Enhancing the sapota [*Manilkara achras* (Mill) Forsberg] yield through intervention of *Arbuscular mycorrhizal* fungi and its associated bacteria

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Abstract

Field experiments were carried out to study the effect of dual inoculation of Arbuscular Mycorrhizal (AM) fungi and its associated bacteria (Pseudomonas putida strain PAN4 – NCBI accession no. HM590707.1) to enhance the yield of sapota (var. DHS-2, Ten-year old sapota plants were supplied with the mixed culture of AM fungi (Funneliformis mosseae, Rhizophagus fasciculatus and Rhizophagus intraradices) with or without P. putida along with two levels of fertigation i.e. 100% recommended dose of fertilizers (RDF) and 75% RDF. AM fungal root colonization and spore proliferation, leaf nutrient concentration and yield were significantly higher with 75% RDF along with AM fungi and P. putida treated plants as compared to other treatments. Plants inoculated with 75% RDF along with AM fungi and P. putida recorded higher AM fungal colonization (45.7%) and AM fungal spore population (61.7%) as compared to 100% RDF. The leaf nutrient concentrations i.e. nitrogen, phosphorus, potash, calcium, magnesium, iron, manganese, zinc and copper increased by 17.4, 38.8, 20.5, 7.1, 10.3, 15.1, 16.6, 25.6 and 4.37% respectively compared to 100% RDF.

The pooled data of yield indicated that application of 75% RDF along with AM fungi and P. putida significantly increased the yield as compared to 100% RDF. It was evident that regular application of AM fungi and its associated bacterium i.e. P. putida with 75% RDF significantly improved the plant nutrient uptake, AM fungal root colonization, spore proliferation and yield in sapota under field conditions.

Keywords: Fertigation, AM fungi, mycorrhiza associated bacteria, dual inoculation, sapota, yield.

Introduction

Arbuscular mycorrhizal (AM) fungi is one of the important beneficial fungi that act as a liaising agent between plants and the surrounding soil[67]. It plays a major role in mobilization of nutrients particularly phosphorus (P)[37,102,104] as well other nutrients like Cu, Zn, from soil to plant through its symbiotic association[4,20]. The beneficial effects of AM fungi in plant growth promotion[64,69,70,98], nutrient mobilization[4,59,84], stress management[13,76,] and enhancement of crop yield[3,84] have been well documented in many crop plants[53].

It was reported that the AM fungal spores and hyphae provide sites for free living bacteria to thrive on their surfaces, which are involved in mycorrhizal establishment in host plants and it was defined as Mycorrhiza associated bacteria (MAB) by Garbaye[34] or mycorrhizal helper bacteria[2,32].These associated bacteria are reported to be involved in enhancing mycorrhizal colonization in host plants by different mechanisms viz., the secretion of volatile compounds[10,93], change in pH of rhizosphere[16], production of cell wall degrading enzymes[100], growth promoting substances etc[2]. The role of AM fungi and the benefits of its associated bacteria in terms of enhancement of mycorrhizal colonization, nutrients mobilization, antagonistic potential and growth promotion were clearly documented in guava and sapota seedlings.[6,9,69,70,75,81,92]

The AM fungal spores exudates contain carbohydrates, amino acids and some unidentified compounds and these will act as stimulants for the growth of mycorrhiza associated bacteria[14,107], but the association may vary from genus to genus. For example, the *Rhizophagus* spp., *Funneliformis* spp. and *Glomus* spp. spore surface are being associated with many beneficial bacteria viz., *P. putida, P. aeruginosa, Brevibacillus* sp, and *Bacillus subtilis. Streptomyces fradiae, S. avermitilis, S. cinnamonensis* and *Leifsonia poae*[70,92], *Paenibacillus brasiliensis*[6], *Rhizobium meliloti*[8] and *B. coagulans*[55]. The above findings clearly indicate the relationship between AMF and their associated bacteria which is of great importance for sustainable agriculture.[2,12,54,57]

There are many scientific evidences which proved that application of AM fungi with P solubilizing bacteria increased plant nitrogen (N) and P uptake as compared to un-inoculated plants (control).[7,46,68] The AM fungi inoculation in Kinnow mandarin was found to modify the rhizosphere favorably to improve soil N availability and the uptake by plant which resulted in better growth, fruit yield, and quality of Kinnow[99]. Many studies have clearly established the mycorrhizal association in improving zinc (Zn) uptake in several plant species[25,31,94]. The P solubilizing bacteria with AM fungi enhance P uptake in plants.[81,108] The *P. putida*

isolated from *F. mosseae* spores from sapota rhizosphere recorded significantly higher zinc phosphate solubilization (206.0 µg ml^{-1}) and zinc oxide (232.4 µg ml^{-1})[69].

Some of findings indicated that inoculation of AM fungi with *Azotobacter* spp. showed positive response on mango plant growth[78]. Similarly, the combined application of AM fungi improved the nutrient content and plant growth in mango[77,91] and pomegranate[80]. This observation indicated that application of AM fungi with its associated bacteria could be useful to alleviate nutrient deficiency in fruit crops.

Recently, a AM fungal package along with their associated bacteria to produce healthy, vigorous guava and sapota seedlings under nursery condition has been developed[69,70] but these effects have not been established under field conditions. In general, most of the scientific findings have evaluated the beneficial effects of AM fungi with plant growth-promoting rhizobacteria (PGPR) bacteria several crop plants[52,68], but very little information is available with its spore associated bacteria especially in perennial fruit crops like sapota. Keeping this in view, the present experiment was conducted to study the effect of AM fungi and its associated bacteria in enhancement of mycorrhizal colonization, nutrient uptake and yield of sapota under field conditions.

Material and Methods

Study site for field experiment: The field experiment was conducted on ten year old sapota (*var*. DHS-2) trees planted with a spacing of 7 m x 7 m in sandy loam soil at the Research Farm of ICAR-Indian Institute of Horticultural Research, Bengaluru (Karnataka, India) situated at 13° 58' N and 78°E at an altitude of 890 meters with an average precipitation of 850 mm annually. Initial soil samples were collected from the field and analyzed for its chemical properties. The soil was slightly acidic in reaction (pH 6.18) with 0.72%, 181.4, 20.6 and 239.2 kg ha^{-1} organic carbon, available nitrogen, phosphorus and potassium respectively. The initial soil micronutrient content was 1233.50, 429.30, 10.73, 10.95, 2.19 and 1.43 ppm of Ca, Mg, Mn, Fe, Zn and Cu respectively.

Inoculums preparation: A mixed inoculum of AM fungi i.e. 78-80 spores g^{-1} substrate (*F. mosseae, R. fasciculatus* and *R. intraradices*) was used throughout the experimentation. The AM fungi used in this experiment was isolated from sapota cropping system and selection of AM fungi and inoculums preparation has been discussed elaborately in our earlier publication[69]. The *P. putida* (Genbank accession number - HM590707) used in this experiment was isolated from the *F. mosseae* spore and the detail of selection of this bacterium was discussed in our earlier publication[69].

The selected bacterium was multiplied in nutrient broth and the inoculum was prepared by using sterilized lignite as carrier material, the inoculum containing 3.2 x 10^9 cfu g^{-1} carrier was used in this experiment. The substrate based 200 g AM fungal inoculum along with 100 g carrier based *P. putida* was applied.

Treatment details: Six treatments viz. T1 - un inoculated control, T2 - fertigation with 100% recommended dose of fertilizer (RDF), T3 - 100% RDF plus AM fungi, T4 - 75% RDF plus AM fungi, T5 - 75% RDF plus AM fungi and *P .putida*, T6 - 100% RDF plus AM fungi and *P. putida* were imposed and each treatment was replicated six times in a randomized block design. In each treatment, twelve plants were randomly selected and the treatments were imposed. The recommended dose of fertilizer was given (400g N:160g P:450g K $tree^{-1}$ $year^{-1}$) through fertigation and each plant received 20 kg of well decomposed farm yard manure (FYM) along with AM fungi and *P. putida*. Drip irrigation was scheduled on daily basis to replenish 80% of evaporation losses[43].

AM colonization, soil physicochemical properties, leaf nutrients and yield analysis in sapota: Rhizosphere soil and root samples were collected from each treatment after six month of imposing the treatments and analyzed for AM fungal colonization[73] and AM fungal spore population by adopting wet sieving and decantation method[38]. Soil pH and electrical conductivity (EC) were measured in a 1:2.5 (w/v) soil/water mixture. Organic carbon was analyzed by the wet oxidation method of Walkley and Black[103], available N was estimated using the method of Subbiah and Asija[89]. Available phosphorus by Bray's extractant - molybdophosphoric blue colour method[45], available K extracted in 1 M NH_4OAc[42] and exchangeable Ca and Mg by versenate (EDTA) titration method are used.[46]

The soil micronutrients viz. Fe, Mn, Zn and Cu were extracted by DTPA extractant and estimated by atomic absorption spectrophotometer[51]. The N content in the leaf samples was analyzed by Kjeldahl method[5]. Phosphorus, potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were estimated by tri-acid mixture (9:4:1 HNO_3: $HClO_4$: H_2SO_4) as given by Jackson[45]. Fruits were harvested from the individual tree at regular intervals and the cumulative yield was expressed as kg $tree^{-1}$. All the observations were recorded for two years and pooled data are presented.

Analysis: Percentage (%) of AM colonization and spore numbers were arcsine and square-root transformed respectively to ensure homogeneity of variance before analysis[39].

Results and Discussion

Effect of AM fungi and *P. putida* on mycorrhizal root colonization in sapota: AM fungal root colonization and spore proliferation in sapota rhizosphere were assessed for two years and the results are presented in figure 1 and figure 2 respectively. In general, AM root colonization and spore population were higher in AM fungi alone or AM fungi plus

P. putida inoculated treatments as compared to 100 % RDF fertilizers. In general, there was a gradual increase of AM fungal colonization in first and second year as compared to initial level of colonization.

Among the treatments, AM fungi plus *P. putida* enhanced 42.5 and 47.8 % higher AM fungal colonization in first and second year respectively compared to only 100 % RDF. The pooled analysis indicated that either 100% or 75% RDF with AM fungi plus *P. putida* recorded 13.1-15.7 % higher AM fungal colonization and 9.2-23.0% spore population as compared to only AM fungi application. This finding clearly indicated the role of *P. putida* in enhancing the mycorrhizal colonization.

It was also observed that there was 11.5% reduction of AM fungal colonization and 25.7% reduction of spore population in 100% RDF plus AM fungi treated plants as compared to 75% RDF plus AM fungi which indicated that higher dose of inorganic fertilizers application may affect the AM fungal colonization. The pooled data of AM fungal spore population in sapota rhizosphere indicated that application of *P. putida* along with AM fungi recorded 54.2-61.7% higher spore population as compared to only 100% RDF.

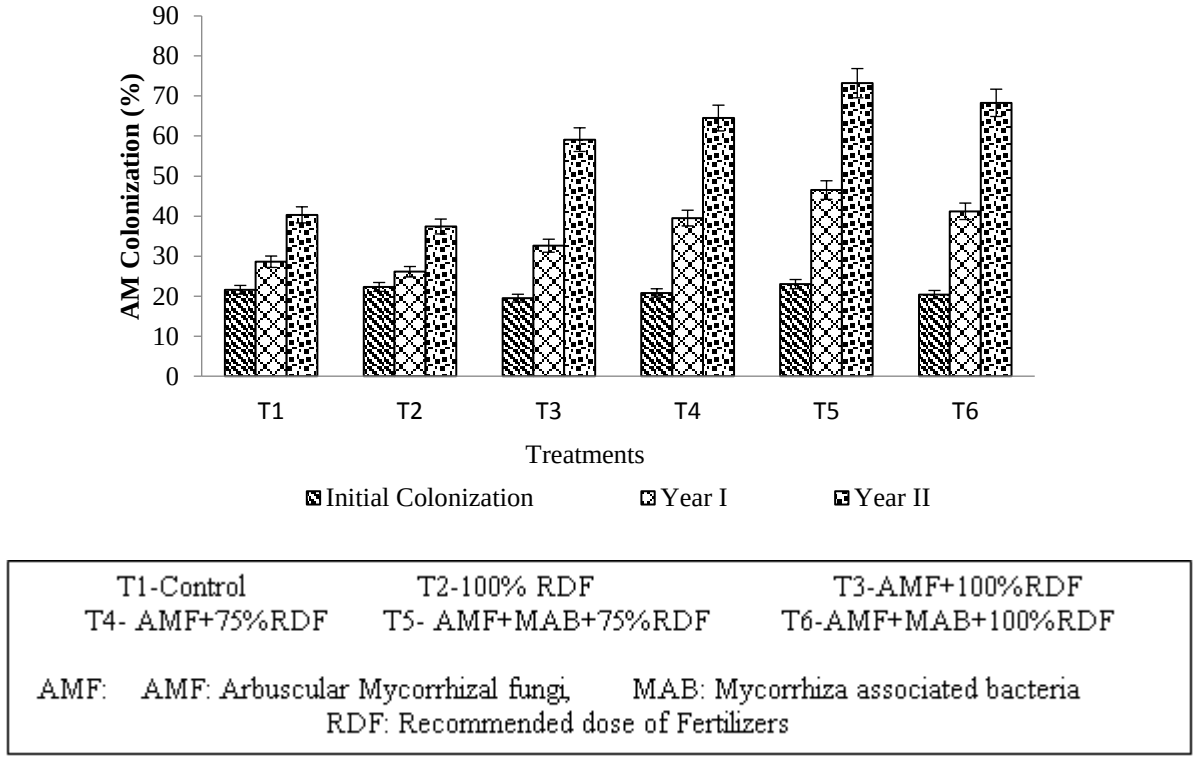

Figure 1: Effect of AM fungi and its associated bacteria on AM fungal colonization in sapota roots under field conditions

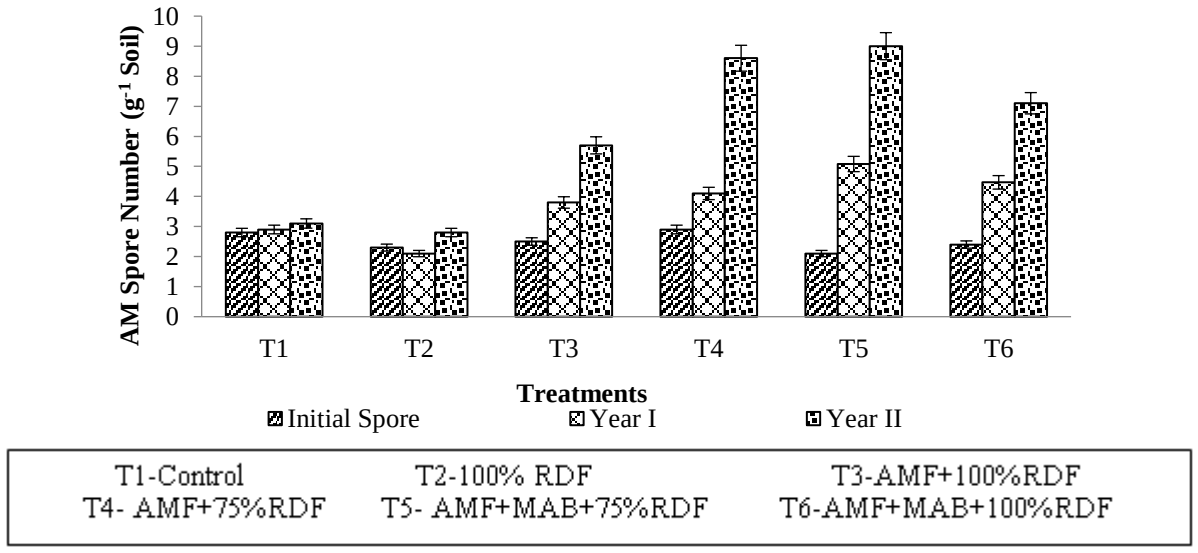

Figure 2: Effect of AM fungi and its associated bacteria on AM spore population in sapota rhizosphere under field conditions.

Mamatha et al[55] reported that dual inoculation of AM fungus with *B. coagulans* increased mycorrhizal root colonization by 46.0 % in papaya plants over control. Likewise, the co-inoculation of *Glomus mosseae* with fluorescent pseudomonad increased mycorrhizal colonization by 41.0 - 42.3% in tomato as compared to control[33]. Panneerselvam and his co-workers[70] have proved the concept in Guava seedlings: They have reported that application of *G. mosseae* with *P. putida* significantly increased the AM fungal colonization (86.2%) as compared to individual inoculants of *G. mosseae* or *P. putida* alone. In red bell pepper, inoculation of *G. mosseae* plus *P. flourescens* resulted in maximum mycorrhizal dependency[30,95].

Xavier and Germida[106] also reported that bacteria isolated from AM fungal spore cell walls were able to promote *G. clarum* spore germination, and similar observations have been recorded by many researchers[35,41,83]. The inoculation of AM fungi with *P. putida* or *P. validus* was observed to significantly increase AM fungal colonization and hyphae development[41].

Similarly, Vivas et al[101] reported that co-inoculation of *G. mosseae* with *B. brevis* increased AM colonization and the development of extra radical mycelium in plants.

The above findings clearly proved that application of AM fungi along with its associated bacteria or plant growth promoting rhizobacteria is essential for better mycorrhizal colonization in host plants. Many scientific findings proved the mechanism of mycorrhiza associated bacteria and their role in enhancing mycorrhizal colonization in host plants.

The secretion of hydrolytic enzymes[21] and metabolites[83] by some beneficial bacteria is involved in enhancement of AM fungal colonization in host plant. The other associated mechanisms for stimulation of mycorrhizal colonization in host roots include the change in pH and production of cell wall degrading enzymes by bacteria.[15]

In some of the experiments, co-inoculation of mycorrhiza with their associated bacteria was found to increase lateral root development and plant growth which was due to the production of auxins or auxin-related substances. This was observed to be the main mechanism for improvement of AM fungal colonization[34,101]. Few studies proved that the bacterial association is required for the proliferation of AM fungal spores. Mugnier and Mosse[60] observed that spore germination of *G. mosseae in-vitro* was possible only in the presence of their associated bacteria *S. orientalis*.

The *P. putida* used in this experiment was characterized by Panneerselvam et al[70] and has been clearly documented. The multi potential growth promoting attributes of this bacterium viz. enzyme, phytohormones, siderophore, HCN production, phosphate and zinc solubilization potential and these attributes might have also enhanced the mycorrhizal colonization in sapota field. They also reported that the mycorrhiza associated bacteria *P. putida* were found to be involved in the stimulation of AM fungal spore proliferation and its colonization in sapota rhizosphere.

The present findings indicated that inclusion of *P. putida* is essential when applying AM fungi for better colonization and sporulation in sapota under field condition.

Effect of AM fungi and *P. putida* on soil pH, EC and OC: The influence of AM fungi and its associated bacteria on pH, EC and OC content in sapota rhizosphere is presented in table 1. The results revealed that the pH ranged from 6.8 to 7.07 and 6.07 to 6.84 in the first and second year respectively, but there was no significant variation among the treatments. In all the treatments, there was a slight decrease in the pH after second year as compared to first year. Similarly, there was no significant variation in the EC among the treatments, but there was a gradual increase in the EC in all the treatments after second year as compared to first year.

The influence of AM fungi on OC content in sapota rhizosphere showed that there was a slight improvement in the OC (0.81-0.84%) in AM fungi alone or AM fungi plus *P. putida* inoculated treatments after first year compared to only 100% RDF treatment (0.72%). After second year, application of AM fungi plus *P. putida* recorded significantly higher OC (0.91-0.95%) as compared to 100% RDF plus AM fungi or 100% RDF alone treatment. The two years pooled data showed that inoculation of AM fungi plus *P. putida* enhanced 13.7-15.7% higher OC content in sapota rhizosphere than only inorganic fertilizers application.

Few reports have indicated that the pH of soils may affect AM fungal colonization in host plants. The optimum pH for colonization of *G. fasciculatum* in maize roots was found to be between 5.6 and 6.2[22,47]. Similarly, in subterranean clover roots, the colonization of *Glomus* sp. (WUM 16) was affected extensively at the highest pH level[1]. In the present investigation, there was a gradual decrease of pH (6.07-6.84) and increase of AM fungal colonization which was noticed in all the treatments after second year, this indicated that slight reduction of pH might be one of the factors for enhancing mycorrhizal colonization in sapota field. The increase in the EC in all the treatments after second year might be due to the regular application of fertilizers and quality of irrigation water.

In this experiment, the enhancement of soil organic carbon in sapota rhizosphere due to AM fungi intervention is one of the positive signals for soil health improvement. It was clearly demonstrated that in agricultural soils, fungi account for 70% of the microbial biomass[71] of which AM fungi may constitute up to 80-90% of the soil fungal biomass[63] and its association in crop plants was considered as one of the important components for C sequestration[86]. AM fungi may act as a large carbon sink and physically protect OC through improved soil aggregation[24,105].

Table 1
Effect of AM fungi and its associated bacteria on soil pH, EC and OC in sapota rhizosphere under field condition

Treatments	pH			EC (dsm^{-1})			OC (%)		
	Year I	Year II	Pooled	Year I	Year II	pooled	Year I	Year II	pooled
T1-Control	7.05	6.84	6.94	0.18	0.28	0.23	0.76	0.71	0.73
T2-100% RDF	6.86	6.31	6.58	0.15	0.30	0.23	0.72	0.78	0.75
T3-AMF+100%RDF	7.07	6.48	6.77	0.20	0.32	0.26	0.81	0.89	0.85
T4-AMF +75%RDF	6.95	6.27	6.61	0.19	0.30	0.24	0.83	0.87	0.85
T5-AMF+MAB+75%RDF	6.75	6.07	6.41	0.18	0.31	0.24	0.84	0.95	0.89
T6-AMF+MAB+100%RDF	6.80	6.38	6.59	0.20	0.31	0.25	0.84	0.91	0.87

AMF: Arbuscular Mycorrhizal fungi, MAB: Mycorrhiza associated bacteria, RDF: Recommended dose of Fertilizers.

Effect of AMF and *P. putida* on leaf nutrient concentrations in sapota: The effect of AM fungi plus *P. putida* on sapota leaf nutrient concentrations (Table 2) indicated that application of AM fungi with 100% or 75% RDF increased leaf nitrogen concentration (1.55-1.56% N) compared to 100% RDF application, but on par with each other. Among the treatments, AM fungi plus *P. putida* with 100% or 75% RDF recorded significantly higher leaf 'N' concentration (1.62-1.72%) compared to only 100% RDF treatment. The second year data on leaf P concentration showed that application of 75% RDF along with AM fungi plus *P. putida* significantly increased leaf P than all other treatments. The pooled data analysis of leaf 'P' indicated that plants treated with AM fungi plus *P. putida* recorded 25-33% higher leaf 'P' concentration compared to only inorganic fertilizers application.

Similar trend was noticed in case of leaf 'K' concentration. The leaf secondary nutrient concentrations *i.e.* Ca and Mg (Table 3) showed that the plants inoculated with AM fungi plus *P. putida* had significantly higher leaf 'Ca' content (1.63-1.89%) than other treatments after first year but this trend was not observed in second year.

Among the treatments, there was no significant variation in leaf 'Mg' after first year, but there was gradual improvement in second year irrespective of treatments. The pooled data indicated that AM fungi plus *P. putida* increased the leaf 'Mg' concentration (10%) compared to only 100% RDF treatment. The leaf micronutrient concentration showed that application of AM fungi plus *P. putida* with 100% or 75% RDF recorded significantly higher leaf 'Fe' (143.95-147.09 ppm), 'Mn' (27.72-27.27 ppm) and 'Zn'(23.89-25.74 ppm) content compared to all other treatments (Table 4). In general, application of AM fungi plus *P. putida* with 75% RDF enhanced leaf Fe, Mn and Zn concentrations. AM fungi or its combination with *P. putida* did not show any significant variation in leaf 'Cu" content.

In general, mycorrhizal association in plants accumulates P, K, Ca, Cu and Mn in the leaf in higher concentrations than non-mycorrhizal plants[48,79]. The AM fungal hyphae can absorb and mobilize the nutrients to plants even few centimeters away from root zone. Though the benefits of AM fungi are more common for immobile nutrients like phosphorus and zinc[46,74], which can also enhance the nitrogen (N) nutrition in host plant[56]. The AM fungal hyphae effectively acquire nitrate (NO_3^-) and ammonium (NH_4^+) from the external medium[9].

Many research findings have proved the beneficial effects of AM fungi in perennial fruit crops[49,61]. Application of AM fungi was found to increase the plant nutrients content in fruit crops[59,84]. Padma and Kandaswamy[66] reported 30.85% increase in the total P in papaya through application of 75% of recommended dose of P along with mixed inoculum (*G. mosseae* + *G. fasiculatum*+ *Gigaspora margarita*) as compared to un-inocualted plants (control). Sukhada[90] also reported a two fold increase in leaf P concentration in papaya inoculated with *G. mosseae* and *G. fasiculatum* than un-inoculated control. In apple, *G. mosseae* inoculation was found to enhance P uptake[84].

Some earlier findings also have clearly proved that application of AM fungi along with PGPR bacteria or its associated bacteria increased the plant nutrient uptake[3,27] but this information is limited in perennial fruit crops like sapota. In tomato, it was found that the inoculation of two bacterial cultures of *P. flourescens* 92rk and P190r along with *G. mosseae* BEG12[33] improved plant mineral nutrition. Xavier and Germida (2003)[106] reported that the bacteria associated with *G. clarum* NT4 spores identified as *B. pabuli* LA3 significantly enhanced shoot N content and P use efficiency in pea plants.

Apart from macronutrients, the mobilization of immobile elements such as zinc and P from the root depletion zone by AM fungi was reported by several workers[36,50,65,96]. In line with the earlier findings, application of AM fungi along with *P. putida* significantly enhanced leaf nutrient concentrations viz. N, P, K, Fe, Zn and Mn in sapota in the present investigation.

Effect of AMF and its MAB *P. putida* on sapota yield: The effect of AM fungi and their associated bacteria i.e. *P. putida* on improvement of sapota yield (Table 5) clearly indicated that 75% RDF with AM fungi and *P. putida* significantly increased sapota yield in the first year (54.83 kg $plant^{-1}$), second year (74.66 kg $plant^{-1}$) and also increased pooled yield (64.74 kg $plant^{-1}$) compared to control (21.15 kg plant-

^{1}), 100% RDF (45.47 kg plant^{-1}), AM fungi+100% RDF (43.68 kg plant^{-1}), AM fungi+75% RDF (41.81 kg plant^{-1}), and AM fungi+MAB+100% RDF (49.75 kg plant^{-1}). So, the pooled analysis of two year yield data revealed that 75% RDF with AM fungi and *P. putida* increased fruit yield as compared to only 100% RDF and control.

Table 2
Effect of AM fungi and its associated bacteria on leaf macronutrient concentration in sapota under field condition

Treatments	N (%)			P (%)			K (%)		
	Year I	Year II	*Pooled	Year I	Year II	*Pooled	Year I	Year II	*Pooled
T1-Control	1.08^{c}	1.23^{c}	1.15^{c}	0.12^{de}	0.10^{e}	0.11^{d}	0.81	0.83^{d}	0.82^{c}
T2-100% RDF	1.38^{b}	1.47^{b}	1.42^{b}	0.10^{e}	0.13^{d}	0.12^{d}	0.83	0.88^{cd}	0.85^{bc}
T3-AMF+100% RDF	1.51^{ab}	1.60^{ab}	1.55^{ab}	0.12^{cd}	0.16^{c}	0.14^{c}	0.88	1.01^{bc}	0.94^{ab}
T4-AMF +75% RDF	1.49^{ab}	1.63^{ab}	1.56^{ab}	0.14^{bc}	0.18^{b}	0.16^{b}	0.92	1.10^{ab}	1.01^{a}
T5-AMF+MAB+75% RDF	1.67^{a}	1.77^{a}	1.72^{a}	0.16^{a}	0.21^{a}	0.18^{a}	0.93	1.21^{a}	1.07^{a}
T6-AMF+MAB+100% RDF	1.57^{a}	1.67^{a}	1.62^{a}	0.14^{ab}	0.19^{b}	0.16^{b}	0.90	1.18^{a}	1.04^{a}

Table 3
Effect of AM fungi and its associated bacteria on leaf secondary nutrient concentration in sapota under field condition

Treatments	Ca (%)				Mg (%)		
	Year I	Year II	*Pooled		Year I	Year II	*Pooled
T1-Control	1.67^{b}	1.73	1.70		0.24	0.26^{d}	0.25^{c}
T2-100% RDF	1.65^{b}	1.71	1.68		0.24	0.28^{cd}	0.26^{bc}
T3-AMF+100%RDF	1.67^{b}	1.76	1.71		0.25	0.30^{bc}	0.28^{abc}
T4-AMF +75%RDF	1.54^{b}	1.62	1.58		0.25	0.35^{a}	0.30^{a}
T5-AMF+MAB+75%RDF	1.89^{a}	1.74	1.81		0.28	0.31^{abc}	0.29^{ab}
T6-AMF+MAB+100%RDF	1.63^{b}	1.59	1.61		0.27	0.33^{ab}	0.30^{a}

Table 4
Effect of AM fungi and its associated bacteria on leaf micronutrient concentration in sapota under field condition

Treatments	Fe (mg/kg)			Mn (mg/kg)			Zn (mg/kg)			Cu (mg/kg)		
	Year I	Year II	*Pooled	Year I	Year II	*Pooled	Year I	Year II	*Pooled	Year I	Year II	*Pooled
T1-Control	111.20	130.17^{c}	120.68^{c}	21.90	21.23^{e}	21.56^{c}	20.80^{bc}	19.32^{d}	20.06^{cd}	8.80^{a}	8.53	8.66
T2-100% RDF	113.73	135.80^{bc}	124.76^{bc}	22.93	22.52^{de}	22.72^{bc}	18.60^{c}	19.68^{cd}	19.14^{d}	7.95^{ab}	9.11	8.53
T3-AMF+100% RDF	121.39	153.93^{a}	137.66^{ab}	22.51	25.08^{cd}	23.79^{bc}	20.09^{bc}	23.30^{b}	21.69^{bc}	7.83^{ab}	8.38	8.11
T4-AMF +75% RDF	121.01	152.09^{ab}	136.55^{abc}	22.44	26.64^{bc}	24.54^{ab}	19.62^{bc}	22.27^{bc}	20.94^{cd}	7.23^{b}	8.56	7.89
T5-AMF+MAB+75% RDF	127.74	166.44^{a}	147.09^{a}	25.50	29.05^{ab}	27.27^{a}	23.30^{a}	28.19^{a}	25.74^{a}	8.70^{a}	9.14	8.92
T6-AMF+MAB+100% RDF	126.86	161.05^{a}	143.95^{a}	24.40	31.05^{a}	27.72^{a}	21.30^{ab}	26.49^{a}	23.89^{ab}	8.10^{ab}	8.23	8.16

Table 5
Effect of AM fungi and its associated bacteria on sapota yield under field condition

Treatments	Fruit Yield (kg/plant) (Year I)	Fruit Yield (kg/plant) (Year II)	*Pooled Yield (kg/plant)
T1-Control	18.58^{c}	23.72^{e}	21.15^{d}
T2-100% RDF	44.21^{b}	46.73^{c}	45.47^{bc}
T3-AM fungi+100% RDF	45.43^{b}	41.94^{cd}	43.68^{c}
T4- AM fungi+75% RDF	44.00^{b}	39.63^{d}	41.81^{c}
T5- AM fungi+MAB+75% RDF	54.83^{a}	74.66^{a}	64.74^{a}
T6-AM fungi+MAB+100% RDF	45.37^{b}	54.14^{b}	49.75^{b}

These findings clearly indicated that application of AM fungi along with its associated bacteria could enhance sapota yield, which might be due to the synergistic effects between bacteria and AM fungi. In this experiment, it was also observed that AM fungi with *P. putida* significantly increased AM fungal root colonization, spore population, leaf nutrient concentrations as compared to only 100% RDF treatments. Studies by Douds et al[29] in the strawberry plants (*Fragaria x ananassa* Duch. cv. Chandler) inoculated prior to planting with a mixed inoculum of different AM fungi species increased the yield by 17% over un-inoculated control.

Similarly, Bona et al[17] reported that co-inoculation of AM fungi with plant growth promoting bacteria in strawberry plants resulted in increased fruit production, larger fruit size as compared to un-inoculated plants. Two years application of AM fungi (*G. mosseae* or *G. faciculatumin*) in mango (*Mangifera indica* L.) on rootstocks of cv. Totapuri showed clear difference in growth and nutrient uptake (i.e. number of branches, leaf P, available soil P, Zn and Cu) than uninoculated control[58]. Thus, the present study has clearly showed that field experiment in sapota plants responded positively and effectively to the dual inoculation of efficient AM fungi along with its associated bacteria *i.e. P. putida*.

Conclusion

The present study has clearly demonstrated that field established sapota plants responded positively and effectively to the dual inoculation of AM fungi with its associated bacteria. Also the present study clearly proved that the combined application of AM fungi (*F. mosseae*, *R. fasciculatus* and *R. intraradices*) and *P. putida* with 75% RDF significantly improved the plant nutrient uptake, AM fungi sporulation, root colonization and yield in sapota under field conditions.

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