



## Research Article

# Bio-intensive management of major soil borne diseases of tomato in Uttarakhand

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**ABSTRACT:** A glasshouse and field experiments were conducted by using *Trichoderma harzianum* (Th 43), *Pseudomonas* spp. (Pf 173) and Jas Mycorrhizal (AMF) for plant growth promotion and management of root rot and wilt disease of tomato. There were eight treatment combinations for glass house as well as field experiment. In glasshouse, maximum germination (91%), shoot and root length (40.83 cm, 9.79 cm), fresh and dry shoot weight (152.00 g, 28.38 g), fresh and dry root weight (9.33 g, 3.90 g), plant vigour index (4301.45) respectively was recorded in treatment combination of Th 43 + Pf 173 + AMF. In experimental field, maximum reduction of disease over control (72.16%) was found in treatment combination of *T. harzianum* (Th 43) + *Pseudomonas* spp. (Pf 173) + Jas Mycorrhiza (AMF). The maximum yield of tomato (486.70 q/ha) was also recorded in same treatment combination.

**Key words:** Growth promotion, Jas mycorrhizal, *Pseudomonas* spp., *Trichoderma harzianum*

(Article chronicle: Received: 16-06-2019; Revised: 11-11-2019; Accepted: 15-11-2019)

## INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.), is the most widely grown vegetable crop in the world (NHB database 2013). Because of its colour, taste, high nutritive value and diversified use, tomato is the most popular vegetable worldwide. Tomato is fairly adaptable, but grows well in warm conditions at an optimal temperature range of 15°C-25°C at an elevation ranging from 1000 to 2000 meter above sea level. High humidity and low temperature reduce fruit set, yield and quality. Very low temperature delays colour formation and ripening, and high temperature, i.e., 30°C, inhibits fruit set, lycopene development and flavour. Tomato crop thrives best in low to medium rainfall with supplementary irrigation during the off-season. Wet conditions increase disease attacks and affect fruit ripening. Well drained soil with good moisture retaining capacity, high organic matter and pH range of 5.0-7.50 are suitable for tomato cultivation (Robert, 2005; Waiganjo *et al.*, 2006). In India, tomato is cultivated in 882 thousand ha area having production of 18,735.9 thousand metric tons with productivity of 21.24 mt/ha. Uttarakhand contributes an area of 9.08 thousand ha with the production of 113.65 thousand metric ton (Indian Horticulture Database, 2014). Diseases of tomato act as the major limiting factor in quality and productivity. Over 200 diseases have been reported to affect

the tomato plants in the world (Watterson, 1986). Soil borne diseases like damping-off of seedlings, wilt and root rot are caused by several species of *Fusarium*, *Pythium*, *Rhizoctonia* and *Verticillium* etc. causing serious losses in tomato crop. These diseases are widely distributed throughout the world and can significantly reduce the yield and quality of tomato crops (Agrios, 2005). Generally chemicals have been used to manage these diseases over the years. In the beginning, use of chemical pesticides led to remarkable improvements in the productivity. However, the non target effect of these pesticides led to the resistance development in pests. residue in food chains leading to deleterious effect on human health and environment. Due to the excessive use of pesticides, cost of crop production has also increased. Pesticides are toxic in nature and are equally harmful to human beings if applied injudiciously (Abhilash and Singh, 2009). Of late misuse of chemicals has become the most serious environmental issue (Leoz *et al.*, 2013). The challenge today is how to achieve not only food security but also food safety by employing effective as well as environmentally benign measure for management of plant pathogens. Biological control can be achieved either by introducing biocontrol agents directly into natural ecosystem or by adopting practices, which favour population build-up of biocontrol agents under natural conditions. Combination of both the approaches is probably ideal. Prominent among

biocontrol agents is *Trichoderma* which has been recognized as bio-pesticide since long to manage plant diseases as well as bio-fertilizer to increase plant growth and development. It strengthens the plant for its reproductive capacity, ability to survive under unfavorable conditions, increased utilization of nutrients, capacity to modify the rhizosphere and initiates strong aggressiveness against phytopathogenic fungi (Harman, 2000).

Fluorescent *Pseudomonads* are known to improve plant growth by a variety of mechanisms including production of siderophores, synthesis of antibiotics, production of plant growth hormones, enhancement of mineral uptake and synthesis of enzymes that regulate plant ethylene levels (Kloepper *et al.*, 1986; Glick, 1995). Equipped with these abilities, fluorescent pseudomonads are also being exploited as potential crop protectants.

Arbuscular Mycorrhizal Fungi (AMF or AM fungi) are found in the roots of about 80-90 percent of plant species and mutually benefit in a typical symbiotic relationship (Wang and Qiu, 2006). They represent an interface between plants and soils, the mycelia of mycorrhiza grow both inside and outside the plant roots. AMF provide soil mineral nutrients (mainly phosphorus and nitrogen), water and pathogen protection to the plant (Bonfante and Genre, 2010). As a consequence of the above mentioned facts, ecological approaches are now being explored for sustainable plant disease management. The challenge today is to achieve food safety and its sustainability besides food security. Hence to achieve this goal this study was carried out to testing *Trichoderma harzianum* (Th 43) and *Pseudomonas* spp. (Pf 173) and Jas Mycorrhiza (Arbuscular Mycorrhizal fungus), alone and in combination for growth promotion and control of major soil born diseases under field condition.

## MATERIALS AND METHODS

### Study site

Glasshouse experiment was conducted in glasshouse of center of advance faculty training, Department of Plant Pathology, College of Agriculture, G. B. Pant University of Agriculture and Technology Pantnagar, U.S. Nagar-263145, Uttarakhand (India). The field experiment was conducted at Vegetable Research Centre (VRC), G.B. Pant University of Agriculture and Technology, Pantnagar. The centre is situated in the foothills of Himalayas at an altitude of 243.84 m above mean sea level at 29° North latitude and 79.3° East longitude. The climate is humid subtropical with maximum temperature ranging from 32°C to 43°C in summer and minimum ranging from 0°C to 19°C in winters. Monsoon occurs from 3<sup>rd</sup> week of June to middle of September. The frost is experienced from last week of December to the end of February.

## Experimental Material

**Seeds:** Tomato seed of variety TO- 1458 (Syngenta India Ltd) was used in the investigation.

**Soil:** Soil collected from Crop Research Center, Pantnagar was used for glasshouse experiment after sterilization.

**Vermicompost:** Vermicompost obtained from Livestock Research Center, Pantnagar was used for colonization of bioagents and as carrier of microbes in field experiment.

**Microbes formulations:** Talc-based formulations of *Trichoderma harzianum* (Th 43) (NCBI Acc. MK044000.1) and *Pseudomonas* spp. (Pf 173) were obtained from Biocontrol Laboratory, Department of Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar. The Jas Mycorrhiza (Arbuscular Mycorrhizal fungus) manufactured by Shri Ram Solvent Extractions Pvt. Ltd, Jaspur, District Udham Singh Nagar, Uttarakhand, was used in the experiment.

## Evaluation of biocontrol agents for growth promotion under glasshouse condition

A glasshouse experiment was conducted to test plant growth promotion effect of Th 43, Pf 173 and JM in a Completely Randomized Design (CRD) with three replications. Ten kg capacity plastic pots were filled with sterilized, well pulverized sandy-loam soil and treated as per given treatments. Untreated soil was kept as control. Fifteen seeds were sown in each pot. Observation on seed germination was recorded on 15 Days After Sowing (DAS). Thereafter, 5 plants per pot were maintained for 45 days to assess growth promotion effect. Watering of pots was done when required to maintain optimum level of moisture. In order to keep the plants free from insect pests, one spray of Metasystox (0.1 per cent) was given at 20 DAS.

### Treatments for glasshouse experiment

1. *Trichoderma harzianum* (Th 43) @ 10 g/kg
2. *Pseudomonas* spp. (Pf 173) @ 10 g/kg
3. Jas Mycorrhiza (JM) @ 100 g/kg
4. Th 43 + Pf 173 @ each 5 g/kg
5. Th 43 + JM @ 10 g + 100 g/kg
6. Pf 173 + JM @ 10 g + 100 g/kg
7. Th 43 + Pf 173 + JM @ 5 g + 5 g + 100 g/kg
8. Control

### Observations

Observation on seed germination was recorded at 15 DAS, while observations on shoot length (cm) and weight (g) and root length (cm) and weight (g) were recorded at 45 DAS.

#### Seed germination (%)

Seed germination (%) was recorded by using following formula

$$\text{Seed germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

### Shoot and root length

After 45 days of sowing, all the 5 plants were uprooted from each replication treatment. The plants were cut at crown level to separate the root and shoot portions. Shoot length (cm) was measured from highest apical bud to crown level and root length (cm) was measured from crown level to highest apical root apex.

### Fresh shoot and root weight

After the measurement of shoot and root length, the fresh shoot and root were weighed (g) separately on electronic balance.

### Dry root and shoot weight

After recording the fresh shoot and root weight, the same plants were kept in hot air oven at 80°C for 24 hrs for complete drying. After drying, root and shoot weight (g) were measured on electronic balance.

### Vigour index

Vigour Index was calculated by using the seed germination per cent and shoot and root length of plant as below:

$$\text{Vigour Index} = (\text{Shoot length} + \text{Root length}) \times \text{Germination per cent}$$

### Field evaluation

The field trial was conducted during Kharif season of 2013-14 at Vegetable Research Center, GBPUAT Pantnagar. Bio-control agents, Th and Pf and Arbuscular Mycorrhizal Fungi (Jas Mycorrhiza), individually and in combinations were applied at the time of transplanting of seedlings. Experiment was set in a Randomized Block Design (RBD) maintaining three replications for each treatment.

### Field preparation

Experimental field was prepared by one deep ploughing followed by three harrowing and leveling leading to fine and well pulverized soil. Before final leveling of the field, NPK was applied in the form of urea (40 kg), single super phosphate (80 kg), muriate of potash (60 kg) as basal application. Urea as nitrogenous fertilizer was applied @ 20 kg/ha each after 30 days of transplanting and 60 days of transplanting as top dressing.

### Seed root and shoot weight

Tomato seeds of cultivar TO-1458 were sown in nursery bed in the month of August 2013 for raising seedlings. After sowing, seed bed was covered with mulch of dry straw to maintain soil moisture. Light irrigations were given at frequent intervals. After germination of seeds, mulch was removed from the seed bed. Standard agronomical practices were followed for raising the seedlings.

### Transplanting

Tomato seedlings were uprooted from nursery beds 25 days after transplanting (DAS). Vermicompost colonized with bioagent was applied in the soil just before transplanting. The plot size was 2.5 x 2.5 m. A spacing of 50 cm between row to row and plant to plant was maintained. Six foliar sprays of mancozeb (INDOFIL) @ 3 g/liter of water were given first at 40 Days After Transplanting (DAT) rest five in fifteen days intervals after first spray to avoid foliar diseases. To keep the experimental field free from insect pests, four sprays of Metasystox (0.1%) were given at 25, 40, 60 and 80 (DAT). The untreated seedlings served as control. The experiment was laid out in a Randomized Block Design (RBD) with eight treatments, each with three replications.

### Treatments for field experiment

1. *Trichoderma harzianum* (Th 43)
2. *Pseudomonas* spp. (Pf 173)
3. Jas Mycorrhiza (JM)
4. Th 43 + Pf 173
5. Th 43 + JM
6. Pf 173 + JM
7. Th 43 + Pf 173 + JM
8. Control

### Mode of application

Bioagents Th and Pf were multiplied on vermicompost (@ 10 g/kg) separately as well in combinations (5 g+5 g/kg). The colonized vermicompost was applied (@ 50 g/plant) in each plant just before transplanting. The Jas Mycorrhiza (@ 100 g/kg) mixed in vermicompost, with or without colonized with bioagents was applied @ 50 g/plant in the soil just before transplanting.

### Observations

Data on disease incidence was recorded 30, 45, 60 Days After Transplanting (DAT) for wilt and root rot diseases after each observation dead plants of tomato were uprooted and destroyed. The disease incidence was calculated using following formula:

$$\text{Per cent Disease Incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

**Yield**

Ripened fruits were harvested at regular intervals from each plot and total yield (q/ha) was calculated.

**Statistical analysis**

The data recorded under different experiments was subjected to Analysis of Variance (ANOVA) by using standard software STPR developed at Pantnagar University.

**RESULTS AND DISCUSSION**

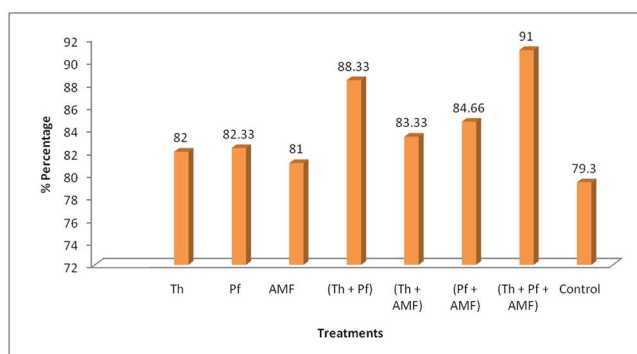
**Effect of bioagents under glasshouse conditions**

Data (Table 1, Fig. 1) revealed that per cent germination in all the treatments was higher than that in control. Maximum germination (91%) was recorded in Th + Pf + JM and was at par with Th + Pf (88.3%) but significantly different from the other treatments and control (79.30%). Root and shoot length, fresh and dry weight of shoot and root were higher in all the treatments as compared to control. Maximum shoot and root length (40.83 cm, 9.79 cm), fresh and dry shoot weight (152.00 g, 28.38 g), fresh and dry root weight (9.33 g, 3.90 g), were recorded in treatment Th + Pf + JM followed by Th + Pf. Maximum plant vigour index (4301.45) was observed in Th + Pf + JM followed by Pf + JM, Th + JM, Th + Pf, and were at par with each other but were significantly higher than control (2931.97) (Fig. 2). The present findings on increase in growth parameters are supported by the findings of Elad *et al.* (2007). Their studies have shown that there was a 30 per cent increase in seedling emergence in plots inoculated with *Trichoderma*. Shores and Harman (2008) reported on overall increased in plant growth of maize when seeds

were treated by *Trichoderma*. Mwangi *et al.* (2011) reported that treatment with *T. harzianum* and AMF significantly enhanced the plant heights and shoot and root dry weight of tomato. Azarmi *et al.* (2011) reported that shoot height, fresh and dry weight of shoot and root of tomato seedlings were significantly increased by the application of *Trichoderma*. Singh *et al.* (2013) observed greater plant growth promotion activity in tomato in the combination of *Trichoderma harzianum* and *Pseudomonas*. Tanwar *et al.* (2013) reported significantly increased chlorophyll a, plant height and root fresh weight, above ground dry weight, head fresh weight, and root nitrogen content in Broccoli in that consortium of AMF + *Trichoderma* + *Pseudomonas*.

**Effect of bioagents under field conditions**

All the treatments were effective in lowering the plant mortality (Table 2) as compared to control. However,



**Fig. 1. Effect of BCAs and AMF on tomato seed germination.** Th = *Trichoderma harzianum*, Pf = *Pseudomonas* spp. AMF = Jas Mycorrhiza

**Table 1: Effect of biocontrol agents and arbuscular mycorrhizal fungi on plant growth promotion in tomato under glasshouse conditions**

S.No.	Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Dry root Weight (g)	Plant Vigor Index
1	<i>Trichoderma harzianum</i> (Th 43)	82.00 (65.18)	35.85	5.64	132.98	7.48	25.00	2.25	3292.57
2	<i>Pseudomonas</i> spp. (Pf 173)	82.33 (65.67)	33.08	6.30	132.67	6.89	23.83	2.13	3319.05
3	Jas Mycorrhiza (JM)	81.00 (64.22)	35.11	6.52	123.17	7.34	25.33	2.05	3366.63
4	Th + Pf	88.33 (70.69)	33.06	5.98	126.07	6.96	24.50	1.82	3452.90
5	Th + JM	83.33 (65.91)	35.00	6.25	131.00	6.78	25.33	1.54	3416.07
6	Pf + JM	84.66 (66.42)	37.03	7.40	141.00	8.21	25.53	2.28	3603.18
7	Th + Pf + JM	91.00 (73.29)	40.83	9.79	152.00	9.33	28.38	3.90	4301.45
8	Control	79.30 (62.96)	32.17	5.21	115.00	6.67	22.66	1.95	2931.97
	CD (5%)	4.24 (4.47)	3.59	1.64	5.19	0.60	3.18	0.53	343.90
	CV (%)	7.19 (6.31)	5.96	6.24	2.48	4.73	5.61	4.85	6.15

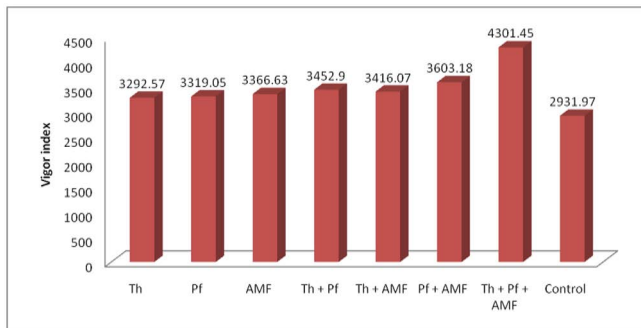
Microbes were directly added in the sterilized soil just before the sowing of seed in pots.

Data average of three replications. Data in parenthesis is angular transformed value.

Doses: Th @10g/kg, Pf @10g/kg, JM @100g/kg, Th + Pf @ each5g /kg, Th + JM @10g + 100g /kg, Pf + JM @10g + 100 g /kg and Th + Pf + JM @5g + 5 g + 100g /kg.

combination treatment *T. harzianum* (Th 43) + *Pseudomonas* spp. (Pf 173) + Jas Mycorrhiza (AMF) was most effective in reducing plant mortality and enhancing the yield (q/ha). At 30 Days After Transplanting (DAT) 74.68%, 45 DAT 71.73% and 60 DAT 72.16% reduction in disease was found over control followed by treatment four combination of *T. harzianum* (Th 43) + *Pseudomonas* spp. (pf 173) where as at 30 days after transplanting (DAT) 62.68%, 45

DAT 55.43% and 60 DAT 52.18% reduction in disease was found over control. The highest yield was also recorded in T-7(486.70 q/ha) followed by T- 4 (374.33 q/ha). Akopru and Demir (2005) reported that reduction in 58 per cent plant mortality caused by *Fusarium* in tomato by the application of AMF and *Pseudomonas fluorescens*. Yigit and Dikilitas (2007) reported 70.20 per cent disease reduction caused by *Fusarium oxysporum f. sp. lycopersici* in tomato by the use of combination of *Pseudomonas fluorescens* and *Trichoderma harzianum* - 22 under green house conditions. Srivastava *et al.* (2010) also reported that the combination of *T. harzianum*, fluorescent *Pseudomonas* and AMF gave significant disease reduction in incidence (70%), then control and increased in yield (20%). Mwangi *et al.* (2011) also reported that Th (P52) and AMF in combination significantly enhanced plant height, root dry weight and reduced wilt pathogen caused by *F. oxysporum f. sp. lycopersici* in tomato. Singh *et al.* (2013) has been reported disease reduction (53.23%) and significant increased in yield in tomato. They also observed that in the combination of *Trichoderma harzianum* and *Pseudomonas* consortium of these bioagents enhanced the plant growth.



**Fig 2. Effect of biocontrol agents and arbuscular mycorrhizal fungi on Plant vigor of tomato under screen house conditions.**

(Th= *Trichoderma harzianum*, Pf = *Pseudomonas* spp. AMF = Jas Mycorrhiza)

In conclusion, the present study confirms that the soil

**Table 2: Effect of different treatments on the management of major soil born diseases (wilt and root rot) of tomato**

S. No.	Treatment details	30 DAT		45 DAT		60 DAT		Production	
		Plant Mortality	Percent decrease over control	Plant Mortality%	Percent decrease over control	Plant Mortality %	Percent decrease over control	Yield (q/ha)	Percent increase over control
1	<i>Trichoderma harzianum</i> (Th 43)	12.66 (18.08)	49.36	14.67 (21.68)	52.17	20.67 (24.09)	46.07	345.33	51.83
2	<i>Pseudomonas</i> spp. (Pf 173)	11.66 (19.98)	53.36	16.67 (24.09)	45.65	19.33 (26.07)	49.56	335.00	50.35
3	Jas Mycorrhiza (AMF)	14.33 (22.21)	42.68	20.00 (26.55)	34.79	22.00 (27.96)	42.60	327.33	49.19
4	<i>Trichoderma harzianum</i> (Th 43) + <i>Pseudomonas</i> spp. (Pf 173)	9.33 (17.77)	62.68	13.67 (21.68)	55.43	18.33 (25.34)	52.18	374.33	55.57
5	<i>Trichoderma harzianum</i> (Th 43) + Jas Mycorrhiza (AMF)	10.00 (18.42)	60.00	15.33 (23.05)	50.02	21.33 (27.50)	44.35	356.00	53.27
6	<i>Pseudomonas</i> spp. (Pf 173) + Jas Mycorrhiza (AMF)	11.33 (19.66)	54.68	16.67 (24.09)	45.56	20.67 (27.03)	46.07	327.33	49.19
7	<i>Trichoderma harzianum</i> (Th 43) + <i>Pseudomonas</i> spp. (Pf 173) + Jas Mycorrhiza (AMF)	6.33 (14.51)	74.68	8.67 (17.09)	71.73	10.67 (19.04)	72.16	486.70	65.82
8	Control	25.00 (29.99)	-	30.67 (33.62)	-	38.33 (38.25)	-	166.33	-
	CD (5%)	2.36 (2.06)	-	2.22 (1.77)	-	2.55 (1.81)	-	4.27	-

Data in parenthesis are angular transformed value, Values are average of three replication DAT= Date after transplanting, Plant Mortality= Due to wilt and root rot of tomato

application of the combination of *Trichoderma harzianum* (Th 43) + *Pseudomonas* spp. (Pf 173) + Jas Mycorrhizal (AMF) was not only very effective for the growth promotion of but also for the management of tomato root rot and wilt pathogens.

#### ACKNOWLEDGEMENTS

Authors are thankful to Joint Director, VRC, Head Department of Plant Pathology, Dean College of Agriculture, G.B. Pant University of Agriculture and Technology, Pantnagar, for providing necessary resources and time to time guidance during the experiment.

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