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TRICHODERMA MEDIATED SEED BIO-PRIMING AGAINST *FUSARIUM* WILT OF TOMATO CAUSED BY *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI* (SACC.) SNYDER AND HANSEN

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ABSTRACT

Tomato (*Lycopersicon esculentum* L. Krust) is one of the prominent vegetable crop grown all over the world. Various number of diseases attacks on the tomato crop causing huge economic losses. Among them, Fusarium wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* (Sacc) W.C. Snyder and H.N. Hansen (1940) is one of the most destructive and economically important disease of tomato causing 30-40% losses in the country. The disease can be managed through various methods such as cultural, chemical and biological methods, including resistant varieties. This study evaluated seed biopriming with *Trichoderma* spp. as a novel approach to enhance plant growth and disease resistance. Results showed that biopriming significantly improved seed germination (95.41%), shoot (48.32 cm) and root length (35.24 cm) and biomass accumulation (shoot: 85.52 g, root: 24.52 g) compared to untreated controls. Disease severity was notably reduced, with the lowest incidence (7.32–10.46%) observed in seeds primed with *T. harzianum* (VAN), outperforming other treatments (8.81–15.43%) and the control (16.38%). Additionally, this treatment maximized yield (1102.80 g/plant), demonstrating its potential as an effective and sustainable disease management strategy.

Key words : Fusarium wilt, Biopriming, *Fusarium oxysporum* f.sp. *lycopersici*, *Trichoderma* spp.

Introduction

Tomato is one of the most versatile crops of solanaceous family, which is widely used as a vegetable all around the world. Some people prefer it raw whereas some like to cook it with vegetables and spices. It is used to prepare soup, salad, pickles, ketchup, purees and sauces, among other foods. Tomato is widely grown crop and acquiring most prominent status at world level due to its rich source of lycopene, lutein, beta-carotene, vitamin A, B complex, C (Ascorbic acid) minerals and dietary fibre content. Beside it's too much importance, the crop is affected by various biotic and abiotic factors resulted in reduction of its quality and quantity. Among them, Fusarium wilt caused by *Fusarium oxysporum* f. sp. l*ycopersici* is the most devastating disease which leads to huge economic loss up to 10-50% (Sivakumar *et al*., 2018). High temperatures are conducive to the development of this disease. The pathogen is soil inhibitant in nature and infects plants through the roots and grows internally through the cortex to the stele, producing xylem browning or blackening and block the xylem vessel due to their physical presence which ultimately reduces vigour of the plant that leads to death of the plant. Several fungicides are available to control this disease effectively

but prophylactic use of these pesticides has led to ground water contamination, hazardous to environment and nontargeted beneficial organism and pesticide residue on soil, food and water can cause mammalian and avian toxicity. Biological control agents (BCAs) for plant diseases are currently being examined as alternatives to the synthetic pesticides due to their perceived increased level of safety and minimal environmental impacts. Seed bio-priming is an innovative skill of seed treatment that assimilates biological (inoculation of seed with beneficial organism to protect seed from pathogenic activities) and physiological facets (seed hydration) of disease control (Reddy, 2013). Generally, it is recently used as an ecofriendly and alternate method for controlling a lot of seed and soilborne pathogens. Hence, the current investigation was carried out to investigate the curative impact of seed biopriming with different isolates of BCAs (*Trichoderma* spp.) on overall growth and yield parameters of tomato plant against Fusarium wilt.

Materials and Methods

Isolation and purification of the test pathogen

During routine observation of the tomato field at student instruction farm of C.S.A. University of Agriculture & Technology, Kanpur, the infected tomato plant was selected and uprooted gently. Stem and roots part of the plant were selected and washed very carefully to remove dirt particles. Brownish coloured stem was cut into small pieces with a sharp scissor, surface sterilized with 0.1% $HgCl_2$ followed by rinsing with sterilized distilled water three times. After that these small pieces are allowed to dry with blotter paper followed by inoculating into petri dishes poured with autoclaved PDA with the help of sterilized forceps. These plates are incubated at 25±1^{0}C in a BOD incubator and check daily. Later the isolate was purified by hyphal tip culture method and identified based on its morpho cultural characteristics. Pathogenicity test was also performed by injecting spore density at 10^6 spore/ml into susceptible tomato seedlings (Dhingra and Sinclair, 1995) to confirm the isolated pathogen.

Isolation and maintenance of biocontrol agents (BCAs)

Soil samples were collected from six district of Uttar Pradesh *viz.* Kanpur Nagar (KN), Varanasi (VN), Unnao (UNN), Farrukhabad (FBD), Kanpur Dehat (KD) and Banda (BDA) and isolated by serial dilution method. Six isolates of *Trichoderma viride* and *Trichoderma harzianum* were isolated on autoclaved PDA and identified based on their morpho-cultural characteristics.

Effect of seed biopriming on different parameters

Seed bio-priming is a novel, beneficial and eco-friendly technique that employs bio-stimulating agents like *Trichoderma* to improve the physiological functioning of seeds and stress resilience. Tomato seeds (Azad T-6) are surface sterilized with 1% sodium hypochlorite and primed with different isolates of *Trichoderma spp.* within petri plates. Primed seeds are then sown in cemented pots in a wire house complex, Department of Plant Pathology, CSAUAT, Kanpur. The entire experiment was carried out in completely randomized design with three replications of each treatment as T_1 = Seed primed with *Trichoderma harzianum* (KN), T_2 = Seed primed with *Trichoderma harzianum* (VAN), T_3 = Seed primed with *Trichoderma harzianum* (UNN), T_4 = Seed primed with *Trichoderma viride* (FBD), T_5 = Seed primed with *Trichoderma harzianum* (KD), T_6 = Seed primed with *Trichoderma viride* (BDA), T_7 = Control.

(Note: KN = Kanpur Nagar, VAN = Varanasi, UNN = Unnao, FBD = Farrukhabad, KD = Kanpur Dehat, and BDA= Banda.)

Seed Germination

After 10 days, total number of germinated seeds were counted and germination percentage was calculated by following formula:

$$\text{Germination \%} = \frac{\text{Number of germinated seed}}{\text{Total number of sown seed}} \times 100$$

Physiological growth parameters

After 40 DAT shoot and root length were measured (cm) from base of the plant to end of its main axis the plant was uprooted gently along with roots and rinse with tap water properly and blotted with paper for the measurement of fresh shoot and root. The weight of fresh root and shoot of plant was calculated in grams followed by drying for 6 hours in hot air oven at 60^{o}C for dry weight of root and shoot (gram) of the plant. The weight was calculated in grams using an electronic balance (Sartorius BT224S).

Determination of disease severity

At 5- and 10-days following inoculation, the incidence and severity of the disease were noted. Three replications were maintained for each treatment for the disease incidence was determined as per scale given by Song *et al.* (2004), where 0 = No visible diseases symptom or vascular browning and leaf yellowing/ withering, 1 = A slight infection, which is about 25% of full scale, one or two leaves becomes yellow, 2 = Moderate infection, two or three leaves becomes yellow, 50% of the leaves

become wilted, 3 = Extensive infection, all plant leaves became yellow, 75% of the leaves becomes wilted and growth is inhibited, 4 = Complete infection, the whole plant leaves become yellow, 100% of the leaves become wilted, and the plants die. Disease severity was measured by the following formula as given by Song *et al.* (2004).

$$\text{Disease severity (\%)} = \frac{(\Sigma \text{ scale} \times \text{number of plants infected}) \times 100}{\text{Highest scale} \times \text{total number of plants}}$$

Yield attributes

Each treated plant's edible fruits were collected twice a week and then weighed with the aid of physical balance. After combining the weight of the fruits at each picking, the total weight of all picking was recorded. Each treatment's crop output was determined independently and data was taken in grams.

Statistical analysis

Complete randomization design (CRD) was used in this experiment. One way ANOVA was used to draw the conclusions and estimated F value will be compared to the probability value that has been tabulated for the appropriate degree of freedom (Fisher and Yates, 1953). Treatment details as per follows T_1 = Seed primed with *Trichoderma harzianum* (KN), T_2 = Seed primed with *Trichoderma harzianum* (VAN), T_3 = Seed primed with *Trichoderma harzianum* (UNN), T_4 = Seed primed with *Trichoderma viride* (FBD), T_5 = Seed primed with *Trichoderma harzianum* (KD), T_6 = Seed primed with *Trichoderma viride* (BDA), T_7 = Control.

Results and Discussion

Isolation and purification of pathogen

Fusarium oxysporum f. sp. *lycopersici* was isolated from the infected tomato stem and identified based on their morpho-cultural characterstics such as white cottony mycelial growth, production of micro conidia (ovoid single celled), macro conidia (slightly curved multi celled and sickle shaped) on elongated conidiophores and chlamydospores on old cultures. The pathogen is confirmed by its disease producing ability on tomato cultivar Azad T-6 by submerging the tomato seedlings into 10^6 spore/ml spore suspension for 10 minutes before transplanting. Initially lower leaves turn yellow followed by tiny brown discolouration and finally wilting occurs after 7-14 days after inoculation.

Efficacy of seed priming with bio-agents on the plant growth parameters and disease severity of fusarium wilt of tomato

Germination percentage

The data presented on the table showed that among all the treatments, the maximum germination percentage was recorded in the treatment T_2 where seed is primed with *Trichoderma harzianum* (VAN) representing 95.41% followed by T_1 treatment as seed was primed with *T. viride* (KN) with 93.70%. Among the treatments the minimum germination percentage was observed in T_4 treatments as seed was primed with *T. viride* (FBD) with 83.53% and rest of the treatments were also superior over control (70.80%). Jisha *et al.* (2013) found that the seed bio-priming and thermo priming both found positive effect directly increases germination percentage of treated seed against over control. Rakshit *et al.* (2015) have been found that biopriming is the process of hydrating seeds and inoculating them with biological control agents to promote seed germination and protect against dangerous microbes.

Table 1 : Effect of seed priming with different bio-agents on their consortial seed germination (%) and seedling mortality (%) in tomato.

Treatments details	Germination (%)	Mortality (%)
T_1 = Seed primed with *Trichoderma harzianum* (KN)	93.70	14.40
T_2 = Seed primed with *Trichoderma harzianum* (VAN)	95.41	10.66
T_3 = Seed primed with *Trichoderma harzianum* (UNN)	89.36	16.66
T_4 = Seed primed with *Trichoderma viride* (FBD)	83.53	19.60
T_5 = Seed primed with *Trichoderma harzianum* (KD)	90.88	15.66
T_6 = Seed primed with *Trichoderma viride* (BDA)	86.16	18.20
T_7 = Control	70.80	20.50
C.D.	3.504	0.692
SE(d)	1.618	0.320
C.V.	2.275	2.368

Shoot and root length

Bio priming with *Trichoderma* spp. have ability to increase shoot and root length of tomato plant. The data in the table shows that the treatments were able to significantly enhance the shoot and root length of tomato plant over control. Among all the treatments, the maximum shoot and root length (90 DAT) was recorded in the treatment T_2 [seed primed with *Trichoderma harzianum* (VAN)] with the value of 48.32 and 35.24 cm, respectively which was followed by T_1 treatment [seed primed with *Trichoderma harzianum* (KN)] having the value 47.15 cm and 33.61 cm, respectively. The minimum shoot and

Table 2 : Effect of seed priming with different isolates of *Trichoderma* spp. on morphological parameters like root and shoot length fresh and dry weight of root and shoot of tomato against Fusarium wilt caused by *Fusarium oxysporum* f.sp *lycopersici*.

Treatments Details	Root length (cm)	Per cent increased over control	Shoot length (cm)	Per cent increased over control	Root weight (g)		Per cent increased over control	Shoot weight (g)		Per cent increased over control
					Fresh	Dried		Fresh	Dried	
T_1 = Seed primed with *Trichoderma harzianum* (KN)	33.61	74.77	47.15	76.98	23.21	3.12	69.29	82.29	16.68	80.53
T_2 = Seed primed with *Trichoderma harzianum* (VAN)	35.24	83.25	48.32	81.38	24.52	3.48	78.84	85.52	17.24	87.60
T_3 = Seed primed with *Trichoderma harzianum* (UNN)	32.29	67.91	45.35	70.23	21.11	2.75	53.97	78.54	15.90	72.31
T_4 = Seed primed with *Trichoderma viride* (FBD)	28.83	49.92	42.78	60.58	18.35	2.57	33.84	74.41	15.17	63.25
T_5 = Seed primed with *Trichoderma harzianum* (KD)	33.09	72.07	46.53	74.66	22.44	2.86	63.67	79.26	16.22	73.89
T_6 = Seed primed with *Trichoderma viride* (BDA)	30.05	56.26	43.86	64.63	19.64	2.68	43.25	76.34	15.54	67.48
T_7 (Control)	19.23		26.64		13.71	2.54		45.58	9.27	
C.D.	1.459		1.942		0.777	0.147		2.561	0.432	
C.V.	2.720		2.558		2.150	2.911		1.942	1.612	

root length was measured in T_4 treatment [seed primed with *Trichoderma viride* (FBD)] with 42.78 cm and 28.83cm, respectively. The seed biopriming with endophytic and rhizosphere microbes to improved plant growth by increasing seed germination, seedling vigour, and fresh weight as well as the percent emergence of radicals, coleoptiles and roots. It also increased root and shoot length, fresh and dry weight (Mnasri *et al*., 2017). Ermiş *et al*. (2016) recorded that solid matrix priming entails soaking solid matrices like bioagents, vermiculite, and sand in osmotic solutions to enable natural imbibition of seed as in soil as resulting promote germination, seedling growth and development. Mahmood *et al*. (2016) showed that biopriming has been shown to increase morphological parameters in a variety of crops. Along with the benefits mentioned above, it also promotes uniform seed germination and better stand establishment.

Fresh and dry weight of shoot and root of tomato

The treatment T_2 where seed primed with *Trichoderma harzianum* (VAN) resulted the maximum fresh shoot and root weight with 82.52 g and 24.52 g, followed by T_1 treatment where seed primed with *Trichoderma harzianum* (KN), produced 82.29 g shoot and 23.21g root weight over control. Similarly, dry shoot and root weight were measured after air dried in hot air oven at 60°C for 20 minutes and found maximum in T_2 (VAN) with 17.24 g and 3.48 g, respectively which was followed by T1 (KN) with 16.68 g and 3.12 g respectively after 90 DAT. Brari (2016) also found that tomato plants treated with *T. harzianum* (N-8) isolate showed a significant stimulatory effect on plant height (70.13 cm) and the dry weight (by 265.42 g) of tomato plants, in comparison to untreated control (54.6 cm and 195.5 g). Mnasri *et al.* (2017) evaluated that the seed biopriming with endophytic and rhizopheric microbes to improved plant growth by increasing seed germination, seedling vigour and fresh weight as well as the percent emergence of radicals, coleoptiles and roots. It also increased root-shoot length and root and shoot fresh and dry weight.

Number of branches and appearance of flowers and fruits

Efficacy of seed priming with bio-agents was tested on the morphological parameters like

Table 3 : Effect of seed priming with different isolates of bio-agents *Trichoderma* spp. on morphological parameters like leaves colour, number of branches, appearance of first flower and first fruit setting of tomato.

Treatments details	Leaves colour	No. of branches (40 DAT)	First appearance of flower (DAT)	First appearance of fruit (DAT)
T_1 = Seed primed with *Trichoderma harzianum* (KN)	Dark green	9.33	27.33	58.66
T_2 = Seed primed with *Trichoderma harzianum* (VAN)	Light green	10.33	26.66	57.66
T_3 = Seed primed with *Trichoderma harzianum* (UNN)	Green green	8.00	29.33	60.33
T_4 = Seed primed with *Trichoderma viride* (FBD)	Pale green	7.33	32.33	62.33
T_5 = Seed primed with *Trichoderma harzianum* (KD)	Dark green	8.33	28.33	59.33
T_6 = Seed primed with *Trichoderma viride* (BDA)	Yellow green	7.66	30.33	61.33
T_7 = Control	Feeble green	6.33	36.00	67.33
C.D.		2.217	4.227	3.014
SE(d)		1.024	1.952	1.392
C.V.		15.305	7.956	2.794

Table 4 : Effect of seed priming with different isolates of *Trichoderma* spp. on disease severity of tomato crop plant against Fusarium wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* at 30, 60 and 90 days after transplanting.

Treatments details	Disease severity (%)		
	30 DAT	60 DAT	90 DAT
T_1 = Seed primed with *Trichoderma harzianum* (KN)	9.56	10.24	12.33
T_2 = Seed primed with *Trichoderma harzianum* (VAN)	7.32	8.65	10.46
T_3 = Seed primed with *Trichoderma harzianum* (UNN)	10.84	12.35	14.37
T_4 = Seed primed with *Trichoderma viride*(FBD)	13.80	15.23	17.28
T_5 = Seed primed with *Trichoderma harzianum* (KD)	9.25	12.84	14.25
T_6 = Seed primed with *Trichoderma viride* (BDA)	12.95	15.02	17.12
T_7 = Control	14.26	16.64	18.25
C.D.	0.444	0.606	0.533
SE (d)	0.205	0.280	0.246
C.V.	2.255	2.636	2.029

number of branches, leaf colour, appearance of flower indicating that among all the treatments, T_2 where seed primed with *Trichoderma harzianum* (VAN) showed maximum number of branches as 10.33 and also resulting early flowering and fruiting in 26.66 and 57.66 DAT, respectively which was followed by seeds primed with *T. harzianum* (KN) as 9.33 branches and flowering and fruiting appears in 27.33 and 58.66 DAT, respectively. The rest of the treatments were also superior over control. Bio-priming with PGPR in various crops having the capacity to produce growth hormones such as auxin, cytokinin, gibberellin etc. which recorded high germination rate, higher shoot and root growth and homogenous crop stands (Noel *et al*., 1996; Verma *et al*., 2001). Shams *et al.* (2023) also found that vegetative growth parameters of cherry tomato plants, such as plant length, plant height, and number of leaves per plant, study depicts the effects of various treatments involving seed-biopriming with *Trichoderma* isolates.

Disease severity

All treatments were able to considerably lessen disease severity as compared to control at 30, 60, and 90 days after transplanting. Among the treatments, the minimum disease severity was recorded in the T_2 treatment, [seed primed with *Trichoderma harzianum* (VAN)] with the value of 7.32, 8.65 and 10.46 per cent at 30, 60 and 90

Table 5 : Effect of seed priming with different isolates of *Trichoderma* spp. on yield potential of tomato crop against Fusarium wilt caused by *Fusarium oxysporum* f.sp. *lycopersici*.

Treatments details	>50 g fruit		25 g to 50 g		< 25 g fruit		Total no. of fruits/pot	Total weight of fruits/pot (g)	% increase over control (by weight)
	No. of fruits	Weight of fruit (g)	No. of fruit	Weight of fruit (g)	No. of fruits	Weight of fruit (g)			
T_1 = Seed primed with *Trichoderma harzianum* (KN)	3.15	217.08	11.32	385.70	16.26	310.51	30.73	1025.350	76.28
T_2 = Seed primed with *Trichoderma harzianum* (VAN)	3.24	197	9.63	329.77	19.56	373.51	32.43	1102.800	89.60
T_3 = Seed primed with *Trichoderma harzianum* (UNN)	3.26	221.92	13.26	454.80	18.28	348.63	34.8	913.29	57.02
T_4 = Seed primed with *Trichoderma viride* (FBD)	2.86	145	8.09	277.49	14.82	282.64	25.77	705.130	21.23
T_5 = Seed primed with *Trichoderma harzianum* (KD)	3.72	210	14.13	484.33	21.40	408.47	41.25	939.480	61.52
T_6 = Seed primed with *Trichoderma viride* (BDA)	2.63	136	14.29	489.87	16.45	313.61	33.37	900.280	54.78
T_7 = Control	2	108	8.5	225.63	13	248	23.5	581.630	
C.D.									39.686
SE(d)									18.326
C.V.									2.547

days age of plant, respectively. T_4 where seed primed with *T. harzianum* (FBD) shows maximum disease severity as from the table it is cleared that biopriming with *Trichoderma* spp. have ability to reduce disease severity and increase shoot and root length, weight, number of branches per plant etc. Barari (2016) has been evaluated native isolates of *Trichoderma species* against *Fusarium oxysporum* f. sp. *lycopersici*, under greenhouse conditions and found that application of *T. harzianum* (N-8) exhibited the least disease incidence (14.75%). Jensen *et al.* (2002) also found that Bio-priming in which specific biological control agents are incorporated into the seed priming process, can be very effective in suppressing many diseases caused by seed and soil borne pathogens.

Yield

The tomato products were harvested and the yield was separately weighted for each treatment. T_2 treatment primed with VAN gives highest fruit yield with 1102.80g followed by T_1 treatment as seed primed with *Trichoderma harzianum* (KN), as 1025.35g fruit yield. The minimum fruit yield was observed in T_4 (705.13g) treatment where seed primed with *T. viride* (FBD) but superior over control (581.63g). Bhatt *et al.* (2015) reported that bio priming has been shown to increase total yield variety of crops, it is because bio-agents treated seed reduce the level of disease incidence. Ravindra *et al.* (2015) found that the yield of tomato crop significantly increased by the combine application of seed treatment with *Trichoderma viride* + soil application of Neem cake powder + foliar spray of Carbendazim.

Conclusion

The study demonstrates that seed biopriming with *Trichoderma* spp. significantly enhances tomato plant growth by improving seed germination, shoot and root development, and biomass accumulation, while effectively suppressing Fusarium wilt disease severity. Compared to untreated controls, all Trichoderma-based treatments reduced disease

incidence, with *T. harzianum* (VAN) showing the most pronounced effect. Importantly, this approach presents an eco-friendly alternative to chemical fungicides, aligning with sustainable agriculture practices by minimizing environmental impact.

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