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Effect of Bio-fertilizers and Organic Amendments on Nutrient Uptake and Soil Microbial Population of Pummelo Seedlings (*Citrus maxima* L) under Nursery Condition

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A B S T R A C T

An experimental was carried out at Regional Horticultural Research and Extension Centre (RHREC), University of Horticultural sciences campus, GKVK, post Bangaluru-560065, during the year 2019 – 20). The nursery was established in potting mixture; sand, soil and farm yard manure in the ratio of 2:1:1 (Sand: Soil: FYM;) taken in polythene bags of size 12 cm x 24 cm. Applied with bio-fertilizers such as *Azospirillum* spp., Phosphorus solubilizing bacteria (PSB), *Pseudomonas flourescens* and Vesicular Arbuscular Mycorrhiza (VAM)@ 6 g per plant soil based bio-fertilizers. Organic amendments such as Panchagavya, Jeevamrutha, Beejamrutha and bio-digested liquid were tested at 5 and 10 per cent (applied through foliar application at 30 days interval). A total of nine treatments were tested in four replications. The results at the end of 120 days showed that the combination of four bio-fertilizers; *Azospirillum, Phosphorus solubilizing bacteria, Pseudomonas flourescens*and VAM had highest Nitrogen per plant (0.636 g) (T_5). However T_3treatment had maximum Phosphorus (0.055 g/plant) and Potassium (0.150 g/plant) with inoculation of *Azospirillum* + PSB+VAM. The maximum bacterial population (4.40 CFU X $10^4 g^{-1}$soil) was found in the treatment *Azospirillum* spp. + PSB +PF + VAM (T_5), followed by T_3 (3.75 CFU X $10^4 g^{-1}$soil). The highest fungal population (2.60 CFU X $10^4 g^{-1}$soil) was noticed in the treatment *Azospirillum* spp. + PSB) + VAM (T_2), followed by T_5 (2.55 CFU X $10^4 g^{-1}$soil), the minimum NPK and microbial population was recorded in control treatment at the end of 120 days in nursery.

Introduction

The Pummelo (Citrus *maxima or citrus grandis*) is the largest fruit among Citrus fruits. It has thickrind and locally it is known as Chakotha, Chakotara etc. In India this fruit is commercially grown on avery limited scale but the trees are found in North Eastern states, North Western Himalayan region, Eastern Uttar Pradesh, Bihar, Karnataka and Kerala. It is used in religious programmes in the country. Pummelo has good nutrition value. It is rich in potassium, fiber and calcium, and pummelo contain vitamin A, B and C and it is an excellent source of potassium. The pummelo are rich in thiamine (B1), riboflavin (B2) and niacin (B3).It is known to help in cases of fever, sore throat, pancreatic cancer,

insomnia, diabetes, fatigue, stomach and also contains some medicinal properties. Pummelo grow in Kerala and Karnataka. The mainly states such as Manipur, Tripura and Assam cultivate the pummelo up to 1,500 meters MSL. The major diseases of pummlo are crown rot, root rot, Scab, Powdery Mildew, gummosis and citrus greening. The major pests are scale, citrus leaf miner, aphids and soft green reported by (Sankar *et al.*, 2014).Efforts in development of pummelo plantation are directed at the availability of superior cultivars that produce adequate flowering to ensure high productivity and fruit quality. Superior cultivars can be obtained through germplasm selection, crossing and biotechnology utilization. Identification of differences among pummelo accessions can be seen based on the characteristics of the fruit, including size and shape of the fruit, seed number, color and texture of flavedo (epicarp), thickness and color of albedo (mesocarp), color and flavor fruit flesh, and aroma of essential oil. This research was done to observe growth and quality of the fruit of three pummelo accessions (Susanto *et al.,* 2018). Pummelo is a tropical citrus fruit. It is known for its large size fruit, with 15-25 cm in diameter and weighs around is 1-2 kg. The plant is spiny if propagated from seed or spineless if propagated vegetative. Its major producer is China followed by United States of America. India is in seventh position having an area of 12,400 ha, with a production of 2, 85,324 tons and productivity of 23.01 t/ha (Anonymous *et al.*, 2013). In India it is grown in far northeastern regions (such as Manipur and West Bengal), and some southern regions such as Karnataka (Bengaluru) and Kerala.

Application of bio-fertilizers helps to improve the soil fertility and crop productivity in several crops through atmospheric nitrogen-fixation, solubilizing insoluble phosphorus and other nutrients. It also improves the seed germination, root proliferation, synthesis of plant growth substances and suppresses the plant diseases (Verma *et al.,* 1993). *Azospirillum* is a non-symbiotic micro aerophilic bacterium commonly found in association with roots of horticultural crops. It has useful characters like high nitrogen fixation capacity and tolerance to high soil temperature. It is nitrogen in the range of 10 – 40 kg per hectare, and also in saving inputs of nitrogenous fertilizers by 20 – 30%. It is also well suited for plants raised through nursery. The phosphate solubilizes included bacteria or fungi but change insoluble form of phosphate to soluble form by producing organic acids. In general about 15 – 25% of insoluble phosphate can be solubilized. These fertilizers play important role in solubilizing insoluble phosphate. About 95 – 99 percent of the total soil phosphorus is insoluble which are indirectly available to the plants. Several soil bacteria; particularly *Pseudomonas* and *Bacillus* and fungi *Pencillium* and *Aspergillus* possess the ability to bring insoluble phosphate in soil into soluble forms by secreting organic acids such as acetic, formic, propionic, lactic, glycolic, numeric and succinic acids. Their inoculum is available in packets of 200 g similar to that of *Rhizobium*. They can be mixed with FYM and applied to soil **(**SankaraRao Karri *et al.,* 2012**).**

Materials and Methods

The experiment on influence of biofertilizers and organic amendments on nutrient uptake and soil microbial population of Pummelo seedlings (*Citrus maxim CV.* Devanahalli) under nursery condition was conducted in poly house at Regional Horticultural Research and Extension Centre, University of Horticultural sciences campus, GKVK, Bangalore 560065 during the period 2019-20.

Pummelo seeds were collected from pummelo plot of RHREC. Seedlings were raised in

polythene bags of (12 x 24) cm with 4 holes punched on the polythene bags from bottom to top to facilitate proper drainage. Then the seeds were sown into the media polybags containing mixtures of Soil, FYM and Sand (2:1:1) and treated with biofertilizers combinations such as *Azospirillum sp*, Phosphorus solubilizing bacteria (PSB), *Pseudomonas fluorescence (PF)* and Vesicular Arbuscular Mycorrhiza (VAM) @ 6 g per seedling (all together) per treatment, these polythene bags were kept under poly-house.at 30 days interval liquid organic manure spanchagavya, jeevamrutha, beejamrutha and bio digester @ 5, 10, 10 and 10 per cent, respectively were applied through foliar application. And observations were recorded at 120 days after germination.

The experiment included nine treatments as follows

T1 Water Spray (control)

T2 *Azospirillum* spp. + Phosphorus solubilizing bacteria (PSB) + *Pseudomonas fluorescens* (PF) (2 g each)

T3 *Azospirillum* spp. +Phosphorus solubilizing bacteria (PSB) + Vesicular Arbuscular Mycorrhiza (VAM) (2 g each)

T4 *Azospirillum* spp. + *Pseudomonas fluorescens*(PF)+Vesicular Arbuscular Mycorrhiza (VAM) (2 g each)

T5 *Azospirillum* spp. + Phosphorus solubilizing bacteria (PSB) + *Pseudomonas fluorescens* (PF)+*Vesicular Arbuscular Mycorrhiza* (VAM)(1.5 g each)

T6 Panchagavya Spray @ 5% after germination

T7 Jeevamrutha Spray @ 10% after germination

T8 Beejamrutha Spray @ 10% after germination

T9Biodigested liquid Spray @ 10% after germination

Results and Discussion

The results in Table 1 (Fig. 1) showed the highest nitrogen uptake (0.636 g) was recorded in the treatment *Azospirillum*, Phosphorus solubilizing bacteria (PSB), *Pseudomonas fluorescens* (PF) and Vesicular Arbuscular Mycorrhiza (VAM) it was on par the *Azospirillum*, Phosphorus solubilizing bacteria (PSB) and Vesicular Arbuscular Mycorrhiza (VAM)(0.575 g), whereas the maximum phosphorus uptake (0.055 g) and potassium uptake (0.150 g) per plant was found in the treatment *Azospirillum*, PF and VAM and it was on par with consortia that contained combinations of *Azospirillum*, *Pseudomonas fluorescens* (PF) and VAM. While the minimum nutrient uptake was recorded un-inoculated control.

The results in Table 2 (Fig. 2) indicates the maximum bacterial population (4.40 CFU X $10^4 g^{-1}$soil) was found in the treatment *Azospirillum* spp. + PSB +PF + VAM (T_5), followed by T_3 (3.75 CFU X $10^4 g^{-1}$soil). The highest fungal population (2.60 CFU X 10^4 g^{-1}soil) was noticed in the treatment *Azospirillum* spp. + PSB) + VAM (T_2), followed by T_5 (2.55 CFU X 10^4 g^{-1}soil) and minimum total microbial population was recorded in T_1 treatment control *i.e.*, water spray.

The evaluation of biofertilizers on nitrogen fixer, phosphate solubilizer, biocontrol agent and phosphorous mobilize in the form of *Azospirillum* spp., *Pseudomonas striata*, *Pseudomonas fluorescens* and VAM respectively had increased nutrient uptake of mango grafts seedlings var. Alphonso under

poly-house condition. Nutrient uptake was significantly superior reported by (Shankarappa *et al.,* 2018).

The effect of farmyard manure and biofertilizers on leaf N, P, K, Fe, Mn and Zn content of Eureka lemon trees. Leaf nitrogen content increased with the application of different treatments as compared to control. The maximum leaf nitrogen content was recorded with combination of 75% NPK + 27.5kg farmyard manure + *Azotobacter* 25g + *Azospirillum* 25g + *Bacillus circulans* 25g and followed by the 50 % NPK + 55 kg farmyard manure + *Bacillus circulans*25g and 75% NPK + 27.5kg farmyard manure + *Azotobacter* 25g. The significant effect of NPK in combination with farmyard manure and biofertilizers was noted on the nitrogen content of Eureka lemon leaves. Exhibited maximum phosphorus content without significant differences.

This may be due to presence of organic manures increased microorganisms population and organic acids which cause better availability of soil phosphorus and better plant uptake, with regard to potassium. This may be due to the balanced nutrients which encourage potassium uptake. These result agree with those obtained by (Sharaf *et al.,* 2011) and El-Sheikh, (2014) on Washington navel orange and lemon trees. These result could be attributed to *Azotobacter, Azospirillum, Bacillus megatherium* and *Bacillus circulans* by increasing leaf nitrogen, phosphorus and potassium content which indicated that the biofertilizers might have created certain microbial environment in the root rhizosphere zone for better uptake of NPK. Such conclusion was confirmed by (Srivastava *et al.,* 2002).

Table.1 Effect of bio-fertilizers and organic amendments on NPK uptake by plant of pummelo seedlings at 120 DAG

Treatments	N uptake (g^{-1} plant)	P uptake (g^{-1} plant)	K uptake (g^{-1} plant)
T_1. Water Spray (control)	0.325	0.029	**0.086**
T_2. *Azospirillum* spp. + Phosphorus solubilizing bacteria (PSB) + *Pseudomonas fluorescens* (PF) (2 g each)	0.395	0.039	**0.107**
T_3.*Azospirillum* spp. + Phosphorus solubilizing bacteria (PSB) + Vesicular Arbuscular Mycorrhiza (VAM)(2 g each)	0.575	0.055	**0.150**
T_4. *Azospirillum* spp. + *Pseudomonas fluorescens* (PF) + Vesicular Arbuscular Mycorrhiza (VAM)(2 g each)	0.519	0.053	**0.137**
T_5. *Azospirillum* spp. + Phosphorus solubilizing bacteria (PSB) + *Pseudomonas fluorescens* (PF) +PF + Vesicular ArbuscularMycorrhiza (VAM) (1.5 g each)	0.636	0.045	**0.134**
T_6. Panchagavya Spray @ 5% aftergermination	0.463	0.042	**0.112**
T_7. Jeevamrutha Spray @ 10% after germination	0.467	0.040	**0.123**
T_8. Beejamrutha Spray @ 10% after germination	0.484	0.042	**0.130**
T_9. Biodigested liquid Spray @ 10% after germination	0.407	0.038	**0.114**
SE.m±	0.009	0.001	**0.001**
CD at 5%	**0.027**	**0.003**	**0.004**

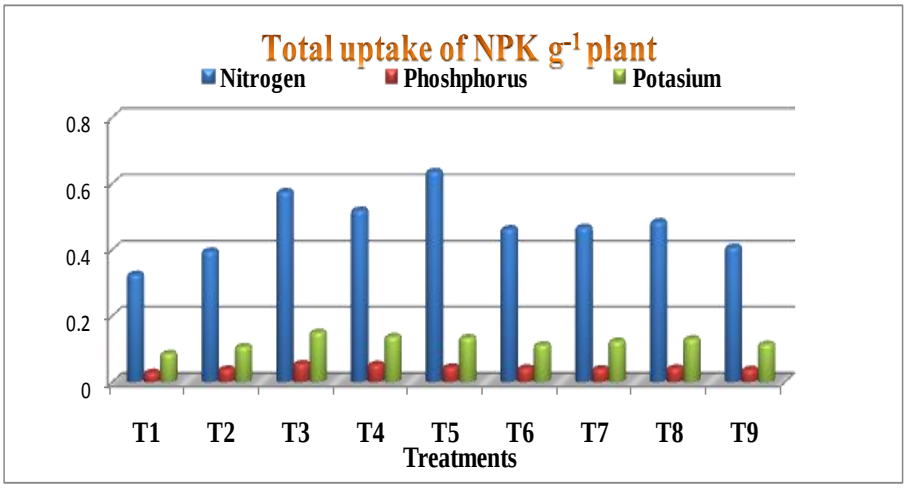
DAG.* Days after germination

Table.2 Effect of bio-fertilizers and organic amendments on soil microbial population at 120 DAG

Treatments	Total bacteria (CFU X $10^4 g^{-1}$ soil)	Total fungi count (CFU X $10^4 g^{-1}$ soil)
T_1. Water Spray (control)	2.27	**1.40**
T_2. *Azospirillum* spp. + Phosphorus solubilizing bacteria (PSB) + *Pseudomonas fluorescens* (PF) (2 g each)	3.07	**2.60**
T_3.*Azospirillum* spp. + Phosphorus solubilizing bacteria (PSB) + Vesicular Arbuscular Mycorrhiza (VAM)(2 g each)	3.75	**2.47**
T_4. *Azospirillum* spp. + *Pseudomonas fluorescens* (PF) + Vesicular ArbuscularMycorrhiza (VAM)(2 g each)	3.30	**2.42**
T_5. *Azospirillum* spp. + Phosphorus solubilizing bacteria (PSB) + *Pseudomonas fluorescens* (PF) +PF + Vesicular Arbuscular Mycorrhiza (VAM) (1.5 g each)	4.40	**2.55**
T_6. Panchagavya Spray @ 5% after germination	3.00	**2.40**
T_7. Jeevamrutha Spray @ 10% after germination	2.67	**2.05**
T_8. Beejamrutha Spray @ 10% after germination	3.05	**2.12**
T_9. Biodigested liquid Spray @ 10% after germination	2.75	**1.80**
SE.m±	0.18	**0.11**
CD at 5%	**0.54**	**0.33**

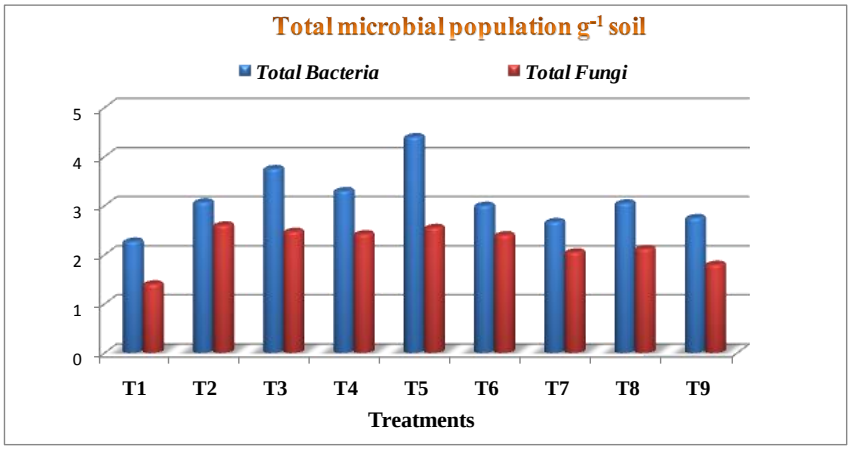
DAG.* Days after germination

Fig.1 Effect of bio-fertilizers and organic amendments on NPK uptake by plant of pummelo seedlings at 120 DAG



DAG.* Days after germination

Fig.2 Effect of bio-fertilizers and organic amendments on soil microbial population at 120 DAG



DAG.* Days after germination

As regard to micronutrients showed that the uptake of Fe and Mn were improved with the application of 75% NPK + 27.5kg farm yard manure + *Azotobacter*25g + *Azospirillum*25g + *Bacillus circulans*25g. While, the Zn content uptake was improved with the application of 50 % NPK + 55 kg farmyard manure + *Bacillus circulans*25g and 75% NPK + 27.5kg farmyard manure + *Azotobacter* 25g. These findings are in line with those of (Selvamani and Manivannan, 2009).

The inoculated pots had been colonized greatly with AM fungi (~50%), while much less root colonization with AM fungi (~10%) has been noted in the control plants. Addition of phosphorus increased sour orange shoot and root DM of both mycorrhizal and un-inoculated plants, although, the pattern of growth response were affected by Phosphorus levels. inoculation with AM fungi increased shoot DM at low and moderate phosphorus rates (15 and 45 mg P kg-¹), while, at high phosphorus rate (90 mg P kg-¹), the beneficial effect of AM fungi inoculation was low reported by (Al Karaki., 2002). Considering that potassium and phosphorus was applied at recommended rates, it is possible that their uptake was enhanced by nitrogen fertilizers which have been reported by mediate the uptake and utilization of potassium, phosphorus and other elements in plants (Brady *et al*., 1984).

The inoculated bacteria were able to persist in the rhizosphere of aonla. In all the treatments population of *Azotobacter*, phosphate solubilizing bacteria and *P.maltophilia* was higher than at the time of sowing. Highest population of *Azotobacter* (6.501 CFU/g), PSB (7.175 CFU/g) and *P.maltophilia* (7.761 CFU/g) was observed in vermicompost enriched *Azotobacter* + PSB + PM4 + AM fungi. These populations were significantly more than the control as well as other treatments reported by (NeetuKhare *et al.*, 2018).

The higher N uptake was observed in the treatment where *Azospirillum* was used with either 100 % or 75 % recommended dose of inorganic fertilizers. P uptake was higher in the treatments where PSB and VAM were used. Lin and Fox (1992) also reported increased P uptake in pot cultured banana in response to mycorrhizal application. In general, higher NPK uptake was observed in the treatments where combination of biofertilizers was applied with 100 % or 75 %

recommended dose of inorganic fertilizers. (Shaimaa, 2017) Reported that biofertilizers combined with mineral fertilizer had more positive effects than both microbial inoculants solely and non-inoculated control. Azotobacter + AM at 75% induced significant increment in growth criteria leaf N, P and K contents, biofertilizers only. Finally, a similar trend was observed regarding the total population of both microbial and Aozotobacter in the rhizosphere region of orange tree, where Azotobacter + AM at 75 and 50% NP stimulated the highest number of total microbial or Azotobacter counts and nitrogenizes activity. On the other hand, the colonization percentage of AMF and number of spores/g soil, as well as the enzyme activities of both dehydrogenase and phosphatase attained higher values from using Azotobacter + AM integrated with 50 and 25% NP application as compared to unioculated control and biofertilizers singly.

In conclusion the highest nitrogen uptake was recorded with combination of *Azospirillum* spp. + Phosphorus solubilizing bacteria (PSB) + *Pseudomonas flourescens* (PF) and VAM, however the maximum phosphorus and potassium uptake in the treatment *Azospirillum* spp. + *Pseudomonas flourescens* (PF) and VAM. Significantly higher bacterial population was found in the treatment *Azospirillum* spp. + PSB +PF +VAM, while the highest fungal population was noticed in the treatment *Azospirillum* spp. + PSB) + VAM at the end of 120 days in experiment.

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