

Comparative study of single culture, bacterial consortium, AMF, and compost manure for plant growth promotion and nutrient uptake of *Solanum lycopersicum var. cerasiforme* (Cherry tomato)

Rinkal K Mulani, Shah Rupal, Priyanka Patel, Meenu Saraf*

Department of Microbiology and Biotechnology, University School of Science, Gujarat University,
Ahmedabad- 380 009, Gujarat.
E-mail: mulanirinkal23@gmail.com

*Corresponding Author

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Abstract

The influence of organic amendment with compost manure, an Arbuscular mycorrhizal (AM) fungal, single culture and bacterial consortium inoculums on plant growth and nutrition value of cherry tomato was studied. A greenhouse experiment was conducted to compare the effects of single bacterial strain, bacterial consortium, AMF and compost manure on growth of Cherry tomato plant (*Solanum lycopersicum var. cerasiforme*). PGPR activity showed maximum phosphate solubilization (61mm) by B1 isolate and Maximum EPS production (49 mg/ml) by A2 isolate. The result revealed the biometric parameters shoot and root length (77.0cm, 25.6cm) whole plant fresh and dried weight (28gm, 19.9gm), chlorophyll A and B content of the leaves (5.1mg/ml, 6.0mg/ml) of whole plant were significantly enhanced by the consortium (*Pseudomonas + Bacillus subtilis*) treatment T10. The maximum N (0.123 ppm), P (0.71 ppm), K (4.8 ppm) and Na (0.72 ppm) were obtained under treatment T10, The maximum uptake of Fe (11.96 ppm),

Zn(4.75ppm), and Cu (0.71ppm) in cherry tomato was recorded by treatment T10 as compare to another treatment and control plant. This approach provided direct information about the application of inoculates, allowed the reduction of chemical inputs and positively influenced tomato quality.

Introduction

Cherry tomato (*Solanumlycopersicum L. var. cerasiforme*) is a cultivated variety of tomato. The variety is generally considered to be similar but not identical to the wild relatives of the domestic tomato. It has become more popular all over the world because of a good source of vitamins A and C, solids content, good taste and fruit set even at high temperature (Premaet *al.* 2011). These compounds have been shown to play a role in the prevention of diseases. Lycopene and other compounds (carotenes, lutein, and B-cryptoxanthin) in tomatoes reduce prostate cancer risk (Giovannucci et al. 1995), cardiovascular pathologies (Limpenset *al.* 2006) and they could be responsible for the lower risk of developing digestive tract pathologies (Franceschiet *al.* 1994). Since tomato plays an important role in human diet, its nutritional quality is of particular concern to consumers throughout the world (Chapagain&Wiesman 2004). The bacteria residing in the rhizosphere of plants and which bring about enhancement in growth and yield of crop plants are widely referred to as plant growth promoting rhizobacteria (PGPR). PGPR can mediate plant growth by different direct and indirect mechanisms (Glick 1995). Several microbial inoculants are used as biofertilizer, which improve the uptake of nutrients like nitrogen, phosphorus, potassium, sulphur, iron, zinc, copper etc. The current trend is the development of a consortium of PGPR which will offer multiple beneficial effects including growth promotion, nutrient value, yield enhancement and protection from diseases and pests. Understanding the interaction between consortium of microbial inoculants and plant systems will pave way to harness more benefits from microbial inoculants for improving plant growth and yield (Raja et al. 2006). Response of plants to colonization by mycorrhizal depends on many biotic and environmental factors. This trial aimed to study the interactions of VAM fungus with rhizosphere soils of cherry tomato plant and their effect, on plant growth, nutrient concentration, and biomass production. Dual inoculation of VAM and Bacterial consortium proved more effective in increasing the growth of different crop plants (Sreeramulu et al., 2002) Arbuscular mycorrhizal are the most common symbiotic association between plants (more than 80%) and soil fungi (Smith & Read 1997) of the Phylum Glomeromycota

(Shewfelt *et al.* 2001) Recent studies have demonstrated that AM fungi can also affect the volatile compounds produced in the leaves (Guerrieriet *al.* 2004) Soil characteristics are improved by the addition of compost manure (Jakobsen 1996; Roset *al.* 2003; Pagliaiet *al.* 2004). Composting has been defined as aerobic microbial activity leading to decomposition of most biodegradable materials, usually mixtures of organic materials, which results in organic residue stability (Valdrighi *et al.* 1996). The addition of compost to soil is considered an environmentally safe and agronomically advantageous process at acceptable operational costs (Dell'Abate *et al.* 2000). Compost manure has a positive effect on soil productivity and soil characteristics (Garcia *et al.* 1991) and its use is suitable mainly for its low risk of toxicity (Witter and Lopez Real 1998). The aim of this work was to evaluate the influence of comparative study of AM fungal-compost manure mixed bacterial inoculum on plant growth of tomato grown in greenhouse; we analyzed the effects on plant growth and morphology, and content of chlorophyll and nutrient value.

Materials and Methods

Sample collection

The five known cultures *Brevibacillus brevis* (A1), *Bacillus lichiniformis* (A2), *Acetobacter calcoaceticus* (A3), *Pseudomonas aeruginosa* (B1), *Bacillus subtilis* (B2) were kindly procured from department of microbiology and biotechnology, Gujarat University. A mycorrhizal fungus, *Glomus intraradices* was collected from BLOO MYCO science lab, Himatnagar, (Gujarat, India). The soil was obtained from a field which was cultivated with cotton, sited in Hanspuragm (Gujarat, India). The compost manure was acquired from animal house.

Compatibility test among bacterial strains

One strain was streaked vertically and other strains were streaked horizontally on nutrient agar media. The plates were incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 72 h (Fukui *et al.* 2010).

PGP Attributes of single culture and consortium

PGP characteristics have the potential to contribute to the development of sustainable agricultural systems. The potential phosphate solubilization of single culture and co-inoculated strain were tested on the solid medium Pikovskaya's medium according to the method of Gravel V

(1977). Exopolysaccharide (EPS) production was checked on liquid medium following method of Modiet al 1989.

Seeds sterilization and bacterilization

Cherry Tomato seeds (*S. lycopersicum* L. var), were collected from Sarthi agro center and were surface sterilized by gently shaking in a 3% H₂O₂ solution for 20 min. After 20 mins decant the H₂O₂ and the seeds with sterile distilled water 3 times. Decant all the distilled water and allow the seeds to dry in sterile clean room. Five single bacterial culture and two bacterial consortia were used for the pot experiment studies.

Pot study of *S. lycopersicum* L. var

The experiment aimed at compare the effects of different inoculants on cherry tomato. Cherry Tomato seed were sown in pots having soil added with single culture, Arbuscular mycorrhizal fungus and compost manure individually. Ten Treatments mycorrhizal (T1) (Mycorrhizal fungal inoculum consisted of a mixture of rhizospheric soil, spores, hypha, and mycorrhizal root fragment. A pot was filled with soil and water as per protocols), compost manure (T2), mycorrhizal +compost manure (T3) *Brevibacillus Brevis* (T4), *Bacillus lichiniiformis* (T5), *Acetobacter* (T6), *Pseudomonas aeruginosa* (T7), *Bacillus subtilis* (T8), *Brevibacillus Brevis* + *Bacillus* + *Acetobacter* (T9), *Pseudomonas* + *Bacillus subtilis* (T10), were performed.). Thirty grams of *Glomus intraradices* (39 spores/g soil) were added in to soil (T1 and T3). compost manure was added 45 gm/Kg of soil (T2 and T3) (sahniet al 2008). All plant treatments were maintained and seeds without treatment were sown in pot as control. All the experiment was cultivated for 3 months (Nov–Jan 2019) in a greenhouse sited in Gujarat University (Ahmedabad, India

Mycorrhizal colonization

1 cm-long piece were cut from each root system and fixed in 70% ethanol, and then stored at 4°C until mycorrhizal colonization analysis. Mycorrhizal colonization and arbuscule abundance were estimated according to Trouvelot et al. (1986). Root pieces were cleared for 30 min at 60°C in 10% KOH, stained with 1% methyl blue in lactic acid and mounted on a slide. The intensity of mycorrhizal colonization was evaluated microscopically.

Plant morphology and growth analysis

The following parameters were determined on 30, 60, and 90 days old plantlets: total plant length (cm), total plant fresh and dried weight (gm), leaf number, Chlorophyll content, mycorrhizal colonization, Arbuscular density and nutrient determination was done. After plant tissue ground, Nitrogen was analyzed using the standard kjelhalh method. The analysis of rest of the elements was carried out by digestion the samples in di-acid digestion mixture ($\text{HNO}_3:\text{HClO}_4$, 9:4). Phosphorus content was estimated as described by Taussky and shorr (1953). Potassium and Sodium content was determined by flame photometry. Micronutrient cations (Zn, Fe, and Cu) concentrations were measured by AAS (Bhargana and Raghupathi, 1993).

Results and Discussion

Bacterial consortium

Compatibility test result showed A1 A2 A3 cultures (CON-1) and B1 B2 cultures (CON-2) were compatible to each other. Absence of zone of inhibition indicates the strains were compatible to grow with one another. Son *et al* (2006) found that synergistic association among of *pseudomonas* and *Bacillus spp.* Combine or mixed inoculants that interact synergistically are currently popular as biofertilizer and bienhancer. Biochemical activities that may increase the beneficial attributes of their physiology (Bashan, 1998)

Characteristic of single culture and consortium for their PGP potential

The Maximum solubilization of P was achieved by *pseudomonas* (49mm) culture. In the mixed cultures, maximum P solubilization was achieved by CON-2 (*Pseudomonas + Bacillus subtilis*) (61mm). The results suggest that the process of P solubilization, which is governed by a complex group of mechanisms, is substantially affected by a mixed culture. The maximum EPS production on liquid medium was recorded in A2 (*Bacillus Lichiniformis*) culture (49mm). In the mixed culture, maximum EPS was proven by (CON-1). Increased EPS production has been reported with *Bacillus Lichiniformis* when grown in mixed culture under in vitro conditions (Janzen and others 1992).

Table: 1 Phosphate solubilization and EPS production by the single culture and consortium.

Culture	Phosphate solubilization(mm)	EPS production (mg /ml)
<i>BrevibacillusBrevis</i>	46	39
<i>Bacillus Lichiniformis</i>	42	49
<i>Acetobacter</i>	39	25
<i>Pseudomonas Aeruginosa</i>	49	35
<i>Bacillus subtilis</i>	30	20
CON-1	54	59
CON-2	61	48

Mycorrhizal colonization

After 3 month of growth, plants treated with mycorrhizal inoculum were colonized treatment T1 and T3 whereas no AM colonization was detected in control and other treatment roots.

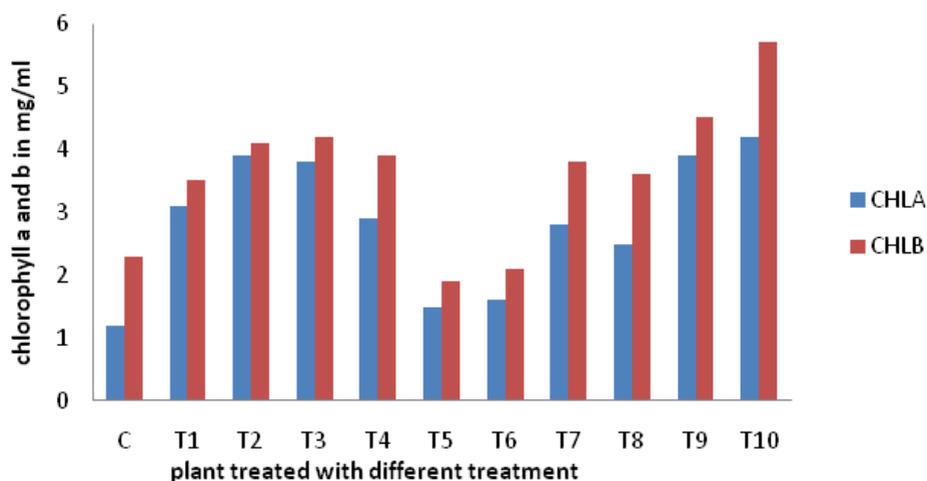
Plant growth parameter

After 3 months of growth, there were a number of differences between plants amended with mycorrhizal inoculums, single culture, bacterial consortium and compost Manure and control plants. Bacterial consortium inoculums (T10) showed significantly increases in leaf number (126), plants shoot length and root length (77cm and 25.6cm) and plant fresh and dried weight (28 gm and 19.9 gm) Table II Chlorophyll content was found in plant there was maximum chlorophyll content (Chl-A and Chl-B) was found in T10 (4.2 mg/ml and 5.7 mg/ml) at 3 month as comparative to other treatment. Outcome of green pigment showed in picture II. Similarly, the treatment with combination of *Pseudomonas + Bacillus spp.* increased the plant growth of tomato (Latha P, 2009) and chilli (Sundaramoorthy S, 2012) more than did individual strain were also reported.

Table: II. Influence of plant morphology soil with AM fungi, compost manure and single culture, bacterial consortium on growth of cherry tomato plant after 3 month of plantation.

Treatments	Shoot length(cm)	Root length(cm)	Fresh weight(gm)	Dried weight(gm)	Leaf Number
Control	27	9.0	06	3.3	33
T1	36	12.0	09	4.0	57
T2	42	14.0	12	5.5	60
T3	54	18.0	16	7.2	43
T4	39	13.0	10	4.5	60
T5	56	18.6	18	6.8	81
T6	45	15.0	15	7.5	69
T7	65	21.6	23	18	84
T8	58	19.3	18	9.5	69
T9	66	22.0	20	13.8	101
T10	77	25.6	28	19.9	126

Picture: II effect of combined application of different treatment on chlorophyll content on cherry tomato plant after 3months of plantation.



Nutrient determination

The analyzed data revealed that the maximum amount of uptake of N (0.123ppm), followed as P (0.71ppm), Na (0.72ppm), Fe(11.96ppm), Zn (4.75ppm), Cu (0.71ppm) by cherry tomato plant was recorded under treatment T10. A perusal of data showed that potassium uptake in cherry tomato plant revealed that the K (6.8 ppm), were found to be the maximum under treatment T7. Nutrient uptake in Cherry tomato plant perusal of data presented in table III. The results on the uptake of nutrient element like Nitrogen , Potassium, Phosphorus, Sodium uptake by cherry tomato plants are in line with those of Mamonova (1978) , Voogt (1993) , Yadav and Batra (1991) andSubbiah and Perumal (1986) , who also observed the nutrient uptake by cherry tomato plants increases with the increasing levels of fertilizer application. Similar results of micronutrientcation (Cu, Fe, Zn,) uptake in Cherry tomato were obtained by Nangliya (2014).

Table: III effect of level of nutrient uptake of cherry tomato plant after 3 month.

atm ents	N it rogen (ppm)	P hosp horus (ppm)	P otassium (ppm)	S odium (ppm)	Iron (ppm)	Zinc(ppm)	C opper (ppm)
ntrol	0.022	0.20	1.9	0.21	3.12	2.85	0.21
	0.080	0.46	6.2	0.48	9.21	3.65	0.32
	0.060	0.39	4.3	0.38	8.91	2.21	0.35
	0.085	0.48	6.1	0.49	4.75	3.55	0.69
	0.028	0.31	2.0	0.52	3.88	4.11	0.93
	0.057	0.38	2.8	0.61	5.33	4.12	0.36
	0.033	0.41	4.3	0.65	6.39	3.12	0.57
	0.091	0.60	6.8	0.71	9.96	2.11	0.61
	0.048	0.34	3.5	0.39	3.96	3.44	0.12
	0.099	0.67	4.5	0.60	4.98	4.24	0.44
	0.123	0.71	4.8	0.72	11.96	4.75	0.71



Picture: I comparative study of the vegetative structure of cherry tomato plant with different treatment.

Conclusion

The present investigation concluded consortium showed the best plant growth promotion and nutrient uptake in *Solanum lycopersicum var. cerasiforme* under pot study. The single culture and bacterial consortium have the ability to produce EPS, solubilized inorganic P. The maximum shoot length, root length, and higher value of plant fresh weight, plant dry weight, and higher value of

chlorophyll contain was showed in treatment T10 (*Pseudomonas Aeruginosa*+*Bacillus Subtilis*) followed by T9 (*BrevibacillusBrevis*, *Bacillus Lichiniformis*, *AcetinobacterCalcoaceticus*,) as compare to another treatment and control plant. Our experiment showed bacterial consortium could be best inoculum as compare to single strain, mycorrhizal and compost manure. Moreover it can be very effective to plant nutrient uptake and plant growth promotion of cherry tomato. The use of bacterial consortium is increasing in agriculture and may offer attractive mechanisms to replace synthetic chemicals and fertilizers.

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