



“EFFECT OF *TRICHODERMA HARZIANUM* AS A BIOFERTILIZER ON TOMATO PLANT (*LYCOPERSICON ESCULENTUM*)”

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ABSTRACT :

Plant growth promotion is one of the indirect mechanisms employed by *Trichoderma* spp. which plays a role in the improvement of plant health. In this study, the fungus *Trichoderma harzianum* was isolated from the soil sample & they were identified by their respective physical tests. A fungus scaled up using CMA media, respectively and was incubated at 28^oc for 42 to 48 hours. The tomato seeds were treated with inoculums as a biofertilizer. The results of biofertilizer were analyzed by their growth pattern such as plant height, number of leaves, and number of branched roots and length of roots. The highest results were recorded in terms of plant growth in 12th and 15th days as compare to control plant growth.

KEYWORDS : Biofertilizer, *Trichoderma harzianum*, Tomato seeds, CMA media and PDA media

1.INTRODUCTION

Fertilizers are widely used to supply essential nutrients for plant to increase yield. Biofertilizer i.e. Biological based product are most advanced agriculture biotechnology products necessary to support developing organic agriculture, sustainable agriculture, green agriculture and non-pollution agriculture. Biofertilizer accelerate certain microbial processes in the soil or rhizosphere which augment the extent of availability of nutrients like nitrogen, phosphorous etc in a form easily assimilated by plant. Biofertilizers are low cost, renewable sources of plant nutrients which supplement chemical fertilizers and their use for agricultural improvement has been a topic of research for a number of years. They can be used either for seed treatment or soil application.

Plant growth promotion is one of the indirect mechanisms employed by *Trichoderma* spp. which plays a role in the biocontrol of various plant pathogens & in improvement of plant health. Treatment with *Trichoderma* generally increases root & shoot growth, reduces the activity of deleterious microorganism in the rhizosphere of plant & improves the nutrient status of the plant. Secretion of hormone-like metabolites & release of nutrients from soil or organic matter, have been proposed as the mechanisms involved in plant growth promotion. *Trichoderma harzianum* is good solubilizer of phosphorus but different strains show wide variation in their ability to solubilize phosphorus. *T. harzianum* was also reported to solubilize Mn₂, metallic zinc, and rock phosphate (mostly calcium phosphate) in a liquid sucrose- yeast extract medium. *Trichoderma* produced chelating metabolites & used redox activity for solubilizing the minerals, both of these mechanisms also play a role in biocontrol of plant pathogens, and they may be part of a multiple component action exerted by *Trichoderma* to achieve effective biocontrol under a variety of environmental of corn with *T.harzianum*, planted in low nitrogen soil resulted in plants that were greener & larger in the early part of the growing season. An increase in the microelement contents of plant was also observed. Symbiotic colonization of roots by *Trichoderma* enhanced root growth, which may be responsible for increased tolerance of plant to biotic & abiotic stresses. Wheat plants raised from *Trichoderma* treated seeds tolerate drought (water stress) better under field condition. Tomato (*Lycopersicon esculentum*) is one of the most

important vegetable crops grown throughout the world and ranks next to potato in terms of the area but ranks first as a processing crop.

2. MATERIALS AND METHODS

2.1 Materials:

2.1.1. Soil Sample Collection: Soil sample have been collected from the field of college of Agricultural Biotechnology, Hatta Ta.Basmat Dist. Hingoli. Desirable fugal strain *Trichoderma harzianum spp.* was isolated in the laboratory from soil sample

2.1.2. Chemicals and Media: PDA (Potato dextrose agar) and CMA (corn meal agar) media used an isolation of fungal strains. The screening of isolated was done with help of Lacto- phenol cotton blue, 70% alcohol etc. have been also used.

2.3. Methods:

2.3.1. Isolation of *Trichoderma Harzianum species* from soil sources through serial dilution technique: For isolation of *Trichoderma spp.*, 1gm of collected soil sample was added aseptically in the 100ml sterile water, a serial dilution technique (10^{-1} to 10^{-10}) was prepared. 1 ml sample of each test tube was pipette out and added onto a Potato Dextrose Agar (PDA) plate and Corn Meal Agar (CMA) and incubated at 28° C for 42 to 48 hours. The culture plates were examined daily. Distinct morphological characteristics were observed for identification and the slant was prepared and stored at 4° C freezer.

2.3.2 Identification of microorganisms:

2.3.3. Mycelia morphology: The characteristics of a mycelium growth (shape, size, spores etc.) were observed. Although fungal growths have many characteristics there are a few basic elements that we can identify.

2.3.4. Fungal identification by staining techniques: For fungal identifications lacto- phenol cotton blue staining technique was used. Take a clean grease fresh slide. Add a drop of mounting fluid that is lacto-phenol cotton blue solution on a slide. Sterilize the needle and cool it then Transfer a mycelium mat on fluid and press it gently so that it easily mixed with the satin. Take clean cover slip and with the help of forceps place the cover slip on *mycelium* mat. Take a blotting paper and wipe the excess stain. Observe under to high power objective of microscope.

2.3.5. Preparation of *Trichoderma harzianum* inoculums: 50ml sterile distilled water was taken in conical flask. 7 day old cultures of *Trichoderma harzianum* were added into conical flask. Thus suspension mix thoroughly using magnetic stirrer

2.3.6. Seeds Treatment: Tomato seeds were sterilized in a 1% bleach solution for 30 min and rinsed thoroughly in sterile distilled water. The seeds were then soaked in 50ml of a suspension of *Trichoderma harzianum* and incubated at 30 min. control seeds were soaked in an equal volume of distilled water. Treated and untreated control seeds were directly sown into the field sowing into the 10cm x 10cm.

3. RESULTS

3.1. *Trichoderma harzianum* Isolated by serial dilution technique:-

1 gm of soil sample added into 100ml of distilled water in conical flask. from this conical flask take 1ml of mixture and transfer it into 9ml of distilled water in another test tube this will give 10^{-1} dilution similarly from 10^{-1} to 10^{-10} .

3.2 Isolation of *Trichoderma species*: The mycelium growth was observed in PDA plate after 3-5 days when the serially diluted samples were placed on PDA media. From mycelium growth, green color conidia was picked, observed under microscope by staining with Lacto phenol cotton blue staining method. The microscopic analysis of the mycelium with spore revealed that the isolate was *Trichoderma harzianum* which was sub cultured and stored in PDA plate at -20° C.



Fig. 3.2.1. Isolation of *Trichoderma harzianum*

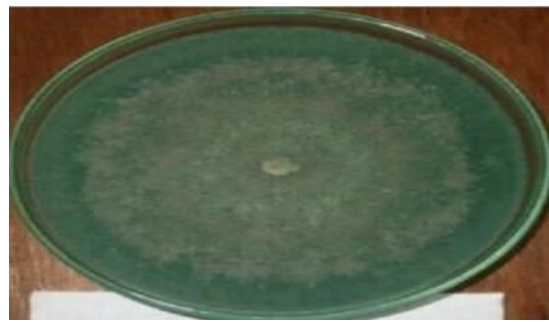


Fig. 3.2.2. Isolation of *Trichoderma harzianum* dark green

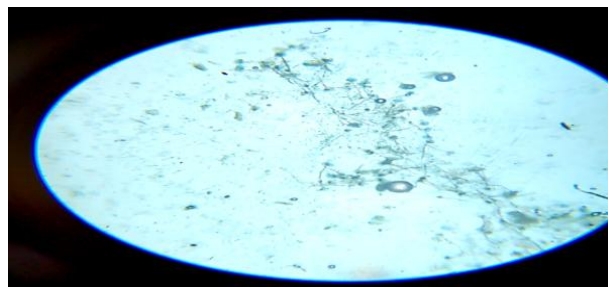


Fig. 3.2.3. Microscopic observation of isolated species

3.3 Morphological characteristics:

1-2 concentric rings with green conidial production. The mycelium production was denser in center then towards the margins.

Table 3.3. Study of morphological characteristics

Sr.No.	Characters	<i>Trichoderma Harzianum</i>
1	Conidial Color	Dark green
2	Conidial Shape	Globule
3	Hyphae	Branched
4	Phialide	Flask shaped
5	Mycelium growth	Dense

3.4. Preparation of *Trichoderma harzianum* inoculums:

50ml sterile distilled water was taken in conical flask. A 7 day old culture of *Trichoderma harzianum* was added into conical flask. Thus suspension mix thoroughly using magnetic stirrer. The Tomato Seeds treated with *Trichoderma harzianum* and untreated (Control) seeds were directly sown on the field.

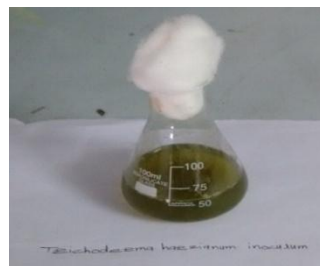


Fig. No. 3.4. *Trichoderma harzianum* inoculums.

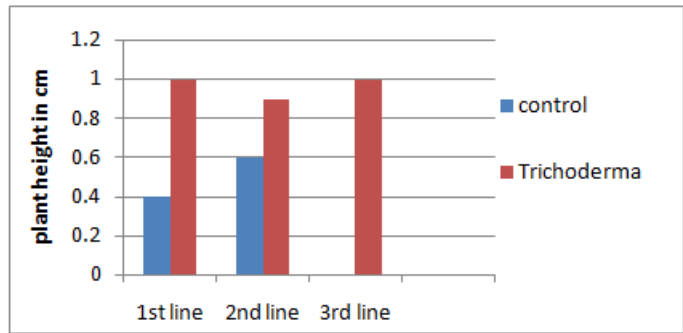


Table No. 3.4 Effect of *Trichoderma Harzianum* on tomato

	CONTROL			TRICHODERMA HARZIANUM (Biofertilizer)		
	1 st line	2 nd line	3 rd line	1 st line	2 nd line	3 rd line
No. of plants	2	3	0	8	7	7
Average height	0.4cm.	0.6cm	0	1.0cm	0.9cm	1.0cm
No. of leaves	2	2	0	2	2	2

The result of Biofertilizer was analyzed by their growth pattern such as plant height, number of leaves and number of branched roots and length of roots. The highest result in terms of plant growth was by *Trichoderma harzianum* against the control plant.



Graph no. 1 4th day Observation

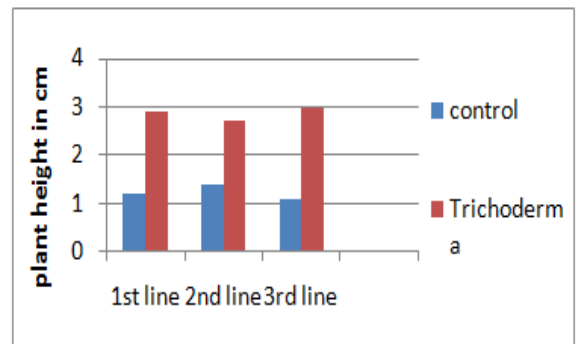


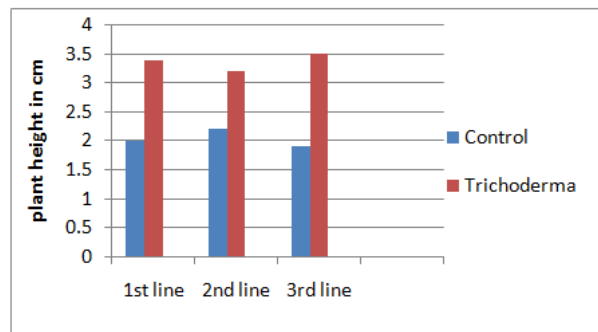
Fig. 3.5 4th Day Observation.

Table No. 3.5 Effect of *Trichoderma Harzianum* on tomato

	CONTROL			TRICHODERMA HARZIANUM (Biofertilizer)		
	1 st line	2 nd line	3 rd line	1 st line	2 nd line	3 rd line
No. of plants	8	8	6	10	9	9
Average height	1.2cm.	1.4cm	1.1cm.	2.9cm.	2.7cm.	3.0cm
No. of leaves	2	2	2	2	2	2



Fig. No.3.6 8th Day Observation



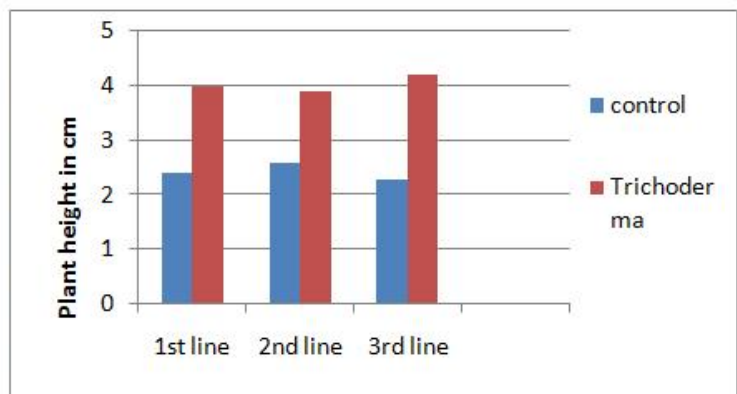
Graph no. 2 8th day observation

Table No. 3.6 Effect of *Trichoderma Harzianum* on tomato

	CONTROL			TRICHODERMA HARZIANUM (Biofertilizer)		
	1 st line	2 nd line	3 rd line	1 st line	2 nd line	3 rd line
No. of plants	8	8	7	10	10	9
Average height	2.0cm.	2.2cm	1.9cm.	3.4cm.	3.2cm.	3.5cm
No. of leaves	2	2	2	4	4	4



FIG. NO. 3.7 12TH DAY OBSERVATION



GRAPH NO.3: 12th DAY OBSERVATION.

Table. No. 3.7 Effect of *Trichoderma Harzianum* on tomato

	CONTROL			TRICHODERMA HARZIANUM (Biofertilizer)		
	1 st line	2 nd line	3 rd line	1 st line	2 nd line	3 rd line
No. of plants	8	8	7	10	10	9
Average height	2.4cm.	2.6cm	2.3cm.	4.0cm.	3.9cm.	4.2cm
No. of leaves	4	4	4	8	8	8

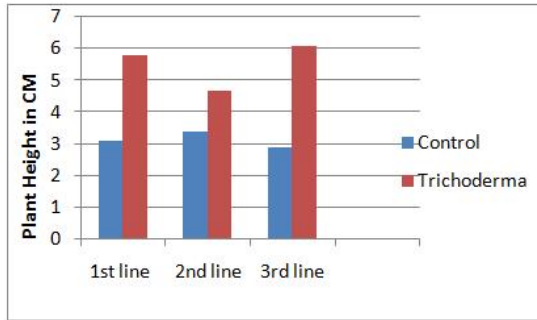


FIG. NO.3.8. 15TH DAY OBSERVATION

GRAPH NO. 4. 15TH DAY OBSERVATION

TABLE NO. 3.8 EFFECT OF TRICHODERMA HARZIANUM ON TOMATO

	CONTROL			TRICHODERMA HARZIANUM (Biofertilizer)		
	1 st line	2 nd line	3 rd line	1 st line	2 nd line	3 rd line
No. of plants	8	8	7	10	10	9
Average height	3.1cm.	3.4cm	2.9cm.	5.8cm.	4.7cm.	6.1cm
No. of leaves	4	4	4	10	10	10
Average height of root	1.8cm.	1.9cm.	2.0cm.	4.1cm	4.2cm	4.4cm
No. of roots	4	4	4	8	8	8

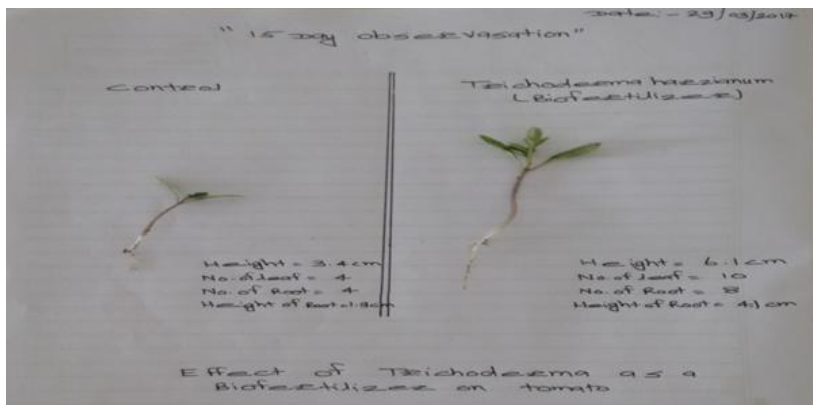


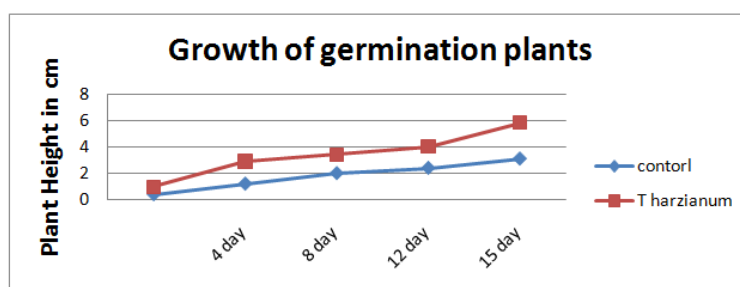
Fig No. 3.9 Effect of Trichoderma harzianum on tomato

Table No. 3.9 Effect of Trichoderma Harzianum on tomato

	Control	Trichoderma harzianum (Biofertilizer)
Height of Plant	3.4 cm	6.1 cm
No. of leaves	4	10
Height of root	1.9 cm	4.1 cm
No. of branched roots	4	8

Table No. 3.10 Effect of *Trichoderma Harzianum* on tomato

Sr. no.	Day	Control (Max. height in cm)	<i>T. harzianum</i> treated tomato plants (Max. height in cm)
1	Seed Germination	0.4cm.	1.0cm
2	4 th	1.2cm	2.9cm
3	8 th	2.0cm	3.4cm
4	12 th	2.4cm	4.0cm
5	15 th	3.1cm	5.8cm

Graph no 6: Effect of *Trichoderma harzianum* on plant growth

4. DISCUSSION:

In the present study, it was clearly observed that the *Trichoderma*-enriched biofertilizer had positive impact on growth and yield of tomato. Maximum yield of tomato plants were increased over control was recorded in 12th day and 15th day. Similarly, the maximum yield was increased over control of tomato was noticed in 15th day, which was analyzed by 5.8 cm height in 1st line and 6.1 cm height in 3rd line. It may be concluded that application of *Trichoderma*-enriched biofertilizer giving higher yields in tomato. Undoubtedly, there is a prospective and potential of *Trichoderma* used as a biofertilizer in crop cultivation to achieve attractive yield.

5. REFERENCES

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