

## RESEARCH PAPER

# Effect of *Pseudomonas fluorescens* and *Trichoderma* spp. on growth, yield and stem rot disease of groundnut

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Effect of *Pseudomonas fluorescens* and *Trichoderma* spp. on stem rot of groundnut incited by *Sclerotium rolfsii* was evaluated in a field experiment conducted during Kharif, 2014-15. Combined seed treatment with tebuconazole, *P. fluorescens* and *Trichoderma* spp. in conjunction with application of *P. fluorescens* and *Trichoderma* spp. to soil and, the treatment in which both, *P. fluorescens* and *Trichoderma* spp were altogether inoculated to seed and soil significantly reduced the intensity and incidence of stem rot disease in groundnut. These two treatments also augmented seed germination, shoot length, branching, dry plant weight and dry pod yield significantly.

**Key words :** *Pseudomonas fluorescens*, *Trichoderma* spp., *Sclerotium rolfsii*, Stem rot of groundnut

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## INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important annual oil seed crop, but maximum productivity has not been reached due to large reduction in yield owing to number of diseases (Mayee and Datar, 1988; Ganesan and Sekar, 2004). Among the soil-borne fungal diseases of groundnut, stem rot caused by *Sclerotium rolfsii* Sacc. is a potential threat to groundnut production and is of considerable economic significance for groundnut grown under irrigated conditions. This disease causes severe damage during any stage of crop growth and yield losses over 25% have been reported by Mayee and Datar (1988), but under severe diseased conditions yield losses range upto 80% (Rodriguezkabana *et al.*, 1975). Methods employed to manage *S. rolfsii* are fungicide applications, solarization, use of antagonistic microorganisms, deep ploughing, crop rotation and incorporation of organic and inorganic residues (Punja, 1985). Chemical fungicides are extensively used in current agriculture; however, it is a fact that its excessive use has led to human health

problems, environmental pollution and development of resistance to fungicide in plant pathogens. Because of this, solemn attempts are needed to identify alternative methods for plant protection, which are more environmentally benign.

Biological methods offer an excellent alternate strategy for effective control of various diseases and plant growth promotional activity. *Pseudomonas* spp. received great attention as biocontrol agent because of their catabolic versatility, excellent root-colonizing abilities and, production of diacetylphloroglucinol (DAPG), pyoluteorin, pyrrolnitrin and phenazines (Chin-A-Woeng *et al.*, 2001; Raaijmaker *et al.*, 2002). They inhibit soil borne plant pathogens by several mechanisms for instance through production of antibiotics, siderophores (Kloepper *et al.*, 1980) and HCN and, competition for space and nutrients. They could serve as promising bioinoculants for agricultural system to increase productivity, since the action of such bacteria is highly specific, eco-friendly and cost-effective. *Trichoderma* spp. are also effective in control of soil and seed borne fungal diseases in several

crop plants (Kubicek *et al.*, 2001), including groundnut (Podile and Kishore, 2002). Major mechanisms involved in the biocontrol activity of *Trichoderma* spp. are competition for space and nutrients, production of diffusible and volatile antibiotics and, hydrolytic enzymes like chitinase and  $\beta$ -1,3-glucanase. These hydrolytic enzymes partially degrade the pathogen cell wall and leads to its parasitization (Kubicek *et al.*, 2001).

In recent years, more emphasis is laid on the combined use of biocontrol agents with different mechanisms of disease control, for improved disease control and also to overcome the inconsistent performance of the introduced biocontrol agents. Keeping this in view, in the present investigation an attempt was made to determine the plant growth promoting ability and control of stem rot disease in groundnut by combined application of antagonistic *Pseudomonas fluorescens* and *Trichoderma* spp.

## RESEARCH METHODOLOGY

The field experiment on *Pseudomonas fluorescens* and *Trichoderma* spp. mediated management of stem rot of groundnut incited by *Sclerotium rolfsii* was conducted during *Kharif*, 2014 at the Instructional Farm of College of Agriculture, Kolhapur (Maharashtra) India. The experiment was conducted in Randomized Block Design (RBD) having three replications, in plots of 3.20 m  $\times$  3.15 m, with 30 cm  $\times$  10 cm spacing. Groundnut variety JL-286 (Phule Unap), susceptible to stem rot disease, was used in the study. Native isolate of *P. fluorescens* was selected for the study based on initial screening in pot culture experiment. Whereas, talc (sodium ammonium silicate) based formulation of *Trichoderma* spp. (consisting of five species *viz.*, *T. viride*, *T. koningii*, *T. hamatum*, *T. harzianum* and *T. longiformum*), containing  $10^8$  cfu  $g^{-1}$ , was obtained from the Division of Plant Pathology and Agricultural Microbiology, College of Agriculture, Kolhapur. The cfu of talc based formulation of *Pseudomonas fluorescens* was  $10^8$  cfu  $g^{-1}$ .

The treatments consisted of combined applications of *Pseudomonas fluorescens* (*Pf*) and *Trichoderma* spp. (*Ts*) as seed treatment (ST) and soil application (SA), along with standard chemical check tebuconazole (Tb) and, combinations thereof. The treatments were: T<sub>1</sub> - ST with Tb, T<sub>2</sub> - ST with *Pf*+*Ts*, T<sub>3</sub> - SA of *Pf*+*Ts*, T<sub>4</sub> - ST with Tb + *pf*+*Ts*, T<sub>5</sub> - ST with Tb + SA of *pf*+*Ts*, T<sub>6</sub> -

ST with *Pf*+*Ts* + SA of *Pf*+*Ts*, T<sub>7</sub> - ST with Tb + *Pf*+*Ts* and SA of *Pf*+*Ts*, T<sub>8</sub> - Uninoculated control and, T<sub>9</sub> - Inoculated control. Seed treatment with *P. fluorescens* and *Trichoderma* spp. was done @ 10 g and 5 g  $kg^{-1}$  seed, respectively; whereas seed treatment with tebuconazole was followed @ 1.5 g  $kg^{-1}$  seed. For combined seed application of tebuconazole and microbial antagonists, the seeds were treated initially with tebuconazole followed by *P. fluorescens* and *Trichoderma* spp. Soil application of *P. fluorescens* and *Trichoderma* spp. was carried out by mixing 2.5 kg talc based formulation of each of the antagonists with 50 kg of farm yard manure (FYM) and sprinkled in the furrows at the time of sowing. Separate uninoculated (without addition of pathogen in field soil) and inoculated controls (with addition of pathogen in field soil) were maintained. For inoculated control treatment, sorghum grain based inoculum of the pathogen ( $10^6$  cfu  $g^{-1}$ ) was added to the soil to a depth of 5 cm in a 10-cm band in the plant row; at a rate of 5  $cm^3$  per 30.5 cm of row at the time of sowing (Ristaino *et al.*, 1994). FYM was applied to the experimental plot @ of 10 t  $ha^{-1}$ . Nitrogen was applied through urea, phosphorus through single super phosphate and potassium through muriate of potash @ 25:50:00 kg  $ha^{-1}$ , respectively. Field data were recorded on seed germination, shoot length, number of branches, dry plant weight, stem rot intensity (using 0-4 scale as per Mahato and Mondal, 2014), stem rot incidence (upto 90 days, at an interval of 15 days, starting from 15 days after sowing), population dynamics of *P. fluorescens* and *Trichoderma* spp. in rhizosphere soil (at 30, 60 and 90 days after sowing) and pod yield.

## RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

### Plant growth parameters:

Results summarized in Table 1 revealed that, the treatment consisting of integrated seed treatment with tebuconazole, *Pseudomonas fluorescens* and *Trichoderma* spp. in conjunction with soil application of *P. fluorescens* and *Trichoderma* spp. (T<sub>7</sub>) had the highest germination (92.89%), shoot length (13.47 cm), number of branches (8.37 plant<sup>-1</sup>) and dry plant weight (6.87 g plant<sup>-1</sup>), which did not differ significantly from

those of the treatment wherein integrated application of *P. fluorescens* and *Trichoderma* spp. was done to both, seed and soil ( $T_6$ ). Germination percentage, shoot length, number of branches and dry plant weight recorded with the latter was 92.59%, 13.39 cm, 8.36 plant<sup>-1</sup> and 6.82 g plant<sup>-1</sup>, respectively. Thus, these two treatments were equally effective and significantly superior over the rest of the treatments in enhancing plant growth parameters. PGPR promote plant growth through direct modes of action like production of plant growth regulators (auxins, cytokinins, gibberellins) and facilitation of the uptake of nutrients (nitrogen fixation, solubilization of phosphorus). Bhatia *et al.* (2008) reported that the fluorescent pseudomonads strains produced fluorescent pigment, siderophore, HCN, IAA, and solubilized phosphate and, had a potential effect on inducing plant growth. The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of plant

pathogens on plants by production of inhibitory substances (antibiotics, antifungal metabolites, iron chelating siderophores and competition for sites on roots) or by increasing natural resistance of the host. Thus, in our investigation, reduction in stem rot incidence due to antagonists might have contributed to plant growth promotion.

### Stem rot intensity and incidence:

Data pertaining to per cent disease index (PDI) and per cent incidence of stem rot as influenced by integrated application of *Pseudomonas fluorescens* and *Trichoderma* spp. were statistically significant (Table 2). Significantly least PDI and stem rot incidence to the tune of 18.33% and 28.86%, respectively were recorded in the plots where integrated seed treatment with tebuconazole, *P. fluorescens* and *Trichoderma* spp. was followed in conjunction with application of *P. fluorescens*

**Table 1: Plant growth parameters of groundnut as influenced by *Pseudomonas fluorescens* and *Trichoderma* spp.**

Treatments	Germination (%)*	Shoot length (cm)	No. of branches plant <sup>-1</sup>	Dry plant weight (g plant <sup>-1</sup> )
T <sub>1</sub> - ST with Tb	81.53 (64.52)	10.56	7.16	5.10
T <sub>2</sub> - ST with <i>Pf</i> + <i>Ts</i>	80.88 (64.04)	10.15	7.08	4.91
T <sub>3</sub> - SA of <i>Pf</i> + <i>Ts</i>	81.34 (64.38)	10.16	7.12	5.07
T <sub>4</sub> - ST with Tb + <i>pf</i> + <i>Ts</i>	85.99 (68.00)	12.06	7.76	5.60
T <sub>5</sub> - ST with Tb + SA of <i>pf</i> + <i>Ts</i>	86.34 (68.29)	12.36	7.78	5.67
T <sub>6</sub> - ST with <i>Pf</i> + <i>Ts</i> + SA of <i>Pf</i> + <i>Ts</i>	92.59 (74.27)	13.39	8.36	6.82
T <sub>7</sub> - ST with Tb + <i>Pf</i> + <i>Ts</i> and SA of <i>Pf</i> + <i>Ts</i>	92.89 (74.71)	13.47	8.37	6.87
T <sub>8</sub> - Uninoculated control	77.63 (61.75)	8.80	6.54	4.23
T <sub>9</sub> - Inoculated control	74.52 (59.67)	7.43	5.90	3.23
S.E. ±	0.76	0.30	0.18	0.15
C. D. (P=0.05)	2.29	0.90	0.54	0.45

\* Figures in parenthesis are angular transformed values

**Table 2: Stem rot intensity and incidence in groundnut as influenced by *Pseudomonas fluorescens* and *Trichoderma* spp.**

Treatments	Per cent disease intensity (PDI)*	Per cent disease incidence*	Reduction over uninoculated control (%)
T <sub>1</sub> - ST with Tb	52.50 (46.42)	55.48 (48.13)	15.28
T <sub>2</sub> - ST with <i>Pf</i> + <i>Ts</i>	50.00 (44.98)	56.87 (48.93)	13.16
T <sub>3</sub> - SA of <i>Pf</i> + <i>Ts</i>	46.50 (42.97)	56.47 (48.70)	13.77
T <sub>4</sub> - ST with Tb + <i>pf</i> + <i>Ts</i>	30.83 (33.70)	44.58 (41.84)	31.92
T <sub>5</sub> - ST with Tb + SA of <i>pf</i> + <i>Ts</i>	27.50 (31.60)	44.34 (41.70)	32.29
T <sub>6</sub> - ST with <i>Pf</i> + <i>Ts</i> + SA of <i>Pf</i> + <i>Ts</i>	19.17 (25.88)	28.98 (32.55)	55.74
T <sub>7</sub> - ST with Tb + <i>Pf</i> + <i>Ts</i> and SA of <i>Pf</i> + <i>Ts</i>	18.33 (25.33)	28.86 (32.48)	55.93
T <sub>8</sub> - Uninoculated control	61.67 (51.74)	65.49 (54.00)	--
T <sub>9</sub> - Inoculated control	81.67 (64.70)	78.92 (62.70)	--
S.E. ±	1.12	1.57	--
C. D. (P=0.05)	3.39	4.70	--

\* Figures in parenthesis are angular transformed values

and *Trichoderma* spp. to soil ( $T_7$ ), which did not differ significantly from those recorded in the treatment wherein *P. fluorescens* and *Trichoderma* spp. were all together inoculated to both, seed and soil (PDI 19.17% and disease incidence 28.98%) ( $T_6$ ). Thus, these two treatments were equally effective and significantly superior over the rest of the treatments in reducing incidence and intensity of the disease. Disease incidence in the former was reduced by 55.93%, whereas that in the latter was reduced by 55.74%, in comparison to uninoculated control. *Trichoderma* are known to penetrate and colonize both the sclerotia and mycelium of *Sclerotium rolfsii* (Henis *et al.*, 1983). Moreover, Franken *et al.* (2002) observed that *Trichoderma* spp. colonize plant roots prior to stimulation of plant growth and provide protection against invasion of infectious foreign organisms. *Trichoderma harzianum* and *T. viride* are active colonizers and the fungi produce antibiotics such as gliotoxin, viridin, cell wall degrading enzymes and biologically active heat stable metabolites such as ethyl acetate (Khan *et al.*, 2004). These substances are involved in disease suppression and plant growth promotion. *Pseudomonas* spp. are also well known for production of broad spectrum antibiotics such as 2, 4-diacetylphloroglucinol and antibiosis was proved to be a major mechanism involved in their biocontrol activity (O'Sullivan and O'Gora, 1992). Some strains of *P. fluorescens* also can produce pyoluteorin in addition to 2, 4-diacetylphloroglucinol, whereas others

synthesize only 2, 4-diacetylphloroglucinol. HCN and siderophores produced by *Pseudomonas* spp. are also involved in their antifungal activity. Further, a combination of *P. fluorescens* and *Trichoderma* found to have an improved biocontrol activity against groundnut stem rot disease (Manjula *et al.*, 2004). Thus, reduced stem rot incidence and intensity, in the present investigation, may be attributed to the facts set forth by the foregoing researchers.

#### Population of antagonists in rhizosphere soil:

Data presented in Table 3 clearly revealed that the population of *Pseudomonas fluorescens* and *Trichoderma* spp. in the rhizosphere soil of groundnut was influenced significantly by the treatments (T), days after sowing (DAS) and interaction (T x DAS). Among the different treatments, the highest population of *P. fluorescens* and *Trichoderma* spp. in the rhizosphere was observed in the treatments viz., integrated seed treatment with tebuconazole, *P. fluorescens* and *Trichoderma* spp. in conjunction with application of *P. fluorescens* and *Trichoderma* spp. to soil ( $T_7$ ) and, integrated seed and soil treatment with both the antagonists ( $T_6$ ). Population of *P. fluorescens* with these two treatments was  $138.16 \times 10^6$  and  $137.52 \times 10^6$  cfu  $g^{-1}$  rhizosphere soil, respectively; whereas that of *Trichoderma* spp. was  $96.44 \times 10^4$  and  $95.95 \times 10^4$  cfu  $g^{-1}$  rhizosphere soil, respectively. The population of *P.*

Treatments	Population of <i>P. fluorescens</i> (x 10 <sup>6</sup> g <sup>-1</sup> soil)				Population of <i>Trichoderma</i> spp. (x 10 <sup>4</sup> g <sup>-1</sup> soil)			
	30 DAS*	60 DAS	90 DAS	Mean	30 DAS*	60 DAS	90 DAS	Mean
T <sub>1</sub> - ST with Tb	67.04	99.62	65.48	77.38	68.79	70.54	54.23	64.52
T <sub>2</sub> - ST with Pf+Ts	85.74	106.99	81.34	91.36	72.83	75.30	68.86	72.33
T <sub>3</sub> - SA of Pf+Ts	99.83	110.42	93.86	101.37	75.69	77.18	70.11	74.33
T <sub>4</sub> - ST with Tb + pf+Ts	103.49	122.93	101.67	109.36	80.80	83.53	73.91	79.41
T <sub>5</sub> - ST with Tb + SA of pf+Ts	112.70	129.87	110.92	117.83	87.26	89.72	79.29	85.42
T <sub>6</sub> - ST with Pf+Ts + SA of Pf+Ts	136.23	152.66	123.67	137.52	98.52	99.22	90.13	95.95
T <sub>7</sub> - ST with Tb + Pf+Ts and SA of Pf+Ts	136.75	153.26	124.47	138.16	98.68	99.25	91.39	96.44
T <sub>8</sub> - Uninoculated control	30.81	35.87	26.35	31.00	21.86	22.15	20.59	21.53
T <sub>9</sub> - Inoculated control	31.80	41.63	29.77	34.40	28.26	29.27	27.95	28.49
Mean	89.38	105.92	84.17	--	70.29	71.80	67.56	--
	S.E.±		C.D. (P=0.05)		S.E.±		C.D. (P=0.05)	
Effect of treatment (T)	0.88		2.49		0.29		0.79	
Effect of days after sowing (DAS)	0.51		1.44		0.16		0.46	
Interaction effect (T x DAS)	1.52		4.31		0.48		1.37	

\* DAS= days after sowing

**Table 4 : Dry pod yield of groundnut as influenced by *Pseudomonas fluorescens* and *Trichoderma* spp**

Treatments	Dry pod yield (q ha <sup>-1</sup> )	Per cent increase in yield over uninoculated control
T <sub>1</sub> - ST with Tb	15.30	17.96
T <sub>2</sub> - ST with Pf+Ts	14.85	14.49
T <sub>3</sub> - SA of Pf+ Ts	15.12	16.57
T <sub>4</sub> - ST with Tb + pf+ Ts	18.17	40.09
T <sub>5</sub> - ST with Tb + SA of pf+ Ts	18.66	43.87
T <sub>6</sub> - ST with Pf+Ts + SA of Pf+ Ts	21.83	68.31
T <sub>7</sub> - ST with Tb + Pf+Ts and SA of Pf+ Ts	22.43	72.94
T <sub>8</sub> - Uninoculated control	12.97	--
T <sub>9</sub> - Inoculated control	08.20	--
S.E. ±	0.06	--
C. D. (P=0.05)	0.17	--

*fluorescens* and *Trichoderma* spp. in the rhizosphere augmented significantly from 30 days after sowing (89.38 x 10<sup>6</sup> cfu g<sup>-1</sup> and 70.29 x 10<sup>4</sup> cfu g<sup>-1</sup>, respectively) to 60 days after sowing (105.92 x 10<sup>6</sup> cfu g<sup>-1</sup> and 71.80 x 10<sup>4</sup> cfu g<sup>-1</sup>, respectively) and decreased at 90 days after sowing (84.17 x 10<sup>6</sup> cfu g<sup>-1</sup> and 67.56 x 10<sup>4</sup> cfu g<sup>-1</sup>, respectively) in all the treatments. Results of the present investigation corroborate with those of Chakravarty and Kalita (2012) who observed that the seed + root + soil application of *P. fluorescens* increased rhizosphere population of the bacteria.

#### Dry pod yield:

Data presented in Table 4 revealed that, significantly highest dry pod yield of groundnut to the tune of 22.43 q ha<sup>-1</sup> was obtained from the plots where integrated seed treatment with tebuconazole, *Pseudomonas fluorescens* and *Trichoderma* spp. was followed in conjunction with application of *P. fluorescens* and *Trichoderma* spp. to soil (T<sub>7</sub>), which did not differ statistically from that recorded in the plots wherein both the antagonists, *P. fluorescens* and *Trichoderma* spp., were all together inoculated to both, seed and soil (21.83q ha<sup>-1</sup>) (T<sub>6</sub>). Thus, these two treatments were found to be equally effective and significantly superior over rest of the treatments in increasing pod yield. Per cent increase in yield with these treatments was 72.94 and 68.31, respectively in comparison to uninoculated control. Maximum performance of the pod yield observed in the groundnut crop treated with *P. fluorescens* and *Trichoderma* spp. can be attributed to minimum stem rot incidence in this crop. Increase in crop yields due to the use of *Trichoderma* as antagonist has been documented in

literature. Mesta and Amaresh (2000) obtained 26 to 30% increased yield of sunflower due to seed treatment with *Trichoderma*. Moreover, Rasu *et al.* (2013) obtained significantly higher top and tuber yield of sugar beet due to combined use of *Trichoderma* and *P. fluorescens* challenged with *Sclerotium rolfsii* than individual treatments. All these earlier reports support the present finding.

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