

## The Beneficial effect of thermochemical organic fertilizer and root endophytic fungi on the growth of tomato (*Solanum lycopersicum* L.)

### ABSTRACT

**Aim:** To study the effect of thermochemical organic fertilizer (TOF) inoculated with beneficial root endophytes on the growth attributes of tomatoes grown in a nursery. Combined impact of TOF and endophytic fungi like *Piriformospora indica* and *Glomus* species on tomato growth parameters such as plant height, shoot length, fresh shoot weight, dry shoot weight, fresh root weight, dry root weight, root volume and root area were assessed.

**Study Design:** The experiment was conducted through a completely randomized design with six treatments and 3 replications

**Place and Duration of Study:** The pot experiment was conducted at the Department of Soil Science & Agricultural Chemistry, College of Agriculture, Vellayani, Kerala Agricultural University between August 2021- September 2021

**Methods:** Sterilized tomato seeds were sown in pottrays filled with different media. Potting mixture containing soil and coir pith compost was taken as a control treatment. Other treatments were TOF with additions of endophytic fungi like *Piriformospora indica* (*P.indica*), *Glomus fasciculatum*, *Glomus mossaea* and mixed *glomus* in TOF. Biometric observations like plant height, shoot length, fresh weight of shoot, dry weight of shoot, fresh weight of root, root dry weight of shoot, root surface area and root volume were observed twenty eight days after sowing.

**Results:** Inoculation with *P. indica* as well as *Glomus sp* in the medium of TOF improved plant growth compared to the uninoculated soil medium. Among all plant growth parameters analyzed, the maximum values were obtained in plants grown in a medium of TOF+ *P. indica*. With respect to root fresh weight, there was no significant difference between treatments of TOF + *P. indica* and TOF+ other *Glomus* species including *G. fasciculatum* and *G. mossaea*. TOF treated with *P. indica* and *G. fasciculatum* outperformed as best in the case of root dry weight compared to all other treatments.

**Conclusion:** Beneficial root endophytes like *Piriformospora indica* and *Glomus sp* grown in thermochemical organic fertilizer were found to have greater potential in promoting the growth of tomato plants in the nursery.

**Keywords:** Thermochemical organic fertilizer, *Piriformospora indica*, *Glomus fasciculatum*, *Glomus mossaea*, root colonization

## Introduction:

“Food security and healthy nutrition of citizens can be achieved only through the enhancement of quality food production. While on the one hand, we concentrate on quantum jumps in production, there occurs a piling up of agricultural bio waste on the other side. An effective and efficient management strategy for waste is the need of the hour. There are different alternative technological options for solid waste management like mulching, fodder, fuel, composting, etc. The conversion of solid waste to organic fertilizer is a desirable option, in light of reports that severe depletion of soil organic matter is a major cause of declining crop productivity” [1]. The patented rapid thermochemical biowaste processing technology [2] enables processing of solid waste to organic fertilizer in less than a day in an environment friendly manner.

“The so produced thermochemical organic fertilizer (TOF) is used as an efficient potting media and is utilized for container cultivation of vegetables in growing bags and pots. Container cultivated tomatoes in a growing medium comprising of thermochemical organic fertilizer, coco peat and topsoil significantly outyielded those grown in conventional growing media of topsoil, sand and farmyard manure” [3]. “The efficiency of thermochemical organic fertilizer on the growth and yield of vegetables and banana has been confirmed in several studies conducted at the Kerala Agricultural University” [4]; [5]. “The carbon content in thermochemical organic fertilizer is considered to be superior in imparting an enhanced rhizosphere priming effect as compared to conventional organic manure sources” [6].

Tomato (*Solanum lycopersicum*) is one of the main vegetable crops worldwide due to its extensive consumption. This plant is a valuable food in the human diet and is known as a protective food because of its unique nutritive value since it is a source of vitamins, carotenoids, lycopene, and antioxidant compounds [7]. The use of beneficial microbes like endophytic fungi as biological agents at the vegetative stage can have advantageous effects. These effects include the promotion of plant growth, biological control of diseases, increases in crop yield, and quality improvement. Plant growth-promoting fungi (PGPF) promote growth through the production of enzymes, phosphate solubilization [8]; [9], siderophore production [10] and, antagonism to phytopathogens [11]. The use of PGPF inoculants as biological agents for the production of vegetable seedlings has been reported by researchers.

“The term endophyte was originally proposed by de Bary in 1866 to define many organisms occurring within plant tissues” [12]. “Endophyte research mostly focuses on endophytic fungi that thrive within plants for at least part of their life cycle without causing overt symptoms” [13]. “Endophytic fungi, viz., *Piriformospora indica* and *Glomus fasciculatum*, colonizing within the roots of many crops asymptotically is well known to promote plant growth, elicit defense responses against pathogens and ameliorate abiotic stresses such as drought and salinity and biotic stress caused by fungi, bacteria and viruses” [14]. “Rhizomicrobiome, the communities of microorganisms surrounding the root of the plant, plays a vital role in promoting plant growth and development” [15]. “The interactions of the rhizomicrobiome are of great importance owing to the diverse root exudates and

reduced carbon that promotes chemotaxis and result in diverse and unique patterns of microbial colonization” [16]. “Microbes of the rhizomicrobiome play key roles in nutrient acquisition and assimilation, improved soil structure, and secreting and modulating extracellular molecules such as hormones, and secondary metabolites leading to enhancement of plant growth” [17].

“*Piriformospora indica*, belonging to the Sebaciniales in the Basidiomycota was discovered in the Thar desert, India and displays an endophytic lifestyle” [18]. “*P. indica* is phylogenetically close to mycorrhizal endosymbionts of orchid and ericoid roots and can colonize the root cells of a broad range of host plants. Positive interactions of *P. indica* are established for many important agricultural and horticultural plants, which allow them to grow under extreme physical and nutrient stress. The fungus promotes plant growth, especially in nutrient-deficient soils, confers tolerance to abiotic (salinity, drought, water, cold, high temperature, and heavy metals) and biotic (root and foliar pathogens) stress, regulates plant growth and development, induces early flowering and enhanced seed production, stimulates the production of active ingredients in medicinal plants, and helps in the hardening of micro propagated or tissue cultured plants” [19]. There are some reports on tomato colonization with *P. indica*. Recent studies have shown that tomato root colonization with *P. indica* resulted in decreased disease severity caused by early blight [19]. Based on previous results, tomato is one of the most important hosts of *P. indica*, and the symbiotic relationship between plant-fungus has positive effects on its growth and development.

Knowledge of the impact of TOF on the root morphological and phenomic characters that nurture the rhizospheric microbial population of beneficial root endophytic fungi is very crucial to understand the depth of rhizospheric exploration and acquisition of essential nutrients. We hypothesized that there would be variability imparted by different TOF – endophytic fungal combinations which enhanced soil nutrient acquisition, root-to-shoot translocation and biomass production in tomato seedlings. In this perspective the objective of this study was to assess the beneficial effect of thermochemical organic fertiliser as a medium for the multiplication of endophytic fungi like *P. indica* and *Glomus* species.

## **2. Materials and methods**

### **2.1. Nursery production of tomato plants**

Potting mixture for growing the tomato seedlings was prepared by mixing topsoil, TOF and coir pith in a ratio of 1:1:1 on a volume basis. The potting mixture thus prepared was used to fill the entire ninety-eight cells in the pro-trays. The different root endophytic fungi were incorporated into the potting mixture as per the various treatment combinations before filling the cavities of the pro-tray. *Piriformospora indica* mycelium was incorporated into the planting medium before filling the pro-tray cavities. For this, the mycelium of the fungal endophyte grown in a 250 ml conical flask containing Potato Dextrose Broth medium was collected by filtering the contents of the flask. Similarly, inoculums of *Glomus fasciculatum* and *Glomus mossaea* were also added separately to the potting media. Tomato seeds were sterilized in 1% sodium hypochlorite aqueous solution for 2 minutes. The seeds were further washed three times with distilled water.

Two seeds were planted in each cell of pro-tray and further maintained to single seedling after germination. Plants were grown in shade house with proper ventilation and sunlight. Seedlings were irrigated with tap water twice daily. Plants were kept for thirty days in the nursery.

## **2.2. Root colonization by endophytic fungi**

The plants treated with endophytic fungi like *Piriformospora indica* and *Glomus sp.* were assessed for root colonization by the corresponding fungi. After one week of germination, plants were uprooted without causing any damage to the roots. The root system was washed in running water to get rid of adhering soil particles. Roots were cut into small bits one cm in length. The bits were boiled in 10% potassium hydroxide (KOH) for 2 minutes. KOH was removed by washing with distilled water. Root bits were directly transferred to the microscopic slide and after staining with lactophenol- trypan blue solution it was examined under a compound bright field microscope. The presence of chlamydospores was taken as a measure of root colonization.

## **2.3. Biometric observations**

Thirty days after seeding, biometric observations of the tomato seedlings were taken by way of destructive sampling. The observations on various biometric attributes recorded included plant height (cm), shoot length (cm), shoot fresh weight (g/plant), shoot dry weight (mg/plant), root fresh weight (g/plant) and root dry weight (mg/plant). Plant height and shoot length were measured using a measuring scale. Plant height was measured as the length from the tip of the main root to the apex leaf. Shoot height is observed as the length from the base of the shoot to the apex leaf. The dry weight was recorded after drying the plant samples for three days at 60 °C in a hot air oven. Root volume was estimated using Archimedes' water replacement principle. Root tissue was submerged in a known volume of water in a measuring cylinder and the volume of water displaced was measured.

## **2.4. Statistical analysis**

The experiment with seedlings raised in the nursery was done in a completely randomized design (CRD). "The F values for treatments were compared with the table values. If the effects were significant, critical differences at a 5% significance level were calculated for effecting comparison among the means. R-package grapesAgri1 was used for data analysis" [21]. Treatments were T1: Soil + coir pith, T2: TOF, T3: TOF + *P. indica*, T4: TOF+ GF, T5: TOF+GM, T6: TOF +Mixed glomus

## **3. Results and Discussion**

Tomato plants grown in the nursery in a medium of soil and compost recorded the lowest values in all the observed parameters as compared to a medium of TOF (Table 1). Among the treatments, T3 consisting of TOF+ *P.india* recorded the highest values of plant height, shoot length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight [22]. The plant height obtained in the best treatment was 13.95 cm which was 2.21 times greater than the control treatment.

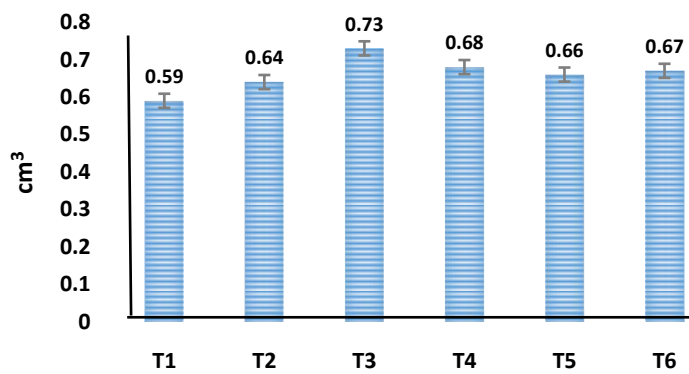
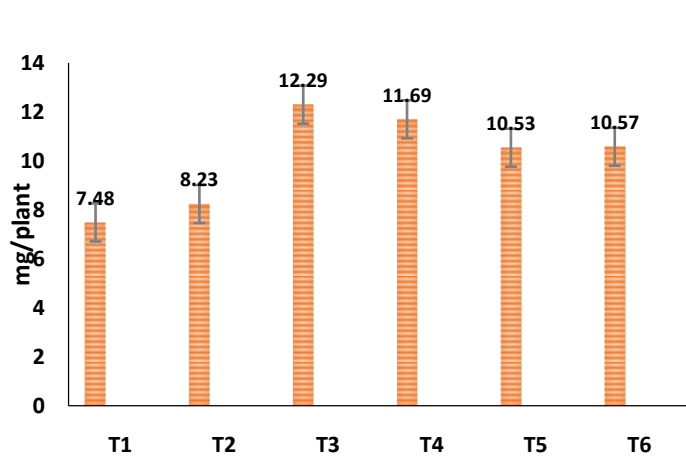
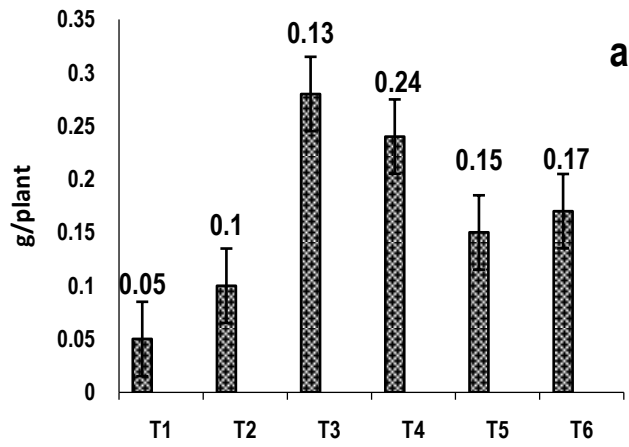
Similar results of 13.98 cm plant height, 1.55 g/plant shoot fresh weight, 85.84 mg/plant shoot dry weight, 0.12 g/plant root fresh weight and 11.64 mg/plant root dry weight in *P. indica* treated plants

were found by [23]. Treatments with glomus addition (T4, T5 and T6) resulted in the second best in the case of plant height. All the glomus treatments were on par with each other. The addition of *Glomus fasciculatum* to TOF gave the second best treatment in parameters like shoot length, shoot fresh weight and shoot dry weight compared to other glomus species. *P. indica* significantly increased tomato plant height, root and shoot dry weight and leaf area compared to non-inoculated plants. The fresh shoot weight of tomato seedlings was almost half that of the tomato seedlings grown in a medium of TOF+ *P.indica*. Fresh root weight was almost the same in plants grown in a medium of *P.indica* alone and also in combination with TOF.

Table 1. Variation in biometric attributes as influenced by different root endophytic fungi in growing media of tomato seedlings

Growing media	Height of plants	Length of shoot	Fresh weight of shoot	Dry weight of shoot
	cm	cm	g/plant	mg/plant
Soil + coir pith (T1)	8.35 ± 0.99 <sup>e</sup>	6.30 ± 0.81 <sup>d</sup>	0.51 ± 0.03 <sup>e</sup>	45.67 ± 0.83 <sup>f</sup>
TOF (T2)	10.54 ± 0.89 <sup>d</sup>	7.91 ± 0.33 <sup>c</sup>	0.55 ± 0.08 <sup>e</sup>	49.57 ± 1.00 <sup>e</sup>
TOF + <i>P. indica</i> (T3)	17.42 ± 0.64 <sup>a</sup>	13.95 ± 0.37 <sup>a</sup>	1.65 ± 0.12 <sup>a</sup>	88.41 ± 1.03 <sup>a</sup>
TOF+ GF (T4)	15.36 ± 0.68 <sup>b</sup>	11.61 ± 0.86 <sup>b</sup>	1.33 ± 0.11 <sup>b</sup>	72.54 ± 1.19 <sup>b</sup>
TOF+GM (T5)	12.58 ± 0.89 <sup>c</sup>	10.65 ± 0.85 <sup>b</sup>	0.84 ± 0.08 <sup>d</sup>	62.49 ± 0.95 <sup>c</sup>
TOF +Mixed glomus (T6)	13.76 ± 0.87 <sup>c</sup>	10.42 ± 0.81 <sup>b</sup>	1.1 ± 0.15 <sup>c</sup>	60.55 ± 0.90 <sup>d</sup>
SEm±	0.48	0.410	0.061	0.57
CD (0.05)	1.49	1.26	0.18	1.76

(TOF: Thermochemical organic fertilizer)



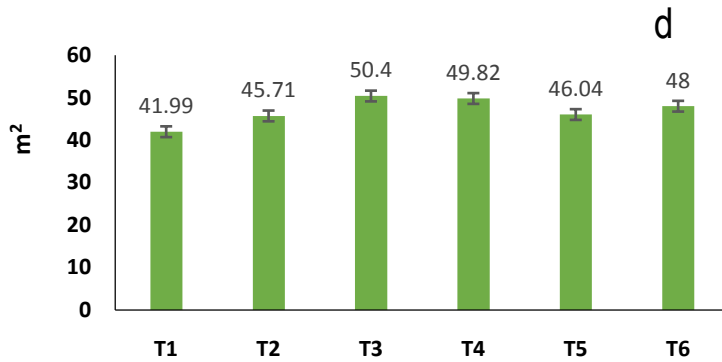


Fig. 1 Effect of different root endophytic fungal combinations with a thermochemical organic fertilizer in growing media on (a) root fresh weight and (b) root dry weight (c) root volume (d) surface area (T1: Soil+ coirpith; T2: TOF; T3: TOF + *P. indica* ; T4: TOF+ GF ; T5: TOF+GM ; T6: TOF +Mixed glomus)

As endophytes play a key role in framing root phenomic characters, observations like root fresh weight and root dry weight is a necessity. In the case of root fresh weight and root dry weight, the treatment with TOF+ *P. indica* recorded the highest value which was also on par with the treatment with TOF+ GF (Fig 1). Treatment with TOF without endophytic fungi yielded the next highest root fresh weight which was again on par with T5 and T6. *P. indica*, the fungal endophyte has been used as a mixed inoculum with PGPR strains for improving growth responses in tomato. The application of a talc based consortium of two strains of *Pseudomonas* with *P. indica* when applied to tomato plants showed an increment of 8.8 fold in dry root weight and 8.6 in dry shoot weight [24]. The root dry weight of *P. indica* colonized tomato plants was up to 58% higher than non-inoculated plants. The roots of the *P. indica* inoculated plants were 54% longer than those of the non-inoculated plants. Similarly, results showed that the shoot length of the inoculated plants increased by 41% compared to the control plants. Furthermore, the results revealed an increase in the shoot dry weight of the inoculated as compared with non-inoculated tomato plants [25]. *P. indica* and mixed-AMF collectively promoted root length, surface area, and volume of *C. oleifera* to a certain extent [26] which is in accordance with the previous findings of Zhang et al. [27]. “The growth parameters of rice seedlings such as shoot and root length, fresh and dry weight, seedling vigor index 1 and 2, root volume and root shoot ratio were found enhanced in *P. indica* inoculated rice seedlings as compared to non-inoculated control seedlings” [28].

“Thermochemical organic fertilizer is capable of contributing to the soil organic carbon pools and an ideal C: N ratio to nurture the soil microbial community in the rhizosphere niche. The constituent organic carbon components of TOF are an effective repository to meet the energy demands promoting microbial diversity and proliferation” [29]. “The indirect effect of the labile carbon fraction of TOF creates a suitable physical environment in the rhizosphere favouring soil respiration. The labile carbon fraction is strongly correlated as a vital driving force for microbial biomass and as a

facilitator of enhanced soil respiration” [30]. “The water soluble and labile fractions of the soil organic carbon pool served as an effective substrate source promoting rhizosphere microbe proliferation. The resultant root zone interactions favored efficient nutrient mineralization, root nutrient acquisition, and translocation to functional metabolic sites for the assimilation of biomass, which subsequently led to superior allocative efficiency in the belowground and aboveground parts of the tomato and amaranthus crops” [31]. These properties of TOF lead to the greater proliferation of beneficial root endophytes like *P. indica* and other glomus species. The co-existence of all such favorable interactions might have created a congenial condition for the effective development and spread of the root system in the TOF + *P. indica* treatment. The highest root volume and the highest root surface area recorded in this treatment bear ample testimony to this fact (Fig 1c, 1d).

*Piriformospora indica*, a novel beneficial root endophyte can be cultivated on various synthetic media outside the host. Mass multiplication of *P.indica* on a suitable and cultivable media is very essential to make it available to the farmers as a promising biofertilizer. A combination of Farm Yard Manure (FYM) and coir pith was standardized as a sustainable, cheaper and easily propagated medium for the rapid growth of *P. indica* and for its mass multiplication [32]. At present research on endophytes mainly focuses on the standardization of medium for the mass multiplication of the same. Multiplication of *P.indica* and glomus species in the medium of TOF creates a synergistic effect on the promotion of adventitious root formation and other biometric parameters such as shoot length, plant height and shoot fresh weight. Application of TOF medium inoculated with these root endophytes, structures roots to suit the nutrient foraging and acquisition. Autoclaved coconut water (ACW) and potato dextrose broth (PDB) as a medium of growth for *P.indica* and the effect of these media on *P.indica* cultivation was tested in a tomato nursery by [33]. The plant height of tomato in a medium grown with *P.indica* in ACW and *P.indica* in PDB gave an average height of 8.39 cm. In comparison to our study, this height is 1.66 times lesser because the TOF is not included in the treatment.

Results of the nursery experiment showed that there were significant differences in plant growth parameters between the effects of thermochemical organic fertilizer inoculated with *P. indica* and Glomus species. Inoculation with *P. indica*, as well as Glomus in the medium of TOF, improved plant growth compared to the uninoculated soil medium. Among all plant growth parameters analyzed, the maximum values were obtained in plants grown in a medium of TOF+ *P.indica*. *P. indica* successfully colonized tomato rootlets in nutrient solution and also positively influenced the growth parameters of tomato similar to that of horticultural potting substrates or sand [34]. With respect to root fresh weight, there was no significant difference between treatments of TOF + *P.indica* and TOF + other Glomus species including *G. fasciculatum* and *G. mossaea*. TOF treated with *P. indica* and *G. fasciculatum* outperformed all the other combinations that were tried as best in the case of root dry weight.

#### 4. CONCLUSION

Beneficial root endophytes like *Piriformospora indica* and *Glomus sp.* grown in thermochemical organic fertilizer were found to have greater potential in promoting the growth of tomato plants in the

nursery. The high labile carbon content of TOF promotes the multiplication of such beneficial microorganisms thereby contributing positively to plant growth. The combination of TOF and endophytes enhanced the root fresh weight and root dry weight. TOF medium multiplied with *P.indica* has a significant positive influence on the growth attributes of tomato grown in nursery. The prospective potential of TOF in serving as a substrate for effective plant establishment, both in terms of belowground and aboveground portions of a solanaceous crop like tomato needs to be harnessed for sustainably attaining higher levels of productivity.

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