Research Article

Enhancement of morphological and physiological parameters in *Vigna radiata* (l.) Wilczek treated with Zinc and *Trichoderma* sp

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Abstract

Background: The growth and development of crop needs optimum ratio of micronutrient in soil. Deficiency or efficiency of these nutrients in soil is affecting the plant growth leads to severe loss in the productivity and yield. For proper growth and yield, it is necessary to maintain soil fertility. Different treatments of $ZnSO_4$ had promotive effects on almost all of the growth, yield and qualitative parameters discussed. **Objective:** Study effect of Zn together with bioagent (*Trichoderma* sp.) that have better response in terms of growth and yield compared to Zn alone. **Material and methods:** A pot experiment of greengram (*Vigna radiata* (L.) was conducted with five different treatments viz. (T3- Soil treatment with zinc, T7- Soil treatment with zinc and antagonist, T11- Soil treatment with zinc and pathogen, T15- Soil treatment with zinc, pathogens and *Trichoderma* sp, Control: not treated) to test the response of greengram (*V. radiata* (L.) plants grown in alluvial soil under warehouse condition. Zinc was applied to the soil in the form of zinc sulphate ($ZnSO_4.7H_2O$) in concentrations (50 mg/kg^{-1}) in which the green gram plants were grown. **Results:** The plant samples were analysed 45 days after sowing. In green gram (*V. radiata* L.*)* plants antagonist with zinc (50 mg kg-1) were also found to be most efficient in increasing all the parameters of growth as Total plant length, Leaf, Internodes distance, Fresh weight , Dry weight and Index vigour compared to control. Maximum increases in plant height and seed weight was most significantly. **Conclusions:** The result of the present study revealed that *Trichoderma* sp had performed best in all the *in vitro* and *in vivo* studies. *Trichoderma* sp can be applied to control soilborne diseases mainly wilt complex disease of greengram (*V. radiata* (L.) significantly with an ecofriendly approach. In addition there significant increase was recorded in yield parameters of greengram (*V. radiata* (L.). *Trichoderma* sp along with zinc can be applied as integrated disease management strategies in greengram (*V. radiata* (L.) for better results but the desired results can also be easily achieved with application of *Trichoderma* without involving any chemical.

Keywords: Zinc, *Vigna radiata, Trichoderma* Sp.

Introduction

Grain legumes have an important role in ensuring nutritional as well as economic security for developing nations. As compared to cereals, they supplement the farmer's income by fetching higher prices when grown as feed crop in many farming systems (Sharma et al*.,* 2016). In cropping systems, food legumes act as important rotation crop with cereals, which reduce soil pathogens and increase the fertility of the soil by supplying nitrogen through nitrogen fixing rhizobacteria thus, economically replace the expensive nitrogenous fertilizers partially (Sharma et al*.,* 2016).

Zinc is one of the important heavy metals, which is needed as a micronutrient for plants for various metabolic processes. However at excessive levels, zinc has the potential to become toxic to plants. Zinc has been used increasingly in different forms like nutrients, fungicide, pesticide or disinfectant. Zinc is a heavy metal known to occur in higher concentrations in a majority of wastes arising from modern industries (Outridge et al*.,* 2011). When present in higher concentrations in the soil and due to its excessive uptake by plants growing on these soils zinc

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might induce toxicity. Usually zinc toxicity leads to chlorosis of young leaves (Hermle et al., 2007). Plants exhibiting Zn toxicity have smaller leaves (Li et al., 2009). In several cases, zinc toxicity has been found to exhibit necrotic lesions on the leaves, eventually leading to entire leaf death (Hermle et al., 2007). In roots, zinc toxicity apparently reduces the growth of main root, induces fewer and shorter lateral roots and yellowing of roots (Li et al., 2009).

The concept of biofertilizers was developed based on the observation that these micro organisms can have a beneficial effect on plant and crop growth (Davidson, 1988). One of the important genuses for biofertilizer producers is *Trichoderma*, a fungus present in nearly all soils. *Trichoderma* sp. thrive in the rhizosphere and can also attack and parasitize other fungi. *Trichoderma* sp. have been known for decades to increase plant growth and crop yield to improve crop nutrition and fertilizer uptake (Yedidia et al., 2001) to speed up plant growth and enhance plant greenness (Harman, 2006), as well as to control numerous plant pathogens (Cuevas et al., 2005). A part of these effects may also be related to the fact that some *Trichoderma* spp. seems to hasten the mineralization of organic materials (Cuevas, 1991), thus probably releasing nutrients from soil organic matter. Positive effects on plant nutrition were also described for other organisms, and many soil bacteria may enhance the mineral uptake of the plant, as for example by the increased solubility of phosphate in the soil solution. *Trichoderma* species play an important role in controlling fungal plant pathogens, especially soil borne fungal pathogens. The use of *Trichoderma*-based products is not only safe for the farmers and consumers but it is also good for the environment. However, much more work needs to be done to develop stable, cost effective, easy to produce and easy to apply formulations (Ha, 2010). There is a great need to develop alternative management systems that would enhance biocontrol ability of *Trichoderma* spp (Cumagun, 2012). So that *Trichoderma* use as biofertilizers in against soil born pathogen.

Some reports are also available on induced deficiency of Mn or Mg or Fe in Zn toxicity (Ellis et al., 2003). The effects of higher concentrations of Zn on plant growth have been studied by many workers (Todeschini et al., 2011). Potarzycki and Grzebisz (2009) reported that zinc exerts a great influence on basic plant life processes, such as (i) nitrogen metabolism uptake of nitrogen and protein quality; (ii) photosynthesis, chlorophyll synthesis, carbon anhydrase activity; reported that Zn-deficient plants reduce the rate of protein synthesis and protein content drastically. Mn is required for biological redo-system, enzyme activation, oxygen carrier in nitrogen Fixation (Romheld and Marachner, 1995). Zinc deficiency in plants is most frequently corrected by application of Zn in soils. In most instances, Zn deficiency in crop species is corrected by applying 4.5 to 34 kg Zn ha^{-1} as broadcast $ZnSO_4$). But limited source of this compound feels a problem of future when the present sources become ended. So that, the present investigation was undertaken to the effect of Zn singly and in combination with bioagent (*Trichoderma* sp.) on growth of green gram (*V. radiata* L.).

Materials and methods

Bio-efficacy of Trichoderma sp in-vitro by dual cultures

Bio-efficacy of *Trichoderma* sp isolates against the soil borne fungi as *Ceratocystis* sp *Curvularia* sp. *Fusarium* sp, *Sclerotinia* sp was tested under *in-vitro* conditions by dual culture technique. All cultures were grown on PDA in Petri plates and incubated at 27 ± 2 ^{0}C for 7 days in three replicates. The colony diameter of test fungus in dual culture with antagonist was measured and growth inhibition was expressed as a percentage of the control. The pathogens and antagonist were also plated singly, which served as control. The radial growth of test fungi was examined daily. All the experiments were conducted in triplicates and data given in tables are average of the three replicates and statistically analyzed for the calculation of mean, standard error (SE).

Antimicrobial activity was expressed in terms of percentage of mycelial growth inhibition and calculated as per formula of Pandey et al., (1982).

In Petri plate,

Percentage of mycelial growth inhibition=dc-dt/dc ×100

Where

dc= Average Diameter of fungal colony in control

dt= Average Diameter of fungal colony in treatment

Amendment of soils using Zn with the plant growth promotion on agricultural crop in pot

To observe the effect of integration of antagonist with compatible metal at their recommended dose compatible with antagonist during *in vitro* experiments, pot trials was conducted with the pathogens antagonist and recommended doses were mixed in pot .The experiments inculcated eight treatments with interaction with pathogen (10^7CFU/ML), antagonist (10^9CFU/ML), and metal. The required population of plants (10 seed per pot) was maintained and pot were hand weeded and irrigated as per standard practices in the area. In pot, three plants of green gram (*V. radiata* L.) were randomly selected and tagged. The observations on growth components at different growth stages and yield components at harvest were

recorded. The height of three plants at different stages in all the treatments were measured from the base of the plant to the tip of the main stem, total number of leaves and branches was recorded of the randomly tagged plants. Fresh weight/dry weight; length of shoot and length of root measured by three randomly selected plants at different crop growth stages. The fungal inoculums (CFU $2.6\times10^{8}gm^{-1}$) formulation (4 kg) was mixed in soil before 4 days of seed sowing. Seed germination, fresh weight/dry weight, length of shoot/root, leaf area, no of branches, seed weight, were measured at 45 days. The yield and its characteristics were recorded at harvest (45 Days). The data was collected from mature plant. The samples were oven dried at 40°C to 45°C to a constant weight for calculating dry weight. At maturity all plants from each pot was harvested. Seeds were threshed, separated, air dried, cleaned and weighed, seed yield was calculated per pot. The resident flora was also observed by dilution plating the soil of selected control and treatment plots on, PDA (with Streptopenicillin 25 mM per plate for fungal population), W A (for Actinomycetes population), NA (for bacterial population). The presence of antagonist was also observed by dilution plating soil, plant root and leaves on selective antibiotic plate. All the experiments were conducted in triplicates and data given in tables are average of the three replicates and statistically analyzed for the calculation of mean, standard error (SE).

Germination percentage and seedling vigour

To observe the effect of antagonist formulation prepared from different agro-waste and their application plants in terms of seedling germination and seedling vigour. The seedling vigour (SV) of one week seedling was calculated as described by Sparg et al. (2005) and germination was calculated as described by Srivastava (2005).

$$\text{Germination (\%)} = \frac{\text{Total number of plants germinated}}{\text{Total number of seed sown}} X100$$

Seedling vigour= (shoot length +root length) × percentage germination (%)

Population of *Trichoderma* as well as microorganisms in soil

To assess the distribution of antagonist formulation applied in the rhizosphere of cucurbit, roots of cucurbit plants were removed from the soil at 10 days interval viz., 10 days, 20 days, and 30 days by the method described by Tsahouridou and Thanassoulopoulos (2002). Loose soil was shaken off soil still adhering to roots was considered to be the rhizosphere soil. The soil segment were transferred in the tubes containing 9 ml of sterile distilled water and shaken vigorously for 10 min. to remove rhizosphere soil Root segments were then removed from the tubes. Tubes containing the soil were then shaken on the rotary shaker for 14 hour at 450 rpm to suspend propagules of antagonist from the rhizosphere soil. Serial dilution was made in sterilized distilled water and then plated on PDA. The plates were then incubated at 27± 2 ºC in dark for 5-7 days. Three replicated for each dilution was maintained.

Primarily identification of the microorganisms isolate was made using the taxonomic keys of Rifai (1969) and Nagamani et al., (2002) and finally conformation of *Trichoderma* spp. by Agharkar Research Institute, Pune was done.

Results and discussion

The growth and development of crop needs optimum ratio of micronutrient in soil. Deficiency or deficiency of these nutrients in soil is affecting the plant growth leads to severe loss in the productivity and yield. For proper growth and yield, it is necessary to maintain soil fertility. Many agricultural agencies and scientific organization suggested to farmer that how to maintain soil fertility and how to manage their loss. Here, study was focused on essential micronutrients zinc, biofertilizer (*Trichoderma* sp). Zn deficiency leads to reduction in growth and yield. The average reduction in radial growth of the pathogens after being subjected to attack by antagonist was observed.

The results indicate that antagonist was effective in suppressing the radial growth of pathogens to varying degree which is presented in **table 1.** The percent reduction in radial growth from 40 % to 82% was observed. There was difference in the average antagonistic ability of antagonist against soil borne pathogens *viz, Ceratocystis* sp, *Curvularia* sp, *Fusarium udam*, *Sclerotinia rolfsii*, in terms of percent inhibition of radial growth. Maximum reduction of growth of the pathogens was shown by selected antagonist, which reduced the radial growth of the all the pathogens by 82.00(±0.056), 68.35(±0.065)' 88.35 (±0.005) and 82.36 (±0.057) respectively. The maximum reduction was observed for *Fusarium udum* followed by *Sclerotinia rolfsii.* There was also significant difference in the way pathogens reacted to fungal antagonist.

Table 1. Antifungal activity of *Trichoderma* sp against test pathogens in dual culture

S.No	Pathogens	% inhibition in dual culture (± SE)
1	*Ceratocystis* sp	82.00(±0.056)
2	*Curvularia* sp	68.35(±0.065)
3	*Fusarium udam*	88.35(±0.005)
4	*Sclerotinia rolfsii*	82.36(±0.057)

Each value is mean of three replicates and Standard Error (±) given along the mean values.

Table 2. Effect of *Trichoderma* sp on green gram (*Vigna radiata* L.*)* in pot trail

S. No	Parameter	On green gram (*V. radiata* L.*)*			
		Treatments			
		T5	T9	T13	Control
1	Germination (%)	90±0.254	65±0.124	85±0.652	90±0.214
2	Vigour index	1800±0.265	671.45±0.124	1326±0.541	119.7±0.264
3	Total plant length (cm)	20±0.001	10.33±0.354	15.6±0.365	13.3±847
4	No. of leaf	3±0.014	3±0.251	4±0.000	3±0.254
5	Internodes distance (cm)	1±0.00	0.9±0.364	1±0.000	1±0.124
6	Fresh weight (gm)	0.60±0.00	0.50±0.241	1.25±0.000	0.55±0.00
7	Dry weight (gm)	0.34±0.214	0.30±0.241	0.85±0.254	0.33±0.264
8	No. of Seed	2±0.241	1±0.142	2±0.255	2±0.365
9	Seed Wight With Follicle	0.37±0125	0.23±0.214	0.40±0.000	0.37±0.014
10	Weight of seed (gm)	0.30±0.265	0.11±0.264	0.35±0.998	0.24±0.214

Each value is mean of three replicates and Standard Error (±SE) is given along the mean values. Statistical analysis of variance (ANOVAs) was done according to the method of D.D Paterson (1939) T5- Soil treatment with *Trichoderma* sp, T9- Soil treatment with Pathogen, T13- Soil treatment with Pathogens and *Trichoderma* sp, Control: not treated

Figure 1. Effect of Zinc Concentration on the growth of plant green gram (*V. radiata* L.*)* with *Trichoderma* sp.

In *V. radiata* L plants, *Trichoderma* sp alone were also found to be most efficient increasing all the parameters of growth as total plant length, leaf number, length of internodes distance, plant fresh weight, Dry weight, Index vigor compared to control. Maximum increases in plant height and weight of seed was most significantly **(Table 2 and Figure 1).** Increase in yield showing to application of Zn is quite obious, as the soil under study was deficient in available Zn (0.54 ppm). Islam et al. (2005) and Singh and Singh (2012) reported similar results in mungbean and chickpea respectively.

In green gram (*V. radiata* L.) plants, *Trichoderma* sp with zinc were also found to be most efficient in increasing all the parameters of growth as total plant length, No of leaves, length of Internodes distance, Fresh weight of plant, Dry weight, Index vigour compared to control. Maximum increases in plant height and seed weight was most significantly **(Table 3).** Increase in yield owing to application of Zn is quite obious, as the soil under study was deficient in available Zn (0.54 ppm). Nitrogen uptake by greengram grain and straw increased significantly with increasing levels of Zn and the highest N uptake was observed. (Sharma et al*.,* 2016)

Isolation of microorganism and disease from pre harvest and post harvest is conducted on green gram (*V.radiata* L.*)* in April to May 2016 in pot trail treatment (**Table 4)**. During the course of study it became evident that *V. radiata* L. plants were found to be not any type of disease The microorganism isolated from these plants were *Alternaria* sp, *Aspergillus* sp, *Curvularia* sp, *Fusarium* sp, *Mucar* sp, *Penicillium* sp, *Pythium* sp, *Rhizoctonia* sp, *Rhizopus* sp, *Sclerotinia* sp. *Verticillium* sp and *Unidentified* sterile

Table 3. Effect of Zinc Concentration on the growth of plant green gram (*V. radiata* L.*)* with *Trichoderma* sp.

S. No	Parameter	On green gram (*V. radiata* L.) Treatments				
		T3	**T7**	**T11**	**T15**	**Control**
1	Germination (%)	80±0.214	55±0.124	80±0.251	80±0.2541	90±0.214
2	Vigour index	1760±0.254	73.15±0.254	1200±0.365	2000±0.000	119.7±0.264
3	Total plant length (cm)	22±0.365	1.13±0.002	15±0.000	25±0.265	13.3±847
4	No. of leaf	3±0.254	2±0.124	3±0.320	4±0.000	3±0.254
5	Internodes distance (cm)	0.4±0.000	1±0.000	1±0.241	3±0.000	1±0.124
6	Fresh weight (gm)	0.72±0.258	0.07±0.001	1.88±0.261	3.05±0147	0.55±0.00
7	Dry weight (gm)	0.60±0.247	0.2±0.000	0.73±0.00	2.06±1.200	0.33±0.264
8	No. of Seed	2±0.00	00±0.000	1±0.000	5±0.254	2±0.365
9	Seed Wight With Follicle	0.33±0.214	00±0.000	0.31±0.220	1.43±0.000	0.37±0.014
10	Weight of seed (gm)	0.23±0.124	00±0.000	0.21±0142	1.24±0.254	0.24±0.214

Each value is mean of three replicates and Standard Error (±SE) is given along the mean values. Statistical analysis of variance (ANOVAs) was done according to the method of D.D Paterson (1939) T3- Soil treatment with zinc, T7- Soil treatment with zinc and *Trichoderma* sp, T11- Soil treatment with zinc and pathogen, T15- Soil treatment with zinc, pathogens and *Trichoderma* sp, Control: not treated

Table 4. Microorganisms isolated from pre harvest and post harvest of plants

Treatment	Microorganisms isolated				Remark
	Soil pH	**Pre harvest**	**Soil pH**	**Post harvest**	
No mix of soil with any metal, pathogens and *Trichoderma* sp	6.7	*Alternaria* sp *Aspergillus* sp *Curvularia* sp *Fusarium* sp *Mucar* sp *Penicillium* sp *Pythium* sp *Rhizoctonia* sp *Rhizopus* sp *Sclerotinia* sp *Verticillium* sp Unidentified sterile mycelia*:	7.0	*Alternaria* sp *Aspergillus* sp *Mucar* sp *Penicillium* sp *Pythium* sp *Rhizopus* sp *Verticillium* sp Unidentified sterile mycelia*:	
Soil mix with zinc concentration	6.7	*Alternaria* sp *Aspergillus* sp *Curvularia* sp *Fusarium* sp *Mucar* sp *Penicillium* sp *Pythium* sp *Rhizoctonia* sp *Rhizopus* sp *Sclerotinia* sp *Verticillium* sp Unidentified sterile mycelia*:	6.5	*Alternaria* sp *Aspergillus* sp *Mucar* sp *Penicillium* sp *Rhizoctonia* sp *Rhizopus* sp Unidentified sterile mycelia*:	
Soil mix with only *Trichoderma* sp	6.7	*Alternaria* sp *Aspergillus* sp *Curvularia* sp *Fusarium* sp *Mucar* sp *Penicillium* sp *Pythium* sp *Rhizoctonia* sp *Rhizopus* sp *Sclerotinia* sp *Verticillium* sp Unidentified sterile mycelia*:	7.6	*Aspergillus* sp *Mucar* sp *Rhizopus* sp *Trichoderma* sp Unidentified sterile mycelia*:	
Soil mix with *Trichoderma* sp and zinc concentration	6.7	*Alternaria* sp *Aspergillus* sp *Curvularia* sp *Fusarium* sp *Mucar* sp *Penicillium* sp *Pythium* sp *Rhizoctonia* sp *Rhizopus* sp *Sclerotinia* sp *Verticillium* sp Unidentified sterile mycelia*:	7.0	*Aspergillus* sp *Rhizopus* sp *Trichoderma* sp Unidentified sterile mycelia*:	Nodule formation

Continue...

Table 4. Continue.....

Treatment	Microorganisms isolated				Remark
	Soil pH	Pre harvest	Soil pH	Post harvest	
Soil mix with only pathogen	6.7	*Alternaria* sp *Aspergillus* sp *Curvularia* sp *Fusarium* sp *Mucar* sp *Penicillium* sp *Pythium* sp *Rhizoctonia* sp *Rhizopus* sp *Sclerotinia* sp *Verticillium* sp Unidentified sterile mycelia*:	7.0	*Aspergillus* sp *Fusarium* sp Unidentified sterile mycelia*:	
Soil mix with pathogen and Zinc concentration	6.7	*Alternaria* sp *Aspergillus* sp *Curvularia* sp *Fusarium* sp *Mucar* sp *Penicillium* sp *Pythium* sp *Rhizoctonia* sp *Rhizopus* sp *Sclerotinia* sp *Verticillium* sp Unidentified sterile mycelia*:	6.8	*Aspergillus spP* *Fusarium spp* Unidentified sterile mycelia	Nodule Formation
Soil mix with pathogen and *Trichoderma* sp	6.7	*Alternaria* sp *Aspergillus* sp *Curvularia* sp *Fusarium* sp *Mucar* sp *Penicillium* sp *Pythium* sp *Rhizoctonia* sp *Rhizopus* sp *Sclerotinia* sp *Verticillium* sp Unidentified sterile mycelia*:	6.5	*Aspergillus* sp *Alternaria* sp *Rhizopus* sp *Trichoderma* sp Unidentified sterile mycelia	
Soil mix with pathogen, *Trichoderma* sp and zinc	6.7	*Alternaria* sp *Aspergillus* sp *Curvularia* sp *Fusarium* sp *Mucar* sp *Penicillium* sp *Pythium* sp *Rhizoctonia* sp *Rhizopus* sp *Sclerotinia* sp *Verticillium* sp Unidentified sterile mycelia*:	7.0	*Aspergillus* sp *Alternaria* sp *Rhizopus* sp *Trichoderma* sp Unidentified sterile mycelia	Nodule Formation

mycelia but after the treatment or post harvest *Aspergillus* sp, *Mucar* sp*, Rhizobium* sp, *Trichoderma* sp and Unidentified sterile mycelia (**Table 4)**. It was also found that Zn and *Trichoderma* sp also affected nodule formation.

Trichoderma sp can be successfully and safely used as both classical and inundative biological control agents. However, we have also seen that fungi as a whole can possess properties that make them potentially hazardous both to the user and to the environment in general. Consequently, the development and use of fungi as biocontrol agents requires an assessment of their potential hazards. In most countries, regulations and registration requirements serve two major purposes: (i) to ensure the safety of the agent; and (ii) to ensure efficacy. Depending upon the strain, the use of *Trichoderma* in agriculture can provide numerous advantages:

- Colonization of the rhizosphere by the BCA ("rhizosphere competence") allowing rapid establishment within the stable microbial communities in the rhizosphere;
- Control of pathogenic and competitive/deleterious microflora by using a variety of mechanisms;
- Improvement of the plant health and
- Stimulation of root growth (Harman et al*.,* 2004).

Trichoderma species play an important role in controlling fungal plant pathogens, especially soil borne fungal pathogens. The use of *Trichoderma*-based products is not only safe for the farmers and consumers but it is also good for the environment. However, much more work needs to be done to develop stable, cost effective, easy to produce and easy to apply formulations (Ha, 2010). There is a great need to develop alternative management systems that would enhance biocontrol ability of *Trichoderma* sp. (Cumagun, 2012).The use of appropriate inoculum production, formulation and application technologies together with quality control checks should also help in this process. Nevertheless, even if reliable BCAs can be produced, they must still be easy to use and cost-effective, or they will either never reach the market-place or not be used by growers.

The result of the present study revealed that *Trichoderma* sp had performed best in all the *in vitro* and *in vivo* studies. *Trichoderma* sp can be applied to control soilborne diseases mainly wilt complex disease of greengram (*V. radiata* L.) significantly with an ecofriendly approach. In addition there significant increase was recorded in yield parameters of greengram (*V. radiata* (L.). *Trichoderma* sp along with, zinc can be applied as integrated disease management strategies in greengram (*V. radiata* L.) for better results but the desired results can also be easily achieved with application of *Trichoderma sp* without involving any chemical.

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