

Original Research Article

Beneficial effect of thermochemical organic fertilizer and root endophytic fungi on growth of tomato

ABSTRACT

Aim: To study the effect of thermochemical organic fertilizer (TOF) inoculated with beneficial root endophytes on the growth attributes of tomato grown in a nursery

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

Place and Duration of Study: This protrag experiment was conducted at the Department of Soil Science & Agricultural Chemistry, College of Agriculture, Vellayani 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 control treatment. Other treatments were TOF with additions of endophytic fungi like *Piriformosporaindica* (*P.indica*), *Glomus fasciculatum*, *Glomus musssaea* 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 on twenty eighth day after sowing.

Results: Inoculation with *P. indica* as well as *Glomus* in the medium of TOF improved the 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 *Piriformosporaindica* and *Glomus* grown in thermochemical organic fertiliser were found to have greater potential in promoting the growth of tomato plants in the nursery.

Keywords: Thermochemical organic fertilizer, *Piriformosporaindica*, *Glomus* species, Tomato nursery, root colonization

Comment [A1]: Include general objective at the beginning, Experimental design used, treatments (quantity and its repetitions), parameters evaluated, analyzes used and the most important results.

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Introduction:

Food security and healthy nutrition of citizens can be achieved only through 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 of wastes 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 tomato in a growing medium comprising of thermochemical organic fertilizer, coco peat and top soil significantly out yielded those grown in conventional growing media of top soil, sand and farmyard manure [3]. The efficiency of thermochemical organic fertilizer on 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 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 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 organism 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*, colonising within the roots of many crops asymptotically is well known to promote plant growth, elicit defence 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 is of great importance owing to the diverse root exudates and reduced carbon that

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Include results of other previous studies, in addition the general objective is not observed at the end of the introduction

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

Piriformosporaindica, belonging to the Sebaciales in the Basidiomycota was discovered in 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 micropropagated or tissuecultured 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 symbiosis relationship between plant-fungus has positive effects on its growth and development.

Knowledge on the impact of TOF on the root morphological and phenomic characters that nurture the rhizospheric microbial population of beneficial root endophyticfungi is very crucial to understand the depth of rhizospheric exploration and acquisition of essential nutrients. We hypothesised that there would be variability imparted bydifferent TOF –endophyticfungal combinations which enhanced soil nutrient acquisition, root to shoot translocation and biomass production in tomato seedlings

2. Materials and methods

2.1. Nursery production of tomato plants

Potting mixture for growing of the tomato seedlings was prepared by mixing top soil, TOF and coirpith in the ratio 1:1:1 on 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 of the cavities of the pro-tray. *Piriformosporaindica* mycelium was incorporated into the planting medium before filling the pro-tray cavities. For this, mycelium of the fungal endophyte grown in a 250 ml conical flask containing Potato Dextrose Broth medium was collected by filtering the contents of flask. Similarly inoculums of *Glomus fasciculatum*, *Glomus mossae* 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 in distilled water for three times.

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More procedurally describe the experiment setup process so that readers can replicate the experiment

parameters evaluated?

list the formulas used

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 thirtydays in the nursery.

2.2. Root colonization by endophytic fungi

The plants treated with endophytic fungi like *Piriformosporaindica* 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 roots. The root system was washed in running water to get rid of adhering soil particles. Roots were cut into small bits of one cm 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- tryphan blue solution it was examined under a compound bright field microscope. Presence of chlamydo spores 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 was measured using measuring scale. Plant height was measured as the length from the tip of main root to the apex leaf. Shoot height is observed as length from the base of shoot to the apex leaf. The dry weight was recorded after drying the plant samples for three days in 60 °C in a hot air oven. Root volume was estimated using Archimedes water replacement principle. Root tissue was submerged in 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 5% significance level were calculated for effecting comparison among the means. R-package grapesAgri1 was used for data analysis [21]. Treatments were T1: Soil + coirpith, 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 medium of TOF (Table 1). Among the treatments, T3 consisting of TOF+ *P.india* recorded highest values of plant height, shoot length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight [22]. 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

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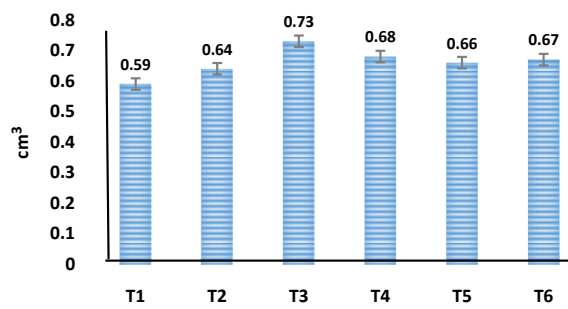
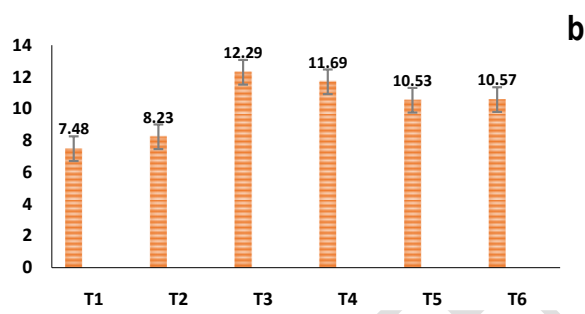
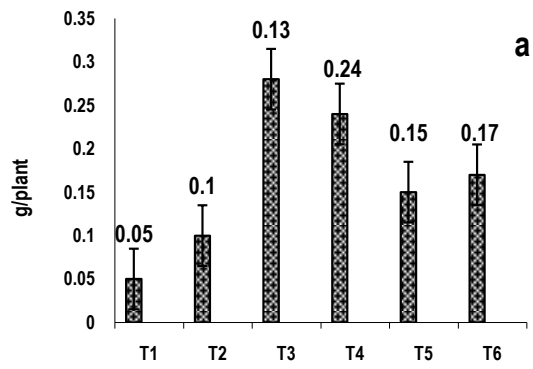
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were found by [23]. Treatments with glomus addition (T4,T5 and T6) resulted in the second best in case of plant height. All the glomus treatments were on par to each other. Addition of *Glomus fasciculatum* to TOF gave the second best treatment in 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. Fresh shoot weight of tomato seedlings is almost half than 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

(TOF:Thermochemical organic fertilizer)

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	8.35 ± 0.99 ^e	6.30 ± 0.81 ^d	0.51 ± 0.03 ^e	45.67 ± 0.83 ^f
TOF	10.54 ± 0.89 ^d	7.91 ± 0.33 ^c	0.55 ± 0.08 ^e	49.57 ± 1.00 ^e
TOF + <i>P. indica</i>	17.42 ± 0.64 ^a	13.95 ± 0.37 ^a	1.65 ± 0.12 ^a	88.41 ± 1.03 ^a
TOF+ GF	15.36 ± 0.68 ^b	11.61 ± 0.86 ^b	1.33 ± 0.11 ^b	72.54 ± 1.19 ^b
TOF+GM	12.58 ± 0.89 ^c	10.65 ± 0.85 ^b	0.84 ± 0.08 ^d	62.49 ± 0.95 ^c
TOF +Mixed glomus	13.76 ± 0.87 ^c	10.42 ± 0.81 ^b	1.1 ± 0.15 ^c	60.55 ± 0.90 ^d
SEm±	0.48	0.410	0.061	0.57
CD(0.05)	1.49	1.26	0.18	1.76



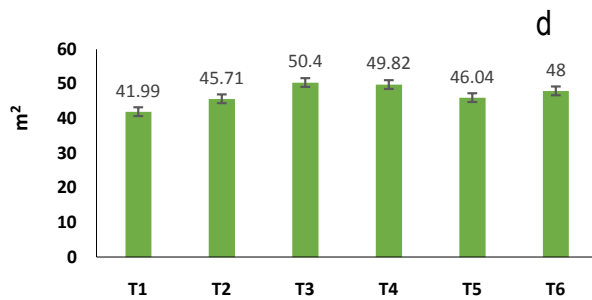


Fig. 1 Effect of different root endophytic fungal combinations with thermochemical organic fertilizer in growing media on (a) root fresh weight and (b) root dry weight (c) root volume (d) surface area

As endophytes play a key role in framing root phenomic characters, observations like root fresh weight and root dry weight is a necessity. In 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 of 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. indic* colonized tomato plants was up to 58% higher than non-inoculated plants. The roots of the *P. indic* inoculated plants were 54% longer than those of the non-inoculated plants. Similarly, results showed that shoot length of the inoculated plants increased by 41% compared to the control plants. Furthermore, the results revealed an increase in 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. indic* 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 is an effective repository to meet the energy demands promoting microbial diversity and proliferation [29]. The indirect effect of labile carbon fraction of TOF creates a suitable physical environment in the rhizosphere favoring soil respiration. The labile carbon fraction is strongly correlated as a vital driving force for the 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 favoured efficient nutrient mineralization, root nutrient acquisition, and translocation to functional metabolic sites for 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 leads to the greater proliferation of beneficial root endophytes like *P. indica* and other glomus species. The co-existence of all such favourable 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 bears ample testimony to this fact (Fig 1c, 1d).

Piriformosporaindica, 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 standardised 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 standardisation 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 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 effect of these media on *P.indica* cultivation was tested in a tomato nursery by [33]. 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 as it lacks TOF in their treatment.

Results of the nursery experiment showed that there were significant differences in plant growth parameters between the effects of thermochemical organic fertiliser inoculated with *P.indica* and Glomus species. Inoculation with *P. indica* as well as Glomus in the medium of TOF improved the 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 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 *Piriformosporaindica* and *Glomus* spp. grown in thermochemical organic fertiliser were found to have greater potential in promoting the growth of tomato plants in the nursery. The high labile carbon content of TOF promotes multiplication of such beneficial microorganisms thereby contributes positively to plant growth. Combination of TOF and endophytes enhanced the root

fresh weight and root dry weight. TOF medium multiplied with *P.indica* have 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 attaining higher levels of productivity in a sustainable manner.

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