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EFFECT OF ENDOPHYTIC MICROBIAL CONSORTIA ON SEEDLING VIGOUR INDEX OF TOMATO

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ABSTRACT

Endophytes are microorganisms that reside within plant tissues without causing harm, establishing symbiotic relationships with their host plants. This abstract explores the effect of developed endophytes in individual and in combinations on growth parameters of tomato cultivar PKM1 such as shoot length (cm), root length (cm), germination per centage and seedling vigour index (SVI). Results demonstrated that endophytic microbial consortia were able to increase the vigour index (562.45) compared to that of chemical treatment (420.22) and control (281.80). Root length and shoot length was recorded to be highest in chemical treatment followed by endophytic microbial consortia treatment which is on par with the check. Highest germination per centage (%) of 80.68% was recorded in the endophytic treatment in which seeds of tomato were treated with isolate TEB-12 (Gram negative cocci).
Keywords **:** Endophytes, Tomato, Fusarium wilt, Paper towel method, Vigour index

Introduction

Tomato (*Lycopersicon esculentum* Mill.), a member of the Solanaceae family, is one of the most cherished vegetables worldwide (Pritesh *et al.,* 2011). As the leading tropical vegetable crop globally (Hadian *et al.,* 2011), it plays a pivotal role in horticulture, contributing to local economies and serving as a crucial dietary staple. Tomatoes enhance both nutrition and livelihoods for rural and urban communities alike (Waiganjo *et al.,* 2006). They are highly versatile, consumed fresh in salads, cooked as vegetables, or processed into products like tomato paste, sauce, ketchup, and juice. Additionally, tomatoes can be preserved through drying.

India ranks as the second-largest producer of tomatoes globally, after China, in both area cultivated and total production. The top tomato-producing countries are China (30.7%), India (11.5%), the USA (8.1%), Turkey (7.0%), and Egypt (5.3%). In India, tomatoes are grown on approximately 7.87 lakh hectares, with an annual production of around 205.72 lakh tonnes (Horticultural Statistics, 2020).

Endophytes are microorganisms, such as bacteria or fungi, that live inside plant tissues without causing any apparent harm to their host. These microorganisms can inhabit various parts of the plant, including leaves, stems, roots, and even seeds, and exist symbiotically or in a mutualistic relationship with the plant. Endophytic microorganisms significantly enhance plant growth and strengthen defenses against pests and pathogens through various mechanisms. They produce and secrete secondary metabolites and biochemicals that neutralize the harmful effects of plant pathogens, including volatile compounds that suppress pathogen growth.

Recent studies have emphasized the effectiveness of using combined inoculants, which consist of a mixture of microorganisms that work synergistically, compared to single bioinoculants. As a result, there is an increasing shift towards developing microbial consortia to enhance crop management practices.

Several endophytes have been successfully isolated and tested for their compatibility with each other and developed into consortium which was used for present study to investigate their efficacy in promoting the growth attributes of tomato through standard roll towel method.

Materials and Methods

The seeds of tomato cv.PKM-1 were sourced from Horticultural college and Research Institute, Tamil Nadu Agricultural University, Periyakulam, Tamil Nadu. These tomato seeds of cv. PKM-1 were treated separately with a chemical (Carbendazim 12% + Mancozeb 63% WP @ 0.15%) and potential endophytes (four endophytic bacteria *viz.*, TEB-4 (*Bacillus safensis*), TEB-12 (Gram negative cocci), TEB-8 (*Pseudomonas aeruginosa*), TEB-15 (*Bacillus subtilis*) and one endophytic fungi TEF-10 (*Trichoderma yunnanense*) separately and with consortium of these endophytes to test their efficacy on seedling vigour index through paper towel method.

The experiment was conducted to assess the impact of 5 selected efficient endophytic isolates individually and in combinations on seed germination and tested for their plant growth promoting ability by the standard roll towel method (ISTA, 1985) in growth chamber.

Surface sterilization and seed treatment of tomato seeds

Tomato seeds were surface sterilized with 0.1% sodium hypochlorite solution for 5 minutes, rinsed with sterile distilled water (SDW) and soaked in endophytic microbial suspension (bacteria – $3x10^8$ cfu ml^{-1} and fungi – $1x10^6$ cfu ml^{-1}) using 1% carboxymethyl cellulose (CMC) for 24h and chemical seed treatment was done using Carbendazim 12% + Mancozeb 63% WP @ 0.15% which was used as check and seeds that were treated with sterile distilled water was kept as control.

Paper /Roll towel method

The seeds were blot dried and placed in wet germination papers and were further incubated at constant growth conditions. Each treatment was replicated four times with 40 seeds per each germination paper. The percentage of germination and seedling vigour index was calculated at an interval of 3 days for a total period of 21 days.

Seedling vigour index (VI) and Germination % were calculated using the following formulae given by Abdul-Baki and Anderson (1973).

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

Seedling vigour index (SVI) = Germination % x Average seedling length (Shoot length + Root length)

Table 1: Treatment details

S. No	Treatments
T_1	TEB-4 (*Bacillus safensis*)
T_2	TEB-8 (*Pseudomonas aeruginosa*)
T_3	TEB-12 (Gram negative cocci)
T_4	TEB-15 (*Bacillus subtilis*)
T_5	TEF-10 (*Trichoderma yunnanense*)
T_6	TEB-4 + TEB-8 + TEB-12 + TEB-15 + TEF-10
T_7	Carbendazim 12% + Mancozeb 63% WP @ 0.15% (check)
T_8	Control

Results and Discussion

The effect of endophytes on seed germination and vigour analysis were carried out by paper towel method (Govender *et al.,* 2008). Tomato seeds which were placed in paper towel were assessed to record their germination per centage. Average seedling length for the germinated seedlings was calculated by recording their root length and shoot at every 3 days interval. Based on the obtained root length, shoot length and germination per centage values, seedling vigour index for each treatment at different intervals was calculated and the results were mentioned as follows.

Effect on Shoot length of tomato

Among all the treatments applied, the combination where tomato seeds were treated with an endophytic microbial consortium resulted in a significant increase in shoot length compared to seeds treated individually with endophytes and is on par with the chemical treatment. All treatments showed improved shoot length over the control, demonstrating their effectiveness in promoting seedling growth.

Of the eight treatments tested at different intervals, the average shoot length was calculated and as mentioned earlier, the results indicated that maximum shoot length of 13.97 cm was recorded in the chemical treatment followed by combination treatment that includes all the endophytic isolates (TEB-4 + TEB-8 + TEB-12 + TEB-15 + TEF-10) in which the shoot length was recorded to be 13.52 cm. An average shoot length of 13.25cm, 12.40cm, 12.17cm, 11.95cm and 11.90cm were observed in treatments with isolates, TEB-15, TEF-10, TEB-4, TEB-8 and TEB-12 respectively. A minimum shoot length of 10.87 cm was recorded in control.

Table 2: Influence of endophytes on shoot length of tomato cv. PKM-1

Treatments	6 days	9 days	12 days	15 days	18 days	21 days
T_1	4.92	6.95	7.07	8.70	9.67	12.17
T_2	5.37	7.10	7.87	8.65	10.02	11.95
T_3	5.47	7.10	7.90	8.52	10.57	11.90
T_4	5.67	7.05	7.92	8.65	10.65	13.25
T_5	5.30	7.17	7.77	8.77	10.57	12.40
T_6	5.90	7.90	7.90	9.27	11.37	13.52
T_7	6.10	8.12	8.20	9.60	12.00	13.97
T_8	4.62	5.92	6.35	7.87	8.37	10.87
C.D.	0.79	0.6	0.89	0.69	0.72	0.77
SE(m)	0.29	0.17	0.37	0.22	0.24	0.28

Effect on root length of tomato

The root length when calculated at different intervals for the eight treatments, the results indicated that highest root length of 14.07 cm was observed in chemical treatment. It was followed by root length of 13.92 cm for the combination treatment (TEB-4 + TEB-8 + TEB-12 + TEB-15 + TEF-10) similar to the findings of the shoot length of tomato. Root length of 13.70 cm was recorded for the isolate TEF-10 (*Trichoderma yunnanense*). and TEB-12 (Gram negative cocci) were on par with each other with root length of 10.35 cm and 10.31 cm respectively. An average root length of 13.30 cm, 12.92 cm, 12.82 cm and 12.65 cm were observed for the isolates TEB-8, TEB-4, TEB-12 and TEB-15 respectively. Minimum root length of 8.45 cm was recorded in control treatment.

Similar study was conducted by Sushma *et al.* (2020) in tomato using endophytic bacteria through standard roll towel method and reported that all the isolates showed maximum root and shoot lengths compared to the control of which isolate EBT18 has shown maximum root length and shoot length of 18.67 cm and 15.91 cm respectively.

Table 3: Influence of endophytes on root length of tomato cv. PKM-1

Treatments	6 days	9 days	12 days	15 days	18 days	21 days
T_1	6.72	7.75	8.75	10.30	11.37	12.92
T_2	7.55	7.65	8.75	10.32	11.72	13.30
T_3	7.25	8.07	8.50	10.52	11.67	12.82
T_4	6.00	7.92	8.40	10.62	11.62	12.65
T_5	7.05	8.62	8.65	10.30	12.07	13.70
T_6	7.75	8.92	9.70	11.55	12.52	13.92
T_7	7.25	8.87	9.77	11.37	12.82	14.07
T_8	4.50	6.50	7.37	8.75	10.37	12.20
C.D.	1.11	1.03	0.96	1.03	0.93	0.97
SE(m)	0.57	0.5	0.43	0.5	0.4	0.44

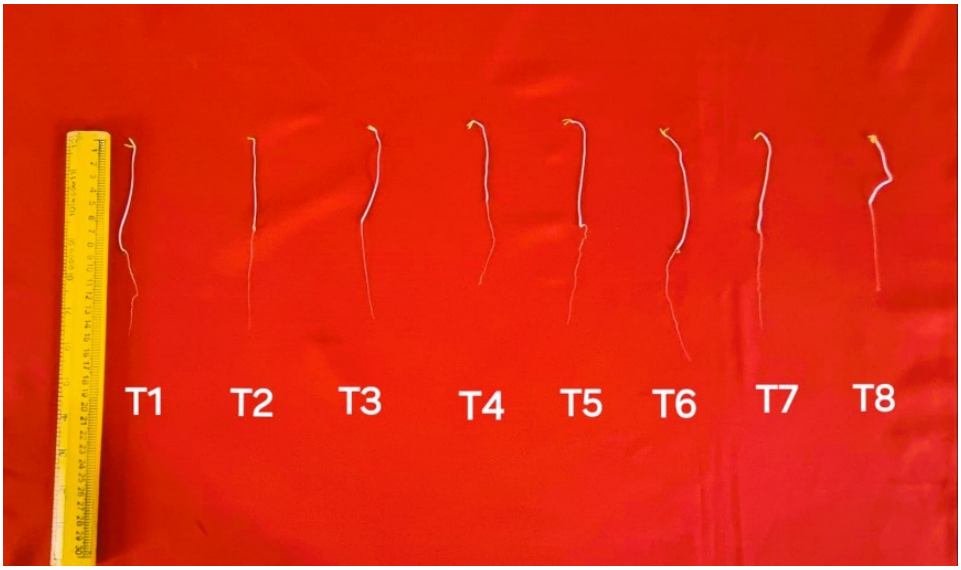

Fig 1: Measurement of growth parameters in tomato seedlings across different treatments

Fig 2: Germinated seedlings in paper towel method in various treatments

(a) T_6 (endophytic microbial consortia) (b) T_7 (chemical treatment) (c) T_8 (control)

Effect on germination per centage of tomato

Of all the eight treatments, the total number of seeds germinated after treatment with endophytes and their consortium were higher in number when compared with the check and control. And among those endophytic treatments, TEB-12 and combination treatment exhibited greater germination percentage of seeds compared with other individual endophytic isolates.

At three days interval, the results showed that germination percentage was recorded highest in the isolate TEB-12 (Gram negative cocci) which is 48.95 % followed by 44.18% in combination treatment (T6). Germination percentages of 41.37%, 40.94%, 38.15 % and 33.56 % was recorded for the isolates TEF-10, TEB-4, TEB-15 and TEB-8 respectively. In chemical treatment, 37.65 % of germination was observed and minimum germination percentage was calculated for the control i.e., 33.54%.

These findings were in agreement with the reports of many who studied and concluded that there has been considerable increase in seed germination % when tomato seeds were treated with endophytes. Haddad *et al*. (2013) reported there is an increased seed germination percentages of about 84% and 100% when tomato seeds were treated with endophytic bacterial strains such as *Bacillus* spp. PF3 and *Pseudomonas* spp. PS1 compared with control.

Effect on Seedling vigour index (SVI) of tomato

Based on shoot length, root length and germination percentage, seedling vigour index of tomato treated with different endophytic isolates was calculated. The combination (T6) in which endophytic

microbial consortium is used was proven to be much efficient in promoting the seedling vigour index when results were assessed.

The results showed that maximum seedling vigour index of 562.45 was recorded for the combination treatment (TEB-4 + TEB-8 + TEB-12 + TEB-15 + TEF-10). It was followed by Isolate TEB-12 with vigour index of 533.95. An average vigour index of 457.30, 432.17, 394.87 and 309.57 were recorded for the isolates TEF-10, TEB-4, TEB-15 and TEB-8 respectively. The vigour index recorded for the chemical treatment was 420.22, while the control showed the lowest index of 281.80.

Table 4: Influence of endophytes on seedling vigour index of tomato cv. PKM-1

Treatments	6 days	9 days	12 days	15 days	18 days	21 days
T_1	234.82	252.72	271.62	332.42	366.32	432.17
T_2	179.42	180.15	203.82	232.35	266.65	309.57
T_3	326.80	345.50	373.90	434.40	507.45	533.95
T_4	199.22	228.85	250.10	293.77	339.20	394.87
T_5	248.62	277.50	288.92	332.27	396.65	457.30
T_6	307.85	325.87	343.97	409.25	466.02	562.45
T_7	227.32	252.70	270.40	311.65	371.37	420.22
T_8	127.50	151.12	168.77	202.85	229.22	281.80
C.D.	80.81	84.97	102.08	118.51	132.07	144.17
SE(m)	3065	3389	4892	6595	8189	9758

Table 5: Effect of endophytes on growth parameters of tomato cv. PKM-1

Treatments	Root Length (cm)	Shoot Length (cm)	Germination %	Seedling Vigour Index (SVI)
T_1	12.92	12.17	79.31 (73.42)	432.17
T_2	13.30	11.95	78.06 (72.40)	309.57
T_3	12.82	11.90	80.68 (74.40)	562.95
T_4	12.65	13.25	78.81 (73.03)	394.87
T_5	13.70	12.40	79.37 (73.46)	457.30
T_6	13.92	13.52	79.87 (73.83)	533.45
T_7	14.07	13.97	78.75 (72.98)	420.22
T_8	12.20	10.87	78.06 (72.40)	281.80
C.D.	0.97	0.77	N/A	144.17
SE(m)	0.44	0.28	1023	9758

Overall findings indicate that combination treatment i.e. endophytic microbial consortia resulted in maximum values of shoot length, root length and seedling vigour index reflecting their efficacy over individual treatments, check and control.

These reports were in alignment with the research works carried out by other researchers who put forward that combined inoculation of endophytic strains produced highest vigour index over single inoculants. Sundaramoorthy *et al.* (2012) reported that combined strains of *Pseudomonas* spp. and *Bacillus* spp. (Pf1+EPCO16) produced significantly higher vigour index (3840.70) of tomato seedlings over individual biocontrol strains Pf1, Py15, Fp7, EPC5 and EPCO16. Chaturvedi *et al*. (2022) also concluded that application of endophytic microbial consortium (*Pseudomona*s spp. and *Bacillus* spp.) has significantly increased the Seedling vigour index (384.0) compared with the control which is 74.0 respectively.

The observed increase in seedling vigour could be attributed to the production of stimulants like indole-3-acetic acid (IAA), which promotes cell division, elongation, and transformation, as noted by Hu (2011) and Shahab *et al*. (2009). Additionally, according to Noel *et al.* (1996), these endophytic bacteria produce other growth-promoting hormones such as cytokinins, ethylene, gibberellins, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase which could have contributed to the enhancement of shoot and root length that ultimately boosted the vigour index. As reported by Gupta *et al.* (2012) the rise in vigour index due to endophytic bacteria may also be due to their phosphate solubilizing capacity as it plays a major role in plant physiological activities such as cell division

and photosynthesis. The growth promoting capacity of endophytic bacteria such as *Pseudomonas* sp. and *Bacillus* sp. may also be due to their ability to produce siderophores and solubilization of phosphate as reported by Rajkumar *et al.* (2005)

Conclusion

To enhance the growth parameters such as germination per centage, root length and shoot length based on the results obtained endophytic microbial consortia can be considered comparatively to that of chemical as it is a sustainable method not only in promoting growth but also protecting the crop from pathogens and pests.

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