + <i>Phomopsis</i>	20.00d (26.56)
2.	<i>T.harzianum</i> + <i>Phomopsis</i>	30.00c (33.21)
4.	<i>B. bassiana</i> + <i>Phomopsis</i>	40.00b (39.23)
5.	<i>Phomopsis vexans</i>	100.00a (90.00)
6.	<i>T. viride</i>	00.00

7.	<i>T. harzianum</i>	00.00
9.	<i>B. bassiana</i>	00.00
10.	Control (untreated)	00.00
11.	Positive control (Blitox 50)	22.5 d(27.5)

Note: Mean values followed by a different letter indicated significant different ($P=0.05$), according to Duncan's multiple range test. (Data in the parentheses are angular transformed value of percentage)

The results presented in the table 2 indicated that *Phomopsis* along with *T. viride* inoculated in the brinjal fruit gave no rot upto 96 hrs (4days) but it causes mild rot (0.8cm) after 5 days. Other two biocontrol agents (*T. harzianum* and *B. bassiana*) gave the fruit up to two days protection from *Phomopsis*. The fruit, is inoculated with only *Phomopsis* shows rotting from 24 hrs and after 5 days it was 4.2 cm. The fruits inoculated with *T. viride*, *T. harzianum* and *B. bassiana* separately show no rot.

The results of three years field trial presented in the table 3 indicated that continuous three years (2013-2016) PDI is lesser (30.25, 35.00 and 28.75 respectively) in the treatment of *T. viride* in comparison with other treatments (*T. harzianum* and Blitox 50) and untreated. The PDI of *T. harzianum* for consecutive three years are 36.56, 42.00 and 42.14 respectively. On the other hand PDI of Blitox treated brinjal in three consecutive years were 32.00, 36.80 and 30.20 respectively. In untreated plots of brinjal the PDI of three years were vary high (70.25,75.45 and 69.55 respectively)in comparison to treated plots. So, *T. viride* is best followed by Blitox 50, *T. harzianum* biocontrol agent combating *Phomopsis* rot of brinjal fruits. The pool data of three years also revealed the same trends. But it is interesting to note that PDI of *T. viride* and Blitox 50 treated plots are statistically similar, so avoiding chemical treatment (Blitox50), *T. viride* may be treated to combat *Phomopsis* rot of brinjal.

The mechanism behind the crop protection by *Trichoderma* spp. lies on the secretion of cell wall degrading lytic enzymes as shown in our earlier experiments (Ghosh et al. 2015). In our earlier work (Ghosh et al. 2015), all three biocontrol agents (*T. harzianum*, *T. viride* and *B. bassiana*) produced lytic enzymes and lysed cell wall preparation of *P. vexans*. It gives clue that biocontrol agents during interaction between this pathogen may have secreted this enzyme to suppress or kill the pathogen. Biological control of pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods and also found that many isolates of *Trichoderma* spp. by producing non-volatile metabolites, volatile metabolites, enzymes which were active against a range of pathogenic fungi (Chet et al., 2009; Barakat et al., 2006; Karthikeyan et al., 2006;Eziashi et al., 2007; Mukhopadhyay, 2009). *T. harzianum* colonizes *S. rolfsii* hyphae, disrupts mycelia growth and kills this pathogen. *Trichoderma* spp. are used as biopesticide, biofertilizer or fertility promoter (Harman et al., 2004; Harmen, 2006; Vinale et al., 2008)

Table 2:-Efficacy of biocontrol agents against *Phomopsis vexans* on *Phomopsis* infected zone of brinjal fruit (incised method).

Sl No.	Biocontrol agents	Rotting (radius in cm) by <i>P. vexans</i> after 24 hrs interval				
		24 hrs	48 hrs	72 hrs	96 hrs	120hrs
1.	<i>T. viride</i> + <i>Phomopsis</i>	0.0	0.0	0.0	0.0	0.8*
2.	<i>T. harzianum</i> + <i>Phomopsis</i>	0.0	0.0	0.8	1.2	2.0
4.	<i>B. bassiana</i> - + <i>Phomopsis</i>	0.0	0.0	1.0	1.6	2.1
5.	<i>Phomopsis vexans</i>	0.6	1.0	2.0	3.0	4.2
6.	<i>T. viride</i>	0.0	0.0	0.0	0.0	0.0
7.	<i>T. harzianum</i>	0.0	0.0	0.0	0.0	0.0
9.	<i>B. bassiana</i>	0.0	0.0	0.0	0.0	0.0
10	Control (untreated)	0.0	0.0	0.0	0.0	0.0
	SEM±					0.001
	CD($P\leq 0.050$)					1.324

*Average of 10 replica

Table 3:-Percentage of disease Index(PDI) of *Phomopsis* rot of brinjal after treatments in three year field trial

SrNo	PDI Treatment	2014	2015	2016	Pool
1	<i>T.viride</i>	30.25c(33.27)	35.00b(36.27)	28.75c(32.39)	31.33c(34.02)
2	<i>T.harzianum</i>	36.56b(37.23)	42.00c(40.40)	40.14b(39.29)	39.56b(38.94)
3	Blitox 50	32.00c(35.06)	36.80b(37.23)	30.20c(33.27)	33.00c(35.06)
4	Untreated	70.25a	75.45a	69.55a	71.75a
	CD(5% level)	4.45	3.94	4.23	4.51
	SEM±	1.67	1.97	2.12	1.56

Note: Mean values followed by a different letter indicated significant different ($P=0.05$), according to Duncan's multiple range test.

Effects of antagonists on seed germination and seedling vigour of Brinjal:-

Brinjal seeds were treated with *T.viride* spore suspension for over night (Fig 2) and minipot test was done for growth promotion activity test of *T.viride* on brinjal (Fig 3). Seed treatment with *T.viride* exhibited the maximum germination(100%) followed by *T.harzianum* (96.20%), while control gave 79.20% (Fig 4). The above result agreed with the finding of Krishanmoorthy (1987) and they reported increased germination & seedling vigour of tomato & chilli respectively by *Trichoderma*. Chaube et. al. (2002) reported that *T.harzianum* increases the root and shoot growth of Chickpea. The mean values of top length, total dry biomass and seed pod numbers of Chickpea showed significant increase when treated with *T.harzianum* in comparison to control (Rai and Singh 2004).



Fig.2:- Seed treatment with spore suspension (Left) & seed germination test (Right)



Fig.3:- Minipot trial (A); growth promotion test-control (B) & treated (C) plants.

The result presented in the table 4 showed that the height (6.00 cm), the dry weight (4.82 gm), no of leaves (3.33) and no of leaf area (36.3 sq cm) of the brinjal plant, treated with *T. viride* (spore suspension 1×10^7 per ml) gave better result in comparison to control (untreated) brinjal plant { the height (4.26 cm), the dry weight (3.53 gm , no of leaves (2.95) and leaf area (32.4 sq cm) }

Table 4:-Calculated t- value, tabulated t- value of shoot, root length and root branches

	Shoot length	Dry wt	Leaf area
Calculated t value	2.73	5.428	3.02
Tabulated t value (P=0.05)	2.048	2.0 48	2.048
Tabulated t value (P=0.01)	2.763	2.763	2.763

Calculated t value of shoot length (2.73), dry weight (5.428) and leaf area(3.02) were greater than tabulated t value (2.048) at P = 0.05 level and also than tabulated t value (2.763) at P = 0.01 level at 28 df. Thus the null hypothesis was rejected. It can be concluded that shoot length, dry weight and leaf area of brinjal of *T.viride* treated plants were statistically significantly different from control (untreated). On the other hand, calculated t value of no of leaves (1.95) was smaller than tabulated t value (2.048) at P = 0.05 level and also than tabulated t value (2.763) at P = 0.01 level at 28 df. (Table 5). Thus the null hypothesis was accepted. It can be concluded that no of leaves (2.95) of *T.viride* treated plants were statistically significantly not different from control (untreated).

The promotion of shoot and root length of brinjal by *T. harzianum* and *Aspergillus niger* was recorded by other workers (Choube et al., 2002). Increased growth by *Trichoderma* sp. was also induced by a diffusible growth regulating factor (Windham et al., 1986). Increased effect of pathogens on the growth of sorghum pre-treated with *T. viride* remarkably increased over the control (Shanmugaiah 2008). Blotter and test tubes experiments were carried out to determine the direct effect of antagonists and pathogen on plant growth parameters. Radicle length, plumule length and vigour index was found better in seedlings which were treated with *T. viride* in blotter tests. Similarly, *T. viride* increased shoot length, root length and vigour index in test tube experiment (Anis et al. 2010).This was indicating that *Trichoderma* species produces plant growth promoting factors (Windham et al., 1986). Different species of *Trichoderma* gained considerable success against pathogenic fungi. *T. harzianum* protects the root system against *F. solani*, *R. solani* and *M. phaseolina* infection on a number of crops (Malik & Dawar, 2003).

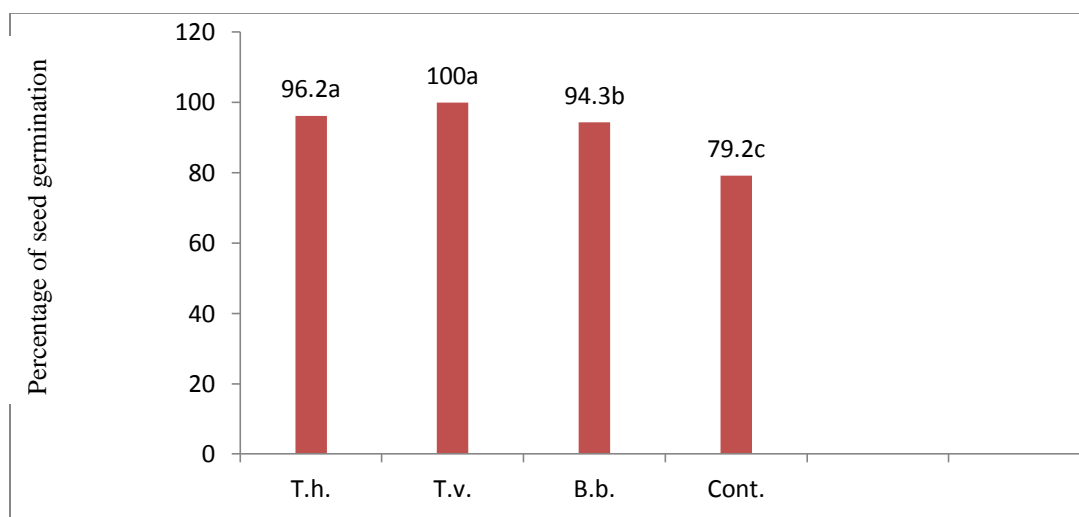


Fig.4:- Effect of biocontrol agent (spore/cell conc. 1×10^7 /ml) on seed germination of brinjal (Temp. $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$).

Note : Mean values followed by a different letter indicated significant different ($P=0.05$), according to Duncan's multiple range test.

Table 5:-Effect of *T. viride* on plant height, dry weight, number of leaves and leaf area of Brinjal

Sl no.	Plant parameter	Treated (<i>T.viride</i>)	Control
1.	Plant height (cm) **	6.00	4.26
2.	Dry weight (gm)	4.82	3.53
3.	No. of leaves	3.33	2.95
4.	Leaf area *(Sq cm)	36.3	32.4

• Average of leaves area of 15 plants.

** Average of 15 plants after 30 days

In our previous experiment (Ghosh & Pal 2017), the effect of 10^8 spore/ml of *T. asperellum* gave maximum (96.00 a) percentage of seed germination of chickpea. In minipot trial, there were 25% increased of percentage of germination of seeds and shoot length but 27% increase of root length and 45% increase of vigour index in compare to control. In microplot trial, 75.25 % and 67.15 % crop protection respectively in two consecutive years were achieved by applying *T asperellum*. Similarly pulse yields and plant dry weight (25.9 & 30.12) were higher in compare with control and both *F.oxysporum ciceri* and *F.o.c.+T.asperellum* treated plots. Microplot field trials *T.asperellum* treated plots gave higher number of pods per plant (47.25 ; 44.50) than control, *F.oxysporum ciceri* treated, and *F.oxysporum ciceri+ T.asperellum* treated plots (8.25 & 12.25). *T. asperellum* treated plants yielded maximum functional nodules. Therefore, *T asperellum* is very active growth promotion including enhancing functional root nodules and fusarial wilt management in chickpea crop. This biocontrol agent (BCA) and Plant growth promotion (PGP) agent in a dose of 10^8 spore /ml by seed dressing may be alternative to chemical fertilizers and fungicides in chickpea cultivation and other crops.

It has also been established that biocontrol agents enhance growth by producing growth stimulating factors (Windham *et al.*, 1986, Ponnurugan and Baby, 2006b). *Trichoderma* has a superior capacity to mobilize and take up soil nutrients compared to other organisms (Chet *etal.*1997). Enhanced growth response of several plants, such as tomato (Ozbayet *al.*, 2004; Vinale *et al.*, 2008), bean (Inberet *al.*, 1994), cucumber (Kleifield and Chet,1992) pepper, lettuce (Vinale *et al.*, 2008) were recorded by application of *Trichoderma*. The application of *Trichoderma* increased both root and shoot growth of plant. There are numerous soil microflora involved in the synthesis of auxins in pure culture and soil. Some of the P-solubilizing bacteria and fungi act as plant growth promoters due to their ability to produce IAA (Souchie *et al.*, 2007).

In conclusion, *T. viride* strain is the best biocontrol agent among these three for controlling *Phomopsis* rot of brinjal and can act as biopesticide along with this crop growth enhancement. Moreover, this investigation showed that the efficacy of *T.viride* to manage the brinjal disease –*Phomopsis* rot is similar to the Blitox 50 chemical fungicide. It also showed that the height, the dry weight, no of leaves and no of leaf area of the brinjal plant, treated with *T. viride* (spore suspension 1×10^7 per ml) gave better result in comparison to control (untreated) brinjal plant. It reflects the potentially of plant growth promotion The recommendation to avoid fungicides and their harmful effect, farmers may alternatively applied *T. viride* spore suspension (10^7 spore /ml) during seedling treatment and four consecutive spray of this suspension at the interval of 15 days after initiation of fruits.

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