Original Research Article

Enhancement of seed germination and seedling vigour in tomato (Solanum lycopersicum L.) by native microbial consortia

ABSTRACT

Several biocontrol agents are being employed as one of the alternative methods to chemical measures in agricultural system to manage plant diseases as well as promoting, plant growth and development.Various studies have shown that combination of two or more beneficial microorganisms can have synergistic effect. The present The present study was conducted in 2019 at Department of Plant Pathology, SASRD, Nagaland University to check out the efficacy of indigenous compatible microbial consortia on plant growth promoting activities like seed germination, seedling vigour index, shoot length, root length, fresh and dry weight of shoot and roots by standard filter paper method Three microbial consortia were prepared using native isolates of Trichoderma and Pseudomonas fluorescens.T_1[(*P. fluorescens* P-7 + *Trichoderma* sp. T-5 + *Trichoderma* sp. T-11), T_2 *(P.fluorescens* P-12 + *Trichoderma* sp. T-5 + *Trichoderma* sp. T-11), T_3 (*P. fluorescens* P-7 + *P.fluorescens* P-12+*Trichoderma* sp. T-5 + *Trichoderma* sp. T-11. The experiment was conducted in completely randomised design (CRD) and five replications were maintained for each treatment.The result showed that, T_3 significantly increased vigour index of tomato seedlings (93.24 %), including germination per cent (20.84 %), shoot length (61%) and root length (59.36 %) over control at 10 DAS. On the basis of findings, all the tested microbial consortia had significantly promoted the plant growth over control where $T_3$was the best among the tested microbial consortia exhibiting plant growth promoting potential for commercial exploitation.

Keywords: *Trichoderma, Pseudomonas fluorescens,* indigenous, commercial

1. INTRODUCTION

Tomato is a member of the Solanaceae family, representing one of the most valuable plant families for vegetable and fruit crops. Tomatoes contain many health-promoting compounds and are easily integrated as a nutritious part of a balanced diet [1]. In addition to consuming the fresh fruits, consumers use tomatoes in processed products such as soups, juices, and sauces [2,3]. The yield of tomato is restricted to a great extent due to various diseases and insect pests associated with tomato cultivation [4]. There is a great need to produce quality and healthy seedlings, which are capable of withstanding adverse abiotic and biotic stresses after transplanting with improved mineral nutrient uptake [5].

Although the use of chemicals to reduce or prevent losses caused by this agent seems simple and successful, the damage inflicted by the residual effects of chemicals on humans and environment should certainly be taken into account. Also unplanned and wide use of fungicides often leads to serious environmental problems besides affecting the health of users and consumers. Moreover,

innovative and safe methods like use of biocontrol agents need to be identified and evaluated for continuous search to develop ecofriendly strategies to reduce the dependence on harmful chemicals [6].

A pre-sowing inoculation of planting material as well as the planting medium with the consortia of beneficial microorganisms is an innovative approach for production of quality and healthy seedlings in horticultural production. A microbial consortium is a carrier based product containing nitrogen fixing, phosphorus and potassium solubilising and plant growth promoting microorganisms in a single formulation. The synergistic effect of the formulated microbes can help in providing healthy and vigorous seedlings and considerably reducing the cost of cultivation by reducing fertilizer requirement of vegetables [7].

Plant-growth-promoting microorganism (PGPM) is a term that applies to all microorganisms (e.g., bacteria, actinomycetes, fungi, and algae) that have a beneficial effect on plant growth through the action of either direct or indirect mechanisms (e.g., mineral nutrition, ethylene reduction, disease suppression) [8]. It has been suggested that, due to the plethora of interactions that can occur when single species are inoculated in the field, positive and consistent results in terms of facilitating plant growth are not always achieved [9]. *Trichoderma* species are common soil-borne filamentous fungi, with some strains capable of establishing beneficial relationships with plants [10,11,12]. Direct plant growth promotion is one of many mechanisms in which Trichoderma spp. enhance plant health, however, the molecular mechanism that underlies this is still unclear [13,14,15]. *Pseudomonas* spp. are important bacteria in agriculture and have been shown to promote growth, protect plants from pathogens and herbivores, play a role in phytoremediation and are a part of the core microbiome of many plants. The success of natural regeneration from seeds and the vigorous growth of native plants could suggest a contribution of microbes to the biological protection of germinated seeds against soil-borne pathogens and to the promotion of plant growth [16-19]. Most studies of PGPMs are based on interactions of single microorganisms with plants, evaluating different parameters of growth and plant health, such as length or weight of the plant or its individual tissues, chlorophyll content, or the nutritional content of its tissues or fruits [20-22]. However, more consistent positive results may be obtained by inoculating plants with microbial consortia containing two or more beneficial microorganisms [23,24].The aim of the present study was to evaluate the *in vitro* potential of two or more interacting biocontrol agents to enhance tomato seeds germination and seedling growth and development.

2. MATERIAL AND METHODS

2.1 Preparation of liquid compatible microbial consortia (CMC)

The present study consist of two native isolates of *Trichoderma* (T-5 and T-11) and two isolates of *Pseudomonas fluorescens* (P-7 and P-12). A 250 ml suspension of each selected native isolates of *Trichoderma* sp. (T-5 and T-11) were prepared from 9 days old cultured PDA medium plates. The plates were rinsed with sterile distilled water and the mycelia were carefully scraped off

the agar with a bent glass rod. This suspension was filtered through filter paper (Whatman No. 1) to separate the spores from the mycelia. The concentration was adjusted to 3.7×10^8 spores/ml with the help of haemocytometer [25]. A 250 ml of each selected native isolates of *Pseudomonas fluorescens* (P-7 and P-12) was prepared by inoculating the strain into King's B broth followed by shaking for 48 hrs (150 rpm) at room temperature. The bacterial suspension was roughly adjusted optically at 1×10^9 cfu/ml (O.D. 600= 1) [26].

From the four different microbial suspensions, three liquid microbial consortia were prepared as follows- T_1[(*P. fluorescens* P-7 + *Trichoderma* sp. T-5 + *Trichoderma* sp. T-11), T_2 *(P.fluorescens* P-12 + *Trichoderma* sp. T-5 + *Trichoderma* sp. T-11), T_3 (*P. fluorescens* P-7 + *P.fluorescens* P-12+*Trichoderma* sp. T-5 + *Trichoderma* sp. T-11 Each of the liquid microbial consortia were prepared by mixing equal volume (1:1:1/ 1:1:1:1 ratio) of each selected isolate following the combinations as mentioned..[27].

2.2 Sterilization of seeds

The healthy seeds of tomato cv. Pusa Ruby were selected for experimental purpose. Tomato seeds were surface sterilized with 1.0 % sodium hypochlorite for 2 min for all treatments followed by three rinsed with sterile distilled water. The *in vitro* experiment was conducted in a complete randomised design (CRD) and five replications were maintained for each treatment. The total five numbers of treatments *viz.,* T_1 (*P. fluorescens* P-7 + *Trichoderma* sp. T-5 + *Trichoderma* sp. T-11), T_2 (*P. fluorescens* P-12 + *Trichoderma* sp. T-5 + *Trichoderma* sp. T-11), T_3 (*P. fluorescens* P-7 + *P. fluorescens* P-12 + *Trichoderma* sp. T-5 + *Trichoderma* sp. T-11), T_4- chemical control and T_5- control were used.

2.3 Wet seed treatment

The surface sterilized seeds were soaked with liquid formulations of consortia [@ 1.0 % or 10 µl/ 1 g seeds; 10 µl formulation of CMC added in 990 µl of sterile distilled water/1 g seed (400 tomato seeds)] and shade dried in laminar air flow for 5 hours. For chemical control treatment The surface sterilized seeds were treated with captan 50 % WP (seed dressing @ 0.3 % or 3mg/1g seed). For control treatment, surface sterilized seeds were soaked in sterile distilled water (@1ml/1g seed) and shade dried in laminar air flow for 5 hours [27]. This experiment was carried out by standard filter paper method (three layered moistened filter papers in Petri plates, 10 seeds/ plate and 20 seeds/replication) [28].

Per cent germination at 10 DAS: Per cent germination was calculated using the following formula – Per cent germination = (No. of seeds germinated / Total no. of seeds sown) X 100

Seedlings shoot length and root length (cm): The root length and shoot length of individual seedlings (10 seedlings/replication) were measured. The shoot length was measured from collar region to the tip of the seedling with the help of a scale and the mean shoot length was expressed in

cm. The root length measured from collar region to the tip of primary root with the help of a scale and the mean root length was expressed in cm.

Seedling vigour index (SVI) : The vigour index of seedlings was calculated by adopting the method suggested by AbdulBaki and Anderson [29] and expressed in number by using the below formula.

SVI = Germination (%) x [Mean shoot length (cm) + Mean root length (cm)].

Fresh weight (mg) of seedling shoot and root: The fresh weight (mg) of root and shoot of individual seedlings (10 seedlings /replication) were measured. By LCD elcetronic weighing balance.

Dry weight (mg) of seedling shoot and root: The dry weight of root and shoot of individual seedlings (10 seedlings/replication) were measured after oven drying at 60° C (when constant weight obtained) for 24 hours.

3. RESULTS AND DISCUSSION

3.1 *In vitro* efficacy of liquid CMC on tomato seedlings

Standard filter paper method was carried out *in vitro* to check the efficacy of liquid consortia on tomato seed germination and seedling vigour index. Per cent germination was recorded at 10 DAS. Tomato seed germination per cent was significantly highest in T_3 (87.00 %) followed by T_2 (85.00 %) and T_1 (82.00 %). The lowest seed germination per cent was observed in control treatment (72.00 %) (Table 1). These results revealed that the T_3 significantly increased seed germination per cent (20.84 %) over control treatment (Table 1).The results were in agreement with the earlier worker, Murthy et al. who reported that the application of liquid consortia of *Trichoderma* spp., significantly increased the tomato seed germination per cent at 10 DAS[30]

3.2 Seedlings shoot length and root length (cm) at 10 DAS

Among the tested treatments, significantly maximum shoot length and root length was observed in seed treated with T-3 (4.83 cm), (5.13 cm) respectively (Table 1) than the other treatment. Minimum shoot length and root length was observed in control treatment (3.00 cm) and (3.22 cm) respectively (Table 1). The T-3 significantly increased shoot length (61.00 %) and root length (59.36 %) over control treatment (Table 2). In agreement to these results, Singh *et al.* (2019)also reported significant increased in shoot length and root length of tomato seedling at 10 DAS by using liquid compatible microbial consortia of *Trichoderma asperellum* and *Pseudomonas fluorescens*[31].

3.3 Seedling vigour index (SVI) at 10 DAS

Among the tested treatments, significantly maximum seedling vigour index was recorded in seed treated with T-3 (865.67) than the other treatment. Minimum seedling vigour index was observed in control treatment (447.84) (Table 1). These results revealed that the T-3 significantly increased

vigour index of tomato seedlings (93.29%) over control treatment (Table 2). The present findings are in harmony with the findings of Manikandan et al. where liquid formulation of *P. fluorescens* Pf1 significantly promoted tomato plant growth compared to untreated control [32.

3.4 Fresh weight (mg) of seedling shoot and root at 10 DAS

Significantly maximum fresh weight of shoot and root was recorded in T-3 (10.79 mg), (0.33mg) and minimum in control (5.11mg) and (0.16 mg), respectively (Table 1). These results revealed that the T-3 significantly increased shoot fresh weight (111.16 %) and root fresh weight (106.25 %) over control treatment (Table 2). The present findings are in agreement with Murthy et al. [31] where liquid consortia of *Trichoderma* spp., significantly increased the fresh weight of shoot at 10 DAS.

3.5 Dry weight (mg) of seedling shoot and root at 10 DAS

Among the tested treatments, significantly maximum dry weight of shoot and root was recorded in T-3 (0.67 mg), (0.40 mg) and minimum in control (0.04mg) and (0.018 mg), respectively (Table 1). The T-3 significantly increased shoot dry weight (67.50 %) and root dry weight (122.23 %) over control treatment (Table 2). Murthy et al. [30] reported that the application of liquid consortia of *Trichoderma* spp. significantly increased the dry weight of shoot at 10 DAS.

Table.1 *In vitro* efficacy CMC on tomato seed germination (%), seedling shoot length, root length, shoot fresh and dry weight, root fresh and dry weight and vigour index at 10 DAS

Treatment	Seed germination %	Seedling shoot at 10 DAS			Seedling root at 10 DAS			Seedling vigour index at 10 DAS
		Shoot length (cm)	Fresh wt. (mg)	Dry wt. (mg)	Root length (cm)	Fresh wt. (mg)	Dry wt. (mg)	
T_1	82.00 (65.12)	4.09	8.59	0.57	4.90	0.28	0.03	738.74
T_2	85.00(67.51)	4.45	9.14	0.59	4.97	0.30	0.03	800.34
T_3	87.00(68.95)	4.83	10.79	0.67	5.13	0.33	0.04	865.67
T_4	75.00(60.41)	3.26	5.45	0.42	3.58	0.18	0.02	513.14
T_5(Control)	72.00 (58.14)	3.00	5.11	0.40	3.22	0.16	0.01	447.84
Sem+-	2.81(2.07)	0.05	0.31	0.02	0.07	0.02	1.32	23.16
C.V. (%)	7.83(7.22)	3.23	9.04	11.06	3.68	19.10	31.69	7.67
CD(p=0.01)	11.30 (8.31)	0.23	1.27	0.10	0.28	0.09	0.01	93.16
CD(P=0.05)	8.29(6.09)	0.16	0.93	0.57	0.21	0.069	0.01	68.31

*Values in parenthesis are angular transformed values.

Table.2 *In vitro* efficacy of CMC on per cent increase of tomato seed germination (%), shoot length, root length, shoot fresh and dry weight, root fresh and dry weight and seedling vigour index at 10 DAS

Treatment	**Seedger. At 10 DAS**	**Per cent increase of plant growth promotion over control**						**Seedling vigour index**
		Seedling shoot			**Seedling root**			
		Length	**Fresh wt.**	**Dry wt.**	**Length**	**Fresh wt.**	**Dry wt.**	
T_1	13.89	36.34	68.10	42.50	52.17	75.00	66.67	64.95

T_2	18.06	48.34	78.86	47.50	54.34	87.50	66.67	78.72
T_3	20.84	61.00	111.16	67.50	59.36	106.25	122.23	93.24
T_4	04.17	08.67	06.65	05.00	11.18	12.50	11.12	14.58
T_5(Control)	-	-	-	-	-	-	-	-

4. CONCLUSION

With the high rate of increasing in world population, our basic requirements ie; food should be made while enhancing sustainability in crop production. Biological means of plant disease management as well as plant growth promotion can be employed as an important alternative to enhance quality and quantity of crop production. The current investigation showed that all the tested native compatible microbial consortia had promoted the growth of tomato seedlings over control at different ranges. The highest seed germination (87%) and seedling vigour (865.67) were recorded in T-3 followed by T-2 and T-1 respectively. These results show their potential to promote seedlings growth as compared to chemical check and control. However, they can be further evaluated to study their potential in field. Moreover, measures to enhance shelf life, doses of applications and awareness among the farmers can be taken to enhance plant growth which in turn promote crop productivity.

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