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Research Article

ESTIMATION OF POPULATION AND EVALUATION OF RHIZOSPHERE MYCOFLORA AND FLUORESCENT PSEUDOMONADS AGAINST *Rhizoctonia solani* UNDER *IN VITRO* CONDITION

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Abstract- Tomato crop is attacked by several soil borne fungal pathogens among which *Rhizoctonia solani* is the most important soil borne pathogen causing symptoms of damping off, crown rot and root rot. To provide bio-efficacy against casual agent, the population of rhizosphere mycoflora and fluorescent pseudomonads was estimated from the soil samples collected from diseased, healthy and luxuriously growing weed plants as colony forming units (cfu) on different culture media and were tested for their antagonistic potential against R. *solani* under *in vitro* condition. Among the sources of rhizosphere soil collected, significantly highest mean cfu count of mycoflora (27.70) and fluorescent pseudomonads (40.75) was observed in case of samples collected from weed plants followed by healthy tomato plants. Among the mycoflora, the isolate M10 was found to be the potential antagonist and was identified as *Trichoderma viride,* whereas the effective pseudomonad isolate P1 was identified as *Pseudomonas fluorescens*.

Keywords- *Rhizosphere isolates, Rhizoctonia solani,* Root rot, *Pseudomonads* and *Trichoderma.*

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Introduction

The tomato (*Solanum lycopersicum L.*) is one of the most widely consumed vegetables in the world, next to potato. Plant diseases constitute a major constraint to crop production often resulting in a great degree of crop losses which may range from slight to 100 per cent [1]. Diseases of tomato caused by fungi, bacteria, viruses, and nematodes can be severe, reduce tomato yield and quality wherever the crop is grown. Among fungal diseases that attack tomato crop early blight, late blight, leaf spots, anthracnose, buckeye rot, wilts and root rots are found to be the important diseases [2]. However, the root rot caused by *Rhizoctonia solani* is the most devastating and destructive disease of tomato and became the major constraint to the profitable production of tomato in India.

As *Rhizoctonia solani* is a soilborne pathogen, the disease caused by it is difficult to manage due to its exceptionally broad host range that includes over 500 plant species including tomato. On the other hand, management of this disease is difficult not only due to its wide host range and soilborne nature but also the long saprophytic survival ability of the pathogen in soil [3].

Though damping off caused by *R. solani in* tomato is very common to see in nurseries, an increased incidence of blight caused by *R. solani* was noticed in recent past in major tomato growing areas of Ranga Reddy district of Andhra Pradesh. The disease was found to appear predominantly during early transplanted to flowering and fruiting stage of the crop causing considerable loss to the crop both in terms of plant stand and yield. However, the disease incidence varied from 12.8 to 33.2% during 2011-12 rabi season under natural field conditions [4].

Keeping the increased incidence of blight on tomato in view, the present study was planned to conduct a systematic study on this disease with following objective.

Objective:

In vitro evaluation of rhizosphere mycoflora and Fluorescent pseudomonads against *Rhizoctonia solani.*

Materials and Methods

In vitro evaluation of rhizosphere mycoflora and fluorescent pseudomonads against *Rhizoctonia solani*

Isolation of rhizosphere mycoflora and fluorescent pseudomonads

To isolate rhizosphere mycoflora and fluorescent pseudomonads, soil samples from different tomato growing fields of major tomato growing areas of Ranga Reddy district were collected. The soil samples were collected from rhizosphere of diseased tomato plants, healthy tomato plants and luxuriously growing weed plants adjacent to the diseased tomato plants. The soil adhering to the roots was collected and mixed to prepare a composite rhizosphere soil.

For the isolation of mycoflora and fluorescent pseudomonads, the serial dilution technique proposed by [5] was followed. About 10 g of soil from sample was taken in a 250 ml conical flask with 90 ml of sterile distilled water. The sample was agitated for 5 minutes and serial dilutions of soil suspensions were prepared.

To isolate mycoflora one ml of 10^{-4} dilution was poured in to sterilized Petri plates containing PDA, TSM, Pikovskaya's agar and Actinomyces isolation agar medium. To isolate bacteria, one ml of 10^{-6} dilution was poured into sterilized Petri plates containing PDA, Nutrient agar, King's medium B base, Pseudomonas agar (for fluorescin), Actinomyces isolation agar medium and Pikovskaya's agar media. The Petri plates were incubated at 26 ± 2°C temperature. The plates were examined daily and colony forming units (cfu) counts were taken on third day for bacteria and fifth day for fungi with the help of 'Quebec' colony counter.

Number of cfu per gram of the soil was calculated by using the following formula

$$\text{Number of cfu g}^{-1}\text{ of soil} = \frac{\text{Colony count on an agar plate X dilution factor}}{\text{Dry weight of the soil}}$$

The individual fungal colonies were directly transferred onto the PDA slants with the help of a sterilized needle. The fungal cultures were further purified by hyphal tip method. Pure cultures of the fungal biocontrol agents were maintained on PDA at 26 ± 2°C by periodical transfers. The fluorescent pseudomonads were purified by streak plate method and maintained on *Pseudomonas* agar (flourescin) by periodical transfers.

Evaluation of antagonistic activity of rhizosphere mycoflora and fluorescent pseudomonads

The antagonistic activity of all the mycoflora and fluorescent pseudomonads isolated from rhizosphere soils was tested against the test pathogen *R. solani* by adopting dual culture technique [6].

Dual culture technique

About 15 to 20ml of melted and cooled PDA medium was poured into Petri plates and allowed to solidify. Five mm discs of both antagonist and the test pathogen were placed in opposite direction on PDA at two cm away from the periphery of Petri plate and four cm apart from each other. Similarly, to evaluate the fluorescent pseudomonads, the bacterial culture was streaked at one end of the of Petri plate (two cm apart from periphery) while at the other end five mm disc of test pathogen was placed. In control plate the test pathogen alone was placed on PDA.

The plates were incubated in a BOD incubator at 26 ± 2°C till the mycelial growth in the control plates covered the entire plate. The radial growth of the pathogen was measured and the per cent inhibition was calculated by adopting following formula.

$$\text{Per cent inhibition over control (I)} = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of test pathogen

C = Radial growth of test pathogen in control (mm)

T = Radial growth of test pathogen in treatment (mm)

Identification of potential antagonistic bioagents

Among all the mycoflora and fluorescent pseudomonads the potential antagonistic bioagents were detected by performing dual culture technique under laboratory conditions.

Of these, the potential antagonistic fungus was identified based on its morphological and cultural characteristics [7] And the effective fluorescent pseudomonads was cultured on *Pseudomonas* Agar (for fluorescin) medium and was tested for its fluorescens using U.V. chamber. Further this potential fluorescent *Pseudomonas* sp. was subjected to Gram's staining [8]. As they were identified as *Trichoderma viride* and *Pseudomonas fluorescens,* they were maintained on their respective media by periodical transfers.

Results and Discussion

In-vitro* evaluation of rhizosphere mycoflora and fluorescent pseudomonads against *Rhizoctonia solani

Control of soilborne plant pathogens by application of fungicides often gives viable success despite the high cost involvement. Hence, there is a need to search for alternative methods of control. Biological control through introduction of microorganisms antagonistic to plant pathogens is one of the important strategy in the management of soilborne plant pathogens. In the present investigation, studies were conducted to know the antagonistic activity of biocontrol agents on *R. solani*.

Estimation of population of mycoflora and fluorescent pseudomonads in soil samples collected from tomato fields

The total population of mycoflora and fluorescent pseudomonads in soil samples collected from three different sources was estimated as colony forming units (cfu) on different culture media and the data is presented in [Table-1 and 2].

Mycoflora population

The results indicated that there was significant difference for colony forming units of mycoflora estimated from different rhizosphere sources on different culture media [Table-1] and [Fig-1].

Among the media used the mean of cfu count was significantly highest on PDA (34.08) followed by *Trichoderma* specific medium (29.68), while that of the least was observed on pikovskaya's agar (12.20) followed by actinomycete isolation agar (14.60) Across the sources of rhizosphere soil significantly highest cfu count was observed in case of weeds (27.70) followed by that in healthy plants (21.21) and diseased plants (20.20). The high cfu count of mycoflora from the rhizosphere soil of weeds present in and around tomato fields may be attributed to their ability to harbor a wide range of microorganisms.

No significant difference for cfu count was observed between diseased plants (12.14) and weeds (12.30) when the mycoflora was estimated on actinomycete isolation agar. Similarly, the cfu counts obtained on *Trichoderma* specific medium were non-significant when they were isolated from healthy (23.86) and diseased (24.20) plants.

Fluorescent pseudomonads population

The data presented in the [Table-2] revealed that, there was significant difference for cfu counts of fluorescent pseudomonads estimated from different soil samples on different culture media used.

Across the media used, significantly highest mean cfu count was recorded on *Pseudomonas* agar medium (56.15) followed by king's medium B base (40.65) and least of that was recorded in pikovskaya's agar (10.03) followed by actinomycete isolation agar (10.86). However, the cfu counts obtained on PDA (33.82) and NA (34.23) are at par with each other.

The rhizosphere soil collected from weed plants resulted in significantly highest mean cfu count (40.75) where as the lowest (21.31) was observed in case of rhizosphere samples collected from diseased plants followed by healthy plants (30.81).

Significantly highest cfu counts from rhizosphere soil of weeds (86.80) and healthy plants (54.80) were recorded on *Pseudomonas* agar medium, while that from the rhizosphere soil of diseased plants (30.14) was observed on king's medium B base medium. However, it was found to be least from all the sources of rhizosphere soil on pikovskaya's agar medium.

No significant difference for cfu counts was observed on PDA (35.50) and NA (35.40) when fluorescent pseudomonads were isolated from the rhizosphere soil of healthy plants while the same trend was observed with actinomycete isolation agar media for cfu counts of rhizosphere soil samples collected from diseased (10.70) and weed plants (10.30).

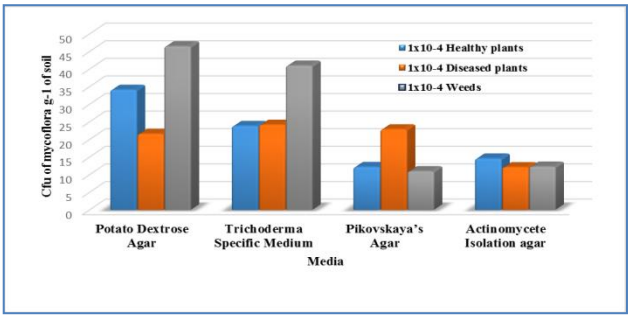

Fig-1 Colony forming units (cfu) of mycoflora formed on different culture media

Similar studies were conducted by [9] who collected soils from five land use types to isolate and identify the mycoflora based on morphological characteristics. The present findings are supported by the results of [10] in which fifteen native *Trichoderma* antagonists were isolated from healthy tomato rhizosphere soil of different geographical regions. Evidences also exist for the isolation of a group of bacteria that are widely distributed and commonly occurring in soils using appropriate media [11].

. **Table-1** *Colony forming units (cfu) of mycoflora formed on different culture media*

	Cfu of mycoflora g-1 of soil (1×10^4)			
Media	**Healthy plants**	**Diseased plants**	**Weeds**	**Mean**
Potato Dextrose Agar	34.14	21.60	46.50	34.08
Trichoderma Specific Medium	23.86	24.20	41.00	29.68
Pikovskaya's Agar	12.20	22.90	11.00	15.37
Actinomycete Isolation agar	14.60	12.14	12.30	13.02
Mean	21.21	20.20	27.70	23.03

Media	CD at 5 %	SE(m)
Source of rhizosphere soil	0.81	0.27
Media X source of rhizosphere soil	0.70	0.24
	1.40	0.48

Table-2 *Colony forming units (cfu) of fluorescent pseudomonads formed on different culture media*

	Cfu of bacteria g-1 of soil (1×10^6)			
Media	**Healthy plants**	**Diseased plants**	**Weeds**	**Mean**
Potato Dextrose Agar	35.50	25.50	40.50	33.82
Nutrient Agar	35.40	24.70	42.60	34.23
King's medium B base	37.00	30.14	54.83	40.65
Pseudomonas Agar (for fluorescin)	54.80	26.85	86.80	56.15
Actinomycete Isolation Agar	11.60	10.70	10.30	10.86
Pikovskaya's Agar	10.60	10.00	9.50	10.03
Mean	30.81	21.31	40.75	30.95

Media	CD at 5 %	SE(m)
Source of rhizosphere soil	0.48	0.16
Media X source of rhizosphere soil	0.34	0.11
	0.83	0.29

The studies of [12] also indicated the same, where the presence of abundant microbial population in weeds rhizosphere contributing to the competitiveness of luxuriantly growing weeds.

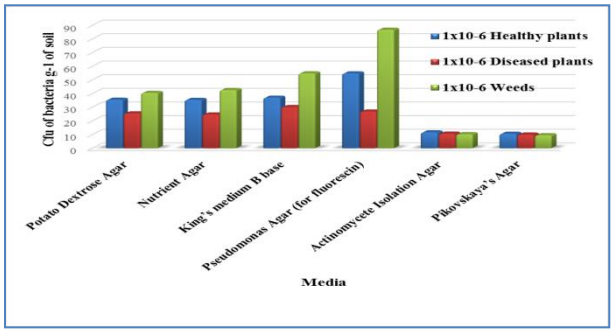

Fig-2 Colony forming units (cfu) of fluorescent pseudomonads formed on different culture media

Antagonistic efficacy of mycoflora and fluorescent pseudomonads

The mycoflora and fluorescent pseudomonads isolated from three different rhizosphere sources were screened against R. *solani* under *in vitro* condition to test their antagonistic potential by dual culture technique. The antagonistic effect of these genera was assessed based on their ability to inhibit the pathogen growth and development on PDA in Petri plates. Across the rhizosphere population tested against *R. solani* a total of 32 fungal isolates and two *Pseudomonas* isolates showed more than 50 per cent inhibition of radial growth of test fungus and were selected for conducting further studies.

The screened rhizosphere isolates of mycoflora and fluorescent pseudomonads were designated serially as M1 to M32 and P1 to P2 respectively. The data pertaining to this study was presented in table of 34 isolates evaluated, M10 showed highest per cent inhibition (79.17) (Plate 4.12) followed by M8 (76.67). Isolate M7 and M9 with growth inhibition of 75.83 per cent were at par with each other which were followed by the isolate P1 (75.41). Isolates M4 and M6 with 74.16 per cent, isolates M5, M26 and M27 with 72.50 per cent, isolates M13, M18 and M21 with 70.00 per cent, isolates M12, M29 and M30 with 69.17 per cent, isolates M1, M17 and M32 with 68.33 per cent, isolates M3, M19 and M20 with 67.50 per cent, isolates M11, M22, M23 and M28 with 66.67 per cent, isolates M14, M15, M24 and M25 with 65.83 per cent and isolates M2 and M31 with 65.00 per cent were found statistically at par with each other.

Isolate M10 (79.17) was significantly different for per cent inhibition over control from all the treatments except M8 (76.67) which is also statistically different from majority of the treatments except M4 (74.17), M6 (74.17), M7 (75.83), M9 (75.83), M10 (79.17) and P1 (75.41). Isolate M16 (71.33) was significantly different with all the isolates except M4 (74.16), M6 (74.16), M5 (72.50), M26 (72.50) and M27 (72.50). Isolate M13, M18 and M21 were statistically different with all the treatments except M5 (72.50), M16 (71.66), M25 (65.83) and M27 (72.50). Isolates M12 (69.17), M29 (69.17) and M30 (69.17) were significantly different from all the treatments except M1, M17 and M32 (68.33) which were statistically different with all isolates except M12, M29 and M30 with growth inhibition of 69.17 per cent. Isolates M3, M19 and M20 with 67.50 per cent inhibition, isolates M11, M22, M23 and M28 with 66.67 per cent inhibition and isolates M14, M15, M24 and M25 with 65.83 per cent inhibition were found non-significant with each other.

Among the mycoflora tested in the present study the isolate M10 was found to be the potential antagonist and was identified as *Trichoderma viride* whereas the effective pseudomonad was identified as *Pseudomonas fluorescens.*

The potentiality of *Trichoderma viride* in suppressing the growth of *R. solani* was represented by several workers earlier and the present results of antagonistic activity of *Trichoderma viride* are in conformity with findings of [13], [14], [15], [16], [17]. And similar studies were conducted by [18], [19], [20] who reported the potentiality of *P. fluorescens in* suppressing the growth of *R. solani*.

Conclusion

The population of rhizosphere mycoflora and fluorescent pseudomonads was estimated from the soil samples collected from diseased, healthy and luxuriously growing weed plants as colony forming units (cfu) on different culture media and were tested for their antagonistic potential against *R. solani* under *in vitro* condition.

Among the mycoflora the isolate M10 was found to be the potential antagonist and was identified as *Trichoderma viride* whereas the effective pseudomonad (P1) was identified as *Pseudomonas fluorescens.*

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