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Effect of different *Trichoderma* isolates on growth, nodulation and yield of Lentil (*Lens culinaris* Medik)

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Abstract
Trichoderma spp. have been widely studied, and are presently marketed as biopesticides, biofertilizers and soil amendments, due to their ability to protect plants, enhance vegetative growth and reduce pathogen populations under numerous agricultural conditions. Current biocontrol studies have confirmed the effectiveness of the *Trichoderma* species against many fungal phytopathogens. In this study, a field experiment was conducted during winter seasons of 2016-17 and 2017-18 at the experimental farm of Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar to know the effect of different *Trichoderma* isolates on growth, biomass and yield of lentil crop. The results indicated that seed treatment with different *Trichoderma* isolates significantly enhanced the field emergence, plant growth, grain weight and nodulation. Among the different *Trichoderma* isolates used, *Trichoderma* isolate 3 proved the best in enhancing field emergence (84.38%), shoot height (35.55 cm), root length (13.10 cm), grain weight (6.35g) and nodule per plant (10.32 no.) compare to untreated in pooled values of two consecutive years in Lentil.

Keywords: Lentil, nodulation, plant growth, *Trichoderma* isolates

Introduction
Lentil is one of the most important protein rich food legume and rank next only to chickpea. Lentil is also called "poor man's meat" (Bhatty, 1988) [2]. Lentil seeds are rich in protein, their mean value is at about 28.5% (Stoilova and Pereira, 1999) [17]. It is grown throughout the northern and central India for grains. India ranked first in area and second in the production with 39.79% and 22.79% of world area and production respectively. The highest productivity was recorded in Croatia (2862 kg/ ha) followed by New Zealand (2469 kg/ ha). Canada rank first in production (41.16%) due to very high level of productivity (1633kg/ ha) as compared to India (611 kg/ha). *(*FAO Statistics, 2014) [5]. During Twelfth Plan (2012-15) the country's area under Lentil was 14.79 lakh hectares with a production of 10.38 lakh tonnes. Madhya Pradesh ranks Ist in acreage e i.e. 39.56% (5.85 lakh ha) followed by UP 34.36 % and Bihar 12.40%. While in terms of production UP ranks Ist at 36.65% (3.80 lakh tonnes) followed by Madhya Pradesh (28.82%) and Bihar (18.49%). The highest yield was recorded by the state of Bihar (1124 kg/ha) followed by W.B. (961 kg/ha) and Jharkhand (956 kg/ha). (DES. 2015-16) [4]. Farmers are applying without thinking many synthetic fertilizers and chemicals for minimising the losses caused by abiotic and biotic stress in lentil crop. The excess uses of these chemicals have been harmful to environment, animal and human beings. Due to negative impact of these chemicals on environment, scientists are searching an alternative of these synthetic chemicals. In recent years, microbes have proved their potential in mitigation of abiotic and biotic stresses in plant. Biological control has become an important aspect of sustainable agriculture (Baker and Paulitz, 1996) [1] and food production. The use of biopesticides and biofertilizers is an alternative for sustaining high production with low ecological impact. Different soil-borne fungi and bacteria are able to colonize plant roots and may have beneficial effects on the plant. Among beneficial microorganisms, *Trichoderma* has emerged as a multifarious microbe to deal with the problem of nutrition, soil salinity, drought, and plant diseases. This fungus has substantial ability to solubilize the soil minerals, produce plant growth hormones, increase the water-use efficiencies, stimulate host defence, and antagonize pathogens, which cumulatively result in a significant improvement in the crop health. *Trichoderma* species are typically known to be soil borne, green-spore ascomycetes that can be associated with the roots of plants as well as in the rhizosphere. *Trichoderma* spp. is the most common mycoparasitic and saprophytic fungi. For the management of seed and soil borne diseases, antagonists from the rhizosphere region of the host plants were isolated. The antagonist cope with plant diseases also provide better nourishment to host plants (Perner *et al*., 2006) [9]. Considering these points, the present study was conducted to find out the most effective *Trichodrma* isolate in enhancing the growth, biomass, nodulation and yield of lentil.

Materials and Methods

The experiment was conducted during *rabi* season 2016-2017 and 2017-2018, at the Research farm of Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar. The Experimental farm is located at an altitude of 52.0 meter from mean sea level, at latitude of 25.98^0 N and longitude of 85.67^0 E. The climate is sub-humid type and monsoon receiving an average annual rainfall of 1234.7 mm mainly during the months - June to October. The experimental plot had a uniform topography. Soil was sandy loam well drained and medium fertility. This experiment was designed in Randomized Block Design with four replicates using KLS 218 cultivar**.** The plot size was 5 x 5 m^2. Field was ploughed then labelled. After layout of experiment, seeds were sown in the last week of November @ 30-40 kg seeds/ ha in each micro plot with the help of hand hoe keeping equal distance of 30 cm row to row. The weeds were removed manually after 45 and 75 days of sowing. The crop was irrigated thrice as per the standard agronomic practices recommended for lentil. Four *Trichoderma* isolates were evaluated. Proportion of emerged seed to the total number of seed sown in a row was counted after 21 days of sowing. Observations were recorded for every row of 5 randomly selected plants. After 90 DAS randomly selected plants were safely uprooted for recording root length, plant height and number of nodules per plant. At the harvest, 10 randomly selected plant pod grain was weighed. The data on field emergence, plant growth, grain weight and nodulation were recorded under various treatments.

Application of *Trichoderma* isolates

Trichoderma isolates were received under the project the STRASA (Stress Tolerant Rice for Africa and South Asia (IRRI), New Delhi, India to study its effect in rice under drought stress. These isolates were also tested in lentil as station trial. Seeds are taken of each treatment and kept for water soaking for 24 hr. After water soaking, bio-control agent treatment was given @ 10gm/ kg seed and applied next day to the seed and untreated seed served as control. All the operations were done in shade and one day before sowing.

Results and Discussion

The performance of *Trichoderma* isolates i.e. *Trichoderma* isolate 1, 2, 3, and 4 have considerable significant positive influence on field emergence, shoot length, root length, grain weight and nodulation compare to control in pooled values of two consecutive years in lentil (Table 1). However, the different *Trichoderma* isolates exhibited their presence by varied level of influence on field emergence, shoot length, root length, grain weight and nodulation. Seed treatment with *Trichoderma* isolates showed positive influence on field emergence and ranged from 73% to 84%. Maximum field emergence was observed in *Trichoderma* isolate 3 (84.00) followed by *Trichoderma* isolate 4 (82.13 %), *Trichoderma* isolate 1 (82.00 %) and *Trichoderma* isolate 2 (81.00%). All these isolates were at par with each other but significantly better over control. The results are in agreement with finding of Singh *et al.* (2016) [15] who reported that seed treatment with native *Trichoderma* isolate (*Th* azad) effectively inhibited the growth of *Fol* (69.23%) under *in vitro* and field conditions and better plant productivity with all the seven seed quality attributes *viz.* germination, shoot length, root length, seedling length, seedling dry weight, vigour index I and vigour index II in lentil. Singh *et al*. (2017) [16] observed significantly maximum germination (83.00%) in lentil by treating seeds with *Trichoderma harzianum* S1+IRRI BMP over control. Various other studies have also reported disease control using *Trichoderma* species, such as Chowdhury *et al.* (2000) [3] found 21.61% to 48.43% increased germination in mungbean, blackgram, pigeon pea and tomato by treating seeds with bioagents; Poddar *et al*. (2004) [10] reported decreased chickpea wilt incidence with isolate of *T. harzianum*. Correspondingly, Siddiqui & Singh (2004) [13] found maximum plant growth, increased transpiration and decreased wilt disease index caused by *F. oxysporum* f. sp. *ciceris* through treatment with *T. harzianum*. Seed treated with *Trichoderm*a isolates also influenced shoot length and ranged varied from 28.18 cm to 35.55 cm. Significant maximum shoot length was observed in *Trichoderma* isolate 3 (35.55cm) followed by *Trichoderma* isolate 1 (33.38 cm) and *Trichoderma* isolate 2 (33.20 cm), these isolates were at par with each other. The minimum shoot length was observed in *Trichoderma* isolate 4 (32.23 cm). The enhanced shoot height may also be due to the improved and faster plant emergence in primed seeds which might have created cooperative competition among the plant for light and resulted in taller plants. Similar results were made by Kumari *et al.* (2018) [7] who reported maximum shoot (47.00 cm) and root (12.80 cm) length in lentil when seed treated with *Trichoderma viride* and *Pseudomonas fluorescence*. Root length is an important trait of lentil and it has positive correlation with yield. Root length was influenced by the different *Trichoderma* isolates in the experiment and root length ranged varied from 10.43 cm to 13.10 cm. The significantly maximum root length was observed in *Trichoderma* isolate 3 (13.10 cm) followed by *Trichoderma* isolate 1 (12.75 cm) and both were at par with each other. The lowest root length was in *Trichoderma* isolate 4 (12.08 cm) but showed better over control. The colonization of *Trichoderma harzianum* in the root resulted in increase in growth of root thus providing enough strength for more nutrient uptake by the roots in fields resulting higher number of grain and 100 grain weight (Masunaka *et al.*, 2011) [8]. Significantly increase grain weight was also observed in seed treated with *Trichoderma* isolates compared to untreated and ranged from 4.70g to 6.35g. Maximum grain weight (6.35g) was observed in *Trichoderma* isolate 3. All the isolates were at with each other in grain weight while significantly better over control. Colonization of *Trichoderma* with lentil roots stimulated plant growth, more nodulation and provided favourable environment in root zone for enhancing root growth, nutrient uptake and seed yield (Harman *et al.*, 2004; Sallam *et al.*, 2008) [6, 11]. The seed priming treatment is known to exert beneficial effect on crop by improving the planting value of seeds. Significantly more number of root nodules per plant was recorded in seeds primed with *Trichoderma* isolate 3 (10.32 no.) followed by *Trichoderma* isolate 1(9.95) and both were at par with each other. However, *Trichoderma* isolate 2 and *Trichoderma* isolate 4 were also significantly higher over control but significantly lower to *Trichoderma* isolate 3 and *Trichoderma* isolate1. Minimum root nodules per plant (7.93 no.) were recorded in untreated control. This result is close agreement with finding of Sharma *et al.* (2012) [12] who reported that seed treatment with *Trichoderma* strain and BPM increases nodulation and plant height. Singh *et al*. (2010) [14] also reported plant growth promotion, nodulation and seed yield in urdbean with *Rhizobium.* The higher root nodules per plant might be due to the fact that molybdenum is needed primarily on the seed coat in order to enhance nodulation with nitrogen fixing bacteria, which requires molybdenum for the proper functioning of the

nitrogen-fixing enzyme nitrogenase, to uphold high rates of biological nitrogen fixation. Therefore, on behalf of this study suggested that all the *Trichoderma* isolates capable to enhance the plant height, nodulation, grain weight and its contributing traits by secreting plant growth promoting substances. That's why the evaluated *Trichoderma* isolates were important contributor to improve plant growth and seed yield in lentil. The use of *Trichoderma* as inoculants biopesticides and biofertilizers is an efficient approach to replace pesticides and chemical fertilizers for sustainable lentil cultivation.

Table 1: Effect of different *Trichoderma* isolates on growth, biomass, yield and nodules of lentil

***Trichoderma* isolates**	**Field Emergence (%)**			**Shoot Length (cm)**			**Root Length (cm)**			**Grain Weight (g)**			**No. of Nodule/ plant**		
	2016-17	**2017-18**	**Pooled**	**2016-17**	**2017-18**	**Pooled**	**2016-17**	**2017-18**	**Pooled**	**2016-17**	**2017-18**	**Pooled**	**2016-17**	**2017-18**	**Pooled**
Trichoderma Isolate1	84.75 (8.30)	79.25 (8.18)	82.00 (8.24)	32.05 (20.03)	34.70 (17.03)	33.38 (18.53	12.05 (24.20)	13.45 (20.62)	12.75 (22.41)	5.29 (29.97)	6.87 (28.89)	6.08 (29.43)	9.60 (28.00)	10.30 (23.35)	9.95 (25.66)
Trichoderma Isolate2	83.00 (6.07)	79.00 (7.84)	81.00 (6.96)	30.80 (15.35)	35.60 (20.00)	33.20 (17.68)	11.95 (23.19)	12.95 (16.14)	12.45 (19.67)	5.28 (29.70)	6.58 (23.45)	5.93 (26.57)	8.90 (18.66)	9.05 (8.38)	8.96 (13.52)
Trichoderma Isolate3	85.50 (9.20)	83.25 (13.65)	84.38 (11.43)	34.55 (29.40)	36.55 (23.27)	35.55 (26.34)	12.40 (27.83)	13.80 (23.76)	13.10 (25.59)	5.60 (37.59)	7.10 (33.20)	6.35 (35.39)	9.90 (32.00)	10.75 (28.74)	10.32 (30.37)
Trichoderma Isolate4	83.50 (6.70)	80.75 (10.23)	82.13 (8.47)	30.70 (14.98)	33.75 (13.82)	32.23 (14.40)	11.25 (15.99)	12.90 (15.69)	12.08 (15.84)	5.11 (25.55)	6.59 (24.59)	5.85 (25.07)	8.85 (18.00)	9.35 (11.97)	9.10 (14.99)
Control	78.25	73.25	75.75	26.70	29.65	28.18	9.70	11.15	10.43	4.07	5.33	4.70	7.50	8.35	7.93
CD (P=0.05)	4.21	5.10	4.07	3.88	3.59	2.44	0.90	0.75	0.61	1.00	0.74	0.75	1.36	1.08	0.71
CV	3.30	4.19	3.26	8.12	6.84	4.87	5.04	3.75	3.31	12.73	7.75	8.48	9.87	7.34	4.96

*Figure parenthesis in per cent increase over control

Conclusion

The result of present study clearly indicates that *Trichoderma* has an important role in enhancing the plant growth, biomass, nodulation and yield of lentil crop. Among the biocontrol agents, *Trichoderma* isolate 3 was found best in enhancing the growth, biomass, and yield of lentil crop. This isolate could be exploited for enhancing the yield under sustainable lentil cultivation.

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