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# Comparative Efficacy of Bioinoculents, Organic Amendments and Pesticides Against *Rhizoctonia solani* Alone on Tomato CV. K-25 under Pot Conditions

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**Abstract:** Studies were undertaken to determine the comparative efficacy of native biocontrol agents viz., *Pseudomonas fluorescens* isolate PS-4*, Trichoderma harzianum* isolate TH-H-3 and *T. virens* isolate TV-K-3 @ $5X10^8$ cfu/g talc; organic amendments viz., neem seed powder @ 250 mg/kg soil and farmyard manure @ 1500 mg/kg soil; carbofuran @ 33.4 mg/kg soil, topsin-M @ 1.4 mg/kg soil and bavistin @ 2.0 mg/kg soil against *Rhizoctonia solani* @ 1.5 g mycelial mat/kg soil on tomato cv. K-25 under pot conditions. Results revealed that all the treatments significantly increased the plant growth, fruit yield and decreased the root infection by the *R. solani* on tomato as compared to untreated inoculated plants. Highest plant dry weight (46.2 g) and fruit yield (213.0 g) was obtained in plants treated with *T. harzianum* followed by bavistin, *P. fluorescens*, topsin-M, *T. virens*, neem seed powder, carbofuran and farmyard manure, respectively. The highest reduction in root infection by the fungus (5.0%) was found in plants treated with *T. harzianum* followed by bavistin, *P. fluorescens*, topsin-M, *T. virens*, neem seed powder, carbofuran and farmyard manure, respectively.

**Key words:** Tomato · *Rhizoctonia solani* · Biocontrol · Organic amendments · Pesticides · Management

## INTRODUCTION

Tomato is one of the most popular and widely grown vegetables in the world. Leading tomato producing countries include China, USA, Turkey, Russia, Italy, Egypt, India, Spain and Mexico. In India, production of tomato was about 11.15 million tonnes, from 0.6 million ha of land with the productivity of 18.6 tonnes/ha and has increased rapidly over the last decade, both in terms of tonnage and area [1]. This crop is attacked by several diseases caused by fungi, bacteria, viruses and nematodes leading to severe crop losses. Among fungal diseases, root rot disease induced by *R. solani* is prevalent. *R. solani* is a pathogen with an exceptionally broad host range that includes over 500 plant species including tomato [2, 3]. The management of this disease is difficult due to long saprophytic survival ability of the pathogen in soil [4]. However, the most common means to control the disease is chemicals, but frequent and indiscriminate use of chemicals often leads hazardous effect on human and also responsible for atmospheric pollution. Reduction or elimination of soil-borne inoculum is the only effective solution to overcome the problem and this may be achieved easily through the use of bioinoculents and organic amendments. Therefore, studies were undertaken to determine the comparative efficacy of naturally occurring bioinoculents, organic amendments and pesticides against *R. solani* on tomato cv. K-25 under pot conditions.

## MATERIALS AND METHODS

**Preparation of Inoculum of *R. solani:*** Pure culture of *R. solani* isolated from the infested plants from the field was maintained on PDA (Potato Dextrose Agar) in Petri dishes at 27±5°C in order to mass-production of the culture. Thereafter, pure culture of the fungus was transferred to flasks containing PDB (Potato Dextrose Broth). The flasks were incubated in a BOD (Biological Oxygen Demand) incubator at a temperature of 27±1°C for 10 days. The known amount of mycelial mat was blended with sterilized distilled water (1:2 w/v) in warring blender for 30 seconds.

**Preparation of Inoculum of *T. harzianum, T. virens* and *P. fluorescens*:** Pure culture of *T. harzianum* isolate TH-H-3 and *T. virens* isolate TH-K-3 isolated from the farmer's field and was maintained on PDA in separate

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Petri dishes at 27±1°C in the laboratory. Mass culture was maintained on PDB in 250 ml Erlenmeyer flasks. Each flask containing 100 ml PDB were plugged with cotton and sterilized by autoclaving for 30 min at 1 kg/cm$^2$ pressure. The flasks were then allowed to cool at room temperature; afterwards each flask was inoculated separately with 1-cm-diameter PDA discs punched from the periphery of actively growing 5-days-old culture of *T. harzianum* isolate TH-H-3. Flasks were then placed in a BOD incubator at 27±1°C and the fungus was allowed to grow for 10 days and after that talc was mixed in culture to maintain the $10^{10}$ cfu/g culture.

Pure culture of *P. fluorescens* isolate PS-4 also isolated from the farmer's field. The culture tubes, each containing 10 ml King's 'B' (Broth) were autoclaved for 30 min at 1 kg/cm$^2$ pressure. After the culture tubes were cooled, each tube was inoculated with a single colony of *P. fluorescens* strain Pf-1 from pure bacterial culture maintained on King's 'B' medium. The culture tubes were then placed in a BOD incubator for 48 h at 30±1°C for the multiplication of *P. fluorescens*. For mass production, 250 ml Erlenmeyer flasks containing 100 ml King's 'B' broth were autoclaved at the same pressure and time as mentioned above. Later, flasks were inoculated with 1.0 ml of *P. fluorescens* cultured broth. The flasks were then kept at 30±1°C in a BOD incubator for 48 h and were shaken twice a day so as to get a uniform growth. Culture was then mixed with talc in the ratio of 1:4 and afterwards the amount of talc was adjusted so that the final cfu of *P. fluorescens* was maintained on $10^{10}$/g culture.

**Experimental Procedure:** Experiment was conducted in 25 cm top diameter earthen pots filled with a mixture of autoclaved sandy loam soil (sand 70%, silt 22% and clay 8%, pH 7.5) and compost (4:1). Twenty-day-old seedlings of tomato cv. K-25 were singly transplanted in all the pots. Various treatments, *Pseudomonas fluorescens, T. harzianum* and *T. virens* @ 0.5x$10^9$ cfu/kg soil; neem seed powder @ 0.25 g/kg soil and farmyard manure @ 1.5 g/kg soil; carbofuran @ 0.033 g/kg soil, bavistin @ 0.0014 g/kg soil and topsin-M @ 0.002 g/kg soil were applied into soil. The neem seed powder, farmyard manure and bioinoculents were applied a week before transplantation, while pesticides were applied a day before transplantation. The treated pots were irrigated as per the need to maintain good soil moisture. Three days after transplantation, roots of tomato were exposed by removing the soil and 1.5 g mycelial mat of *R. solani* culture/kg soil grown on PDB. It was mixed in soil around the exposed roots, afterwards roots were covered gently with sterilized soil.

**Recording of Data:** Picking of ripened fruits was done 110 day after inoculation. The number of fruits and weight were taken separately from each treatment and replicate. Thereafter plants were carefully uprooted from pots and the roots were washed in running tap water to remove the adhering soil particles. Excess water was removed with blotting paper. Plant growth was determined by measuring shoot height, fresh and dry weights of shoot and roots. For determining dry weight, the shoot and roots were dried in an oven at 60°C for 24 h. The percent reduction in plant growth over un-inoculated control was also calculated. The washed roots were cut into pieces of 1.0 cm length and treated with 10% KOH solution and kept at 90°C in a hot air oven for 1 h. The root pieces were then washed again with distilled water, acidified and stained with trypan blue (0.05% in lactophenol) [5]. Ten stained root pieces were taken separately from the samples of individual locality and mounted on a slide in lactophenol and observed under microscope. The portion of length of root pieces, which showed the presence of hyphae of fungi, was estimated. The percent root infection was calculated by measuring the infected portion in relation to total length of root pieces [6]. Data were analyzed by analysis of variance [7] and significant differences among treatments were tested by the least significant difference test (LSD) at probability levels of 5% (LSD 0.05) and 1% (LSD 0.01).

## RESULTS

Data presented in Table 1 regarding the comparative efficacy of different additives against *R. solani* indicated that all the treatments significantly ($P \leq 0.05$) increased the plant growth and fruit yield of tomato cv. K-25 as compared to untreated inoculated plants. Effect of various treatments on plant dry weight and fruit yield was mostly significant ($P \leq 0.05$). However, the highest improvement in plant dry weight (46.2 g) and fruit yield (213.0 g) was found in plants treated with *T. harzianum* followed by topsin-M, *P. fluorescens,* bavistin, *T. virens*, neem seed powder, carbofuran and farmyard manure, respectively.

The extent of root infection by the *R. solani* was decreased by the application of various treatments as compared to untreated inoculated plants. The highest reduction in root infection by the fungus (5.0%) was

Table 1: Comparative efficacy of biocontrol agents, organic additives and pesticides on disease development, plant growth and fruit yield of tomato cv. K-25 inoculated with *Rhizoctonia solani* (7.5 g mycelium/5 kg soil) under pot conditions[a]

| | | | Plant length (cm) | | | Plant fresh weight (g) | | | Plant dry weight (g) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | Number of fruits | Fruit weight (g) | Shoot height | Root length | Total | Shoot | Root | Total | Shoot | Root | Total | Percent root infection[c] |
| Untreated-uninoculated Control | 10.6 | 225.0 | 46.3 | 27.6 | 73.9 | 176.3 | 37.7 | 214.0 | 37.5 | 10.9 | 48.4 | - |
| Untreated inoculated control | 8.4 | 173.0 | 37.0 | 23.2 | 60.2 | 140.0 | 31.1 | 171.1 | 29.1 | 8.9 | 38.0 | 25.0 |
| | (20.8)[b] | (23.1) | (20.1) | (15.9) | (18.5) | (20.6) | (17.5) | (20.0) | (22.4) | (18.3) | (21.5) | |
| *T. harzianum* | 10.2 | 213.0 | 44.8 | 27.0 | 71.8 | 169.7 | 37.3 | 207.1 | 35.5 | 10.7 | 46.2 | 5.0 |
| | (3.8) | (5.3) | (3.2) | (2.2) | (2.8) | (3.7) | (1.1) | (3.2) | (5.3) | (1.8) | (4.5) | |
| *T. virens* | 9.8 | 205.5 | 42.6 | 26.2 | 68.8 | 163.9 | 35.9 | 199.8 | 34.2 | 10.3 | 44.5 | 8.5 |
| | (7.5) | (8.7) | (7.8) | (5.1) | (6.9) | (7.0) | (4.8) | (6.6) | (8.8) | (5.5) | (8.1) | |
| *P. fluorescens* | 10.0 | 210.0 | 43.5 | 26.4 | 69.9 | 167.3 | 36.6 | 203.9 | 34.9 | 10.5 | 45.4 | 7.5 |
| | (5.7) | (6.7) | (6.0) | (4.3) | (5.4) | (5.1) | (2.9) | (4.7) | (6.9) | (3.7) | (6.2) | |
| Farm yard manure | 8.8 | 183.5 | 38.8 | 24.0 | 62.8 | 149.1 | 32.8 | 181.9 | 31.0 | 9.4 | 40.4 | 21.0 |
| | (17.0) | (18.4) | (16.2) | (13.0) | (15.0) | (15.4) | (13.0) | (15.0) | (17.3) | (13.8) | (16.5) | |
| Neem Seed Powder | 9.8 | 203.5 | 41.6 | 25.5 | 67.1 | 161.7 | 35.7 | 197.4 | 33.8 | 10.2 | 44.0 | 11.0 |
| | (7.5) | (9.6) | (10.2) | (7.6) | (9.2) | (8.3) | (5.3) | (7.8) | (9.9) | (6.4) | (9.1) | |
| Carbofuran | 9.0 | 185.5 | 39.0 | 24.2 | 63.2 | 152.0 | 33.5 | 185.5 | 31.7 | 9.6 | 41.3 | 20.0 |
| | (15.1) | (17.5) | (15.8) | (12.3) | (14.5) | (13.8) | (11.1) | (13.3) | (15.5) | (11.9) | (14.7) | |
| Topsin-M | 10.0 | 211.0 | 43.7 | 26.3 | 70.0 | 168.7 | 36.9 | 205.6 | 35.2 | 10.6 | 45.8 | 6.5 |
| | (5.7) | (6.2) | (5.6) | (4.7) | (5.3) | (4.3) | (2.1) | (3.9) | (6.1) | (2.8) | (5.4) | |
| Bavistin | 9.8 | 203.0 | 42.9 | 26.0 | 68.9 | 164.3 | 36.2 | 200.5 | 34.3 | 10.4 | 44.7 | 7.5 |
| | (7.5) | (9.8) | (7.3) | (5.8) | (6.8) | (6.8) | (4.0) | (6.3) | (8.5) | (4.6) | (7.6) | |
| $LSD_{0.05}$ | 0.38 | 8.93 | 1.83 | 1.23 | 3.68 | 7.83 | 1.53 | 9.21 | 1.69 | 0.52 | 2.10 | 1.00 |
| $LSD_{0.01}$ | 0.52 | 12.06 | 2.49 | 1.66 | 4.93 | 10.57 | 2.07 | 12.43 | 2.28 | 0.70 | 2.84 | 1.35 |

[a]Each value is an average of five replicates

[b]Figures in parentheses are percent reduction over untreated uninoculated control

[c]Percent root infection in root by *R. solani*

noted in plants treated with *T. harzianum* followed by topsin-M, bavistin, *P. fluorescens*, *T. virens*, neem seed powder, carbofuran and farmyard manure, respectively (Table 1).

## DISCUSSION

All the treatments significantly increased the plant growth and fruit yield and reduced the root infection by the *R. solani.* The efficacy of these treatments against *R. solani* has also been reported by other workers [8- 18]. *T. harzianum* was found highly effective followed by topsin-M, *P. fluorescens,* bavistin, *T. virens*, neem seed powder, carbofuran and farmyard manure, respectively. *Trichoderma* spp., when applied as seed and soil treatment provide long term protection against soil borne pathogens and enhance the plant growth [19, 20 a, b]. Earlier, Elad [21] also advocated for the various mechanisms responsible for the efficacy of *Trichoderma* spp. against phytopathogenic fungi. The disease controlling efficacy of *Trichoderma* spp. is also due to their ability to grow rapidly and to colonize the infection courts, thus, competing with the pathogens in soil [22]. Besides, these mycoparasitic fungi are able to attack sclerotia or mycelium of soil borne of the pathogens and reduce inoculum in the soil [23-26]. On the other hand, the fungitoxic activity of topsin-M against *R. solani* has been claimed due to its conversion into methyl-2-benzimidazole carbamate (MBC), it may be in response to its transformation to ethyle-2-benzimidazole carbamate (EBC). However, the MBC is the actual fungitoxic moiety of topsin-M. This fungicide also acts by interfering in DNA synthesis/nuclear or cell division [27, 28]. Whereas, Kataria and Grover [29] opined that topsin-M causes inhibition of respiration and synthesis of DNA.

However, the antagonistic potential of *P. fluorescens* (PS-4) against *R. solani* could be attributed to the production of antibiotics [31], siderophore production [32, 33] and due to induced systemic resistance [34]. Besides these, suppression of root pathogens is also due

to competition for food and ability to colonize the roots [35]. Moreover, *P. fluorescens* produces plant growth promoting substances, thereby enhancing plant growth and yield [36, 37]. Bavistin was found effective in controlling root colonization by fungus. It inhibits the nuclear division of fungi by inactivating the spindle, which is composed of microtubules. Various scientists have also been reported, bavistin as an important control measure against *R. solani* [18, 38].

It is concluded that biocontrol agents were highly effective and organic amendments were moderately effective against *R. solani*. Therefore, further research is needed for their combined use in integrated management of *R. solani*.

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