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## *In vitro* evaluation of antagonist's agents against seed-borne fungal diseases of tomato (*Solanum lycopersicum* Mill.)

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### Abstract

Tomato (*Solanum lycopersicum* Mill.) is the most popular vegetable crop grown in the world, crop offers significant nutritional advantages, as it contains significant source of dietary lycopene,  $\beta$ -carotene, vitamin C and antioxidant properties in a low energy dense food. Several human studies indicated a relationship between a high intake of tomato products and a decreased risk of several types of cancer, atherosclerosis and cardiovascular diseases. Crop is severely affecting many of seed-borne fungal infections. These seed-borne diseases cause both quality and quantity of the seed and may lead to seed abortion or elimination of germination capacity. In order to know the effective bio-agents against this pathogen, the experiment were conducted by different powder formulations of antagonists. The powder formulations of antagonists' viz., *Trichoderma harzianum* Rifai, *Pseudomonas fluorescens* Migula, *Bacillus subtilis* Cohn and VAM fungi were taken for seed treatment to test their efficacy in providing protection against seed borne infection under *in vitro* conditions by rolled towel method. Among the four bioagents tested, *Pseudomonas fluorescens* at concentration of 0.8 per cent showed least seed infection with maximum per cent germination and vigour index compared to *Trichoderma harzianum*.

**Keywords:** Seed-borne, tomato, bioagents, trichoderma, pseudomonas and vigour index

### Introduction

Tomato (*Solanum lycopersicum* Mill.) is the most popular vegetable crop grown in the world, crop offers significant nutritional advantages, as it contains significant source of dietary lycopene,  $\beta$ -carotene, vitamin C and antioxidant properties in a low energy dense food (Britt and Kristin, 2011). Several human studies indicated a relationship between a high intake of tomato products and a decreased risk of several types of cancer, atherosclerosis and cardiovascular diseases (Cecilia *et al.*, 2010). Recently, this crop is recognized as a model for plant-pathogen interactions (Arie *et al.*, 2007). In India, area under cultivation is 7.77 lakh hectares with the production of 182.86 lakh tonnes (Anon., 2015). In Karnataka, tomato with a production of 20.34 lakh tonnes (Anon., 2015).

Several diseases affecting tomato are caused by fungi, bacteria, viruses and nematodes and many of them are seed-borne in nature. These seed-borne pathogens are known to cause economically important diseases like early blight, late blight, Fusarium wilt, Septoria leaf spot, damping off and fruit rot. Seed-borne fungi are of considerable importance due to their influence on the overall health, germination and final crop stand in the field. The infected seeds may fail to germinate, or transmit disease from seed to seedling and/or from seedling to growing plant (Islam and Borthakur, 2012). The disease caused by seed-borne fungi may lead to seed abortion or elimination of germination capacity. Thus, the disease control programme is important at each stage of growth. Before the seeds are removed before radical emergence. The bacterial bio-control agent may multiply substantially on seed during biopriming (Callan *et al.*, 1990). Seed encapsulation with beneficial microorganisms is now becoming common.

### Material and Method

#### *In vitro* evaluation of bioagents by rolled towel method

The powder formulations of antagonists' viz., *Trichoderma harzianum* Rifai, *Pseudomonas fluorescens* Migula, *Bacillus subtilis* Cohn and VAM fungi were taken for seed treatment to test their efficacy in providing protection against seed borne infection under *in vitro* conditions by rolled towel method. These bioagents were procured from IOF (Institute of Organic Farming), UAS, Dharwad. Seeds of moderately infected tomato variety PKM-1 were treated with different bioagents at the rate of 0.4 and 0.8 per cent concentrations. The seeds were shaken along with bioagents for 20 minutes in mechanical shaker for uniform application and then stored in separate boxes for 24 hours.

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The treated seeds were tested in 3 replications of 100 seeds by employing rolled paper towel method. The treatments were laid out in factorial CRD design. Seeds without treatment served as control. These paper towels were incubated at 25±2 °C for seven days under 12 h light and 12 h darkness. The per cent germination and per cent infection were recorded after seven days of incubation. Vigour index was calculated by the following formula, given by Abdul and Anderson (1973) [1] *i.e.*

Vigour Index = Seed germination (%) × Seedling length (Shoot + Root length (cm))

$$\% \text{ seed germination} = \frac{\text{No of seed germinated}}{\text{Total no. of seed used}} \times 100$$

$$\% \text{ seed infection} = \frac{\text{No of seed infected}}{\text{Total no. of seed used}} \times 100$$

## Result

### *In vitro* evaluation of bioagents by Rolled towel method

Four bioagents were tested for their efficacy in the management of seed borne fungal infections of tomato (variety: PKM-1) by using rolled towel method, as described in 'Material and Methods' and results are presented in Table 1, Fig. a & b and Plate A. The four bioagents tested in two concentrations, *i.e.*, 0.4 and 0.8 per cent for their efficacy in

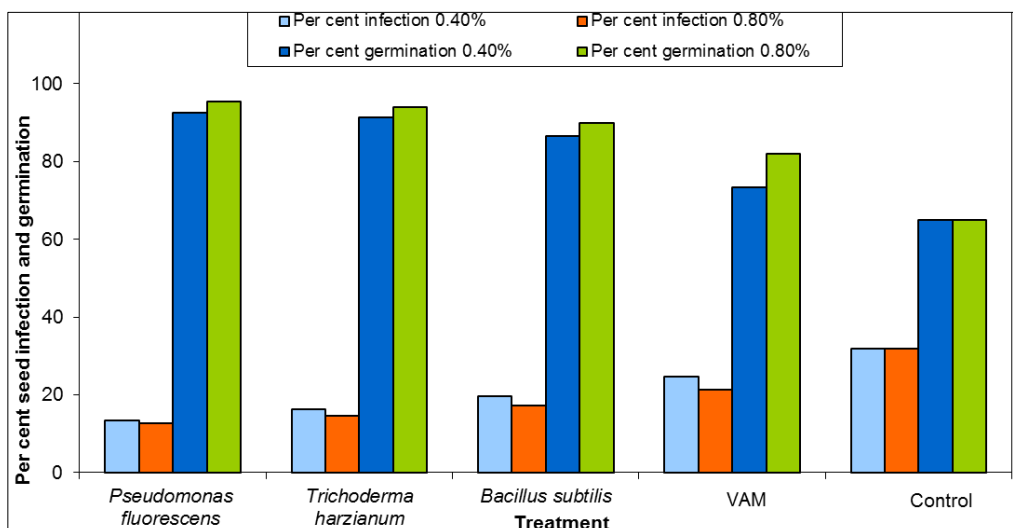
the management of seed-borne fungal infection. Seed treatment with *Pseudomonas fluorescens* showed least seed infection of 13.00 per cent followed by *Trichoderma harzianum* with per cent seed infection of 15.50 per cent but they were found to be on par with respect to per cent germination (94.00 and 92.67 per cent) and vigour index (2031.17 and 2006.78) respectively.

Seed treatment with *Pseudomonas fluorescens* at 0.8 per cent differed significantly over seed treatment with *Trichoderma harzianum* @ 0.8 per cent with respect to per cent seed infection (12.67% and 14.67%) per cent germination (95.33% and 94.00%). Seed treatment with *Pseudomonas fluorescens* at 0.4 per cent differed significantly with seed treatment of *P. fluorescens* at 0.8 per cent with respect to per cent seed infection (13.33% and 12.67%), per cent germination (92.67 and 95.33%) and vigour index (1955.47 and 2106.87). Similarly in case of *T. harzianum* @ 0.4% which differed significantly with seed treatment of *T. harzianum* @ 0.8% with respect to per cent seed infection, per cent germination and vigour index. Seed treatment with VAM fungi was found ineffective as it resulted in seed infection of 23.00 per cent, with a germination of 77.67 per cent and vigour index of 1424.78, but was found significantly superior over control, which exhibited a seed infection of 32.00 per cent, with germination and vigour index of 65.00 per cent and 1027.83 respectively.

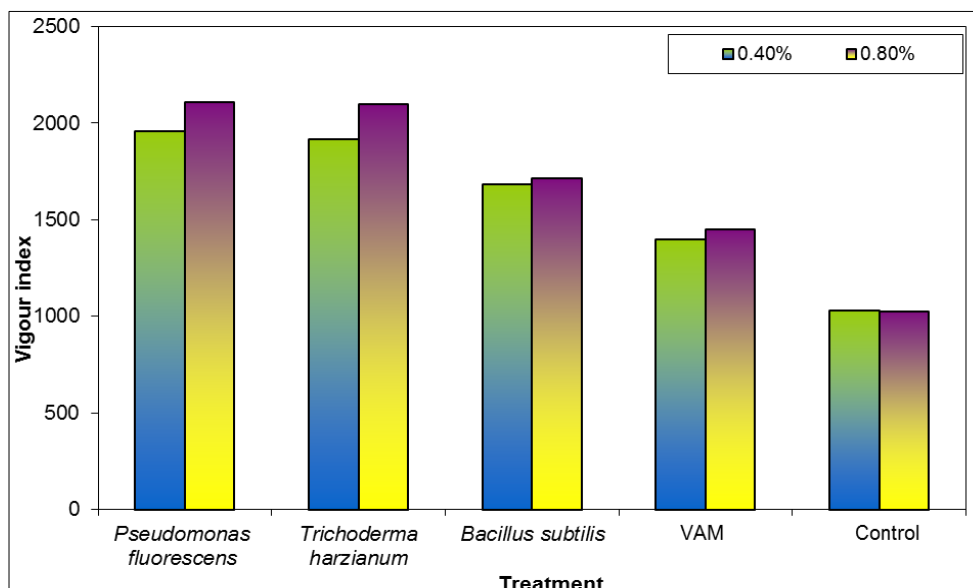
**Table 1:** *In-vitro* evaluation of bioagents against seed-borne fungal diseases of tomato

Treatments	Per cent infection			Per cent germination			Vigour index		
	0.40%	0.80%	Mean	0.40%	0.80%	Mean	0.40%	0.80%	Mean
<i>Pseudomonas fluorescens</i>	13.33 (21.41)*	12.67 (20.85)	13.00 (21.13)	92.67 (74.43)	95.33 (77.58)	94.00 (76.01)	1955.47	2106.87	2031.17
<i>Trichoderma harzianum</i>	16.33 (23.83)	14.67 (22.52)	15.50 (23.17)	91.33 (72.92)	94.00 (75.85)	92.67 (74.39)	1914.70	2098.87	2006.78
<i>Bacillus subtilis</i>	19.67 (26.31)	17.33 (24.60)	18.50 (25.46)	86.67 (68.64)	90.00 (71.57)	88.33 (70.10)	1684.47	1713.00	1698.73
VAM fungi	24.67 (29.78)	21.33 (27.50)	23.00 (28.64)	73.33 (58.91)	82.00 (64.90)	77.67 (61.90)	1398.17	1451.40	1424.78
Control	32.00 (34.45)	32.00 (34.45)	32.00 (34.45)	65.00 (53.73)	65.00 (53.73)	65.00 (53.73)	1029.00	1026.67	1027.83
Mean	21.20 (27.42)	19.60 (26.28)	20.40 (26.85)	82.80 (65.50)	86.27 (68.25)	84.79 (66.84)	1596.36	1679.36	1637.86
	T	C	T x C	T	C	T x C	T	C	T x C
SEM	0.27	0.17	0.37	0.55	0.35	0.78	24.74	15.65	34.99
CD at 1%	0.78	0.49	1.11	1.63	1.03	2.31	72.98	46.16	103.21

\*Figures in parentheses indicate arcsine values



**Fig 1a:** Efficacy of bioagents as seed dressers on seed-borne fungal infections and seed quality parameters of tomato



**Fig 2b:** Efficacy of bioagents as seed dressers on seed-borne fungal infections and other seed quality parameters of tomato

## Discussion

### *In vitro* evaluation of bioagents by Rolled towel method

Among the bioagents, *Pseudomonas fluorescens* at 0.8% differed significantly over *Trichoderma harzianum* with respect to per cent seed infection and per cent germination and further *P. fluorescens* at 0.8% differed significantly over 0.4% with respect to per cent seed infection, per cent germination and vigour index and similarly *T. harzianum* @ 0.8% differed significantly over 0.4% with respect to per cent seed infection, per cent germination and vigour index. Hence these two bioagents *i.e.*, (*P. fluorescens* and *T. harzianum*) were selected for further field evaluation @ 0.8 per cent concentration.

VAM fungi were found to be least effective in eliminating seed-borne infections. Similar results were obtained by El-Mougy *et al.* (2012)<sup>[2]</sup> who reported that seed treatment with *Trichoderma harzianum*, *T. Viride*, *T. hamatum*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Saccharomyces cerevisiae* were effectively control of *Alternaria solani*, *Fusarium solani*, *F. oxysporum*, *Rhizoctoniasolani*, *Sclerotium rolfsii*, *Macrophomina phaseolina* and *Pythium sp.* in tomato seeds. Ganie *et al.* (2013)<sup>[4]</sup> studied the bio efficacy of seed treatment with three bioagents viz., *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluoresce* sunder *in vitro* against *Alternaria solani* (Ellis M. B. 1971)<sup>[3]</sup> Jones and Grout causing early blight of tomato. Among them they noticed that *Pseudomonas fluorescens* showed significantly higher seed germination (71.85%) followed by *T. harzianum* (65.93%) and *T. viride* (58.65%).

Mumtaz *et al.* (2012)<sup>[5]</sup> reported the efficacy of seed treatment with bio control agents (*Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis*) in controlling seed borne fungi like, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani* and *Alternaria alternata* (Fr.) Keissler in tomato seeds Sundaramoorthy and Balabaskar (2013)<sup>[6]</sup> studied the effect of fifteen native isolates of *Trichoderma* species against *Fusarium oxysporum f. sp. lycopersici* which causes Fusarium wilt of tomato under *in vitro* and *in vivo* conditions. The results revealed that *Trichoderma harzianum* (ANR-1) isolate effectively inhibit the radial mycelial growth of the pathogen (53%) compared to all other isolates.

Vandna and Priya (2014) studied the effeicancy of *Trichoderma harzianum* as seed treatment against wilt disease

of tomato caused by *Fusarium oxysporum f. sp. lycopersici* showed maximum seed germination (78.33%) and disease control (66.53%).

Among the four bioagents tested for their efficacy in overcoming seed borne fungal infections of tomato with *Pseudomonas fluorescens* @ 0.8% showed least per cent seed infection with maximum per cent germination followed by *Trichoderma harzianum* @ 0.8%. Seed treatment with *Pseudomonas fluorescens* at 0.8% differed significantly over *Pseudomonas fluorescens* at 0.4% with respect to per cent seed infection, per cent germination and vigour index.

Biological control through the use of antagonistic microorganisms plays a key role in modern agriculture as it is cheap and eco-friendly. Such management options would help in preventing the pollution and also health hazards. Hence in the present investigations an attempt has been made to evaluate the effect of bioagents on seed borne fungal infections of tomato.

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