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Impact assessment of growing media and bioinoculents on growth and bud take of rough lemon

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ABSTRACT

Efficacy of arbuscular microbial inoculations in relation to organic growing media on growth and bud take of citrus nursery were investigated in the current study. Three soil solarized organic growing media Soil, Soil + Farmyard manure and Soil + Vermicompost enriched with bioinoculants *Glomus mossae, Gigaspora* and *Acaulospora,* consortium of Arbuscular Mycorrhizal fungi (AMF) and uninoculated was kept as control. The combinational effect of soil + vermicompost enriched with consortium of AMF recorded the maximum seedling height (68 cm), diameter (6.81 mm), number of leaves (94.23) and plant biomass (58.82%). The root growth attributes exhibited maximum length and diameter of tap root (45.88 cm and 7.12 mm respectively) and number of secondary roots (50.02). Total microbial count was also recorded maximum with soil + vermicompost supplemented with consortium of AMF. Kinnow budded on rough lemon rootstock attained maximum budding success (88.73 %) with combinational application of soil + vermicompost fortified with Consortium of AMF. The same combination furthermore registered maximum length (20.04 cm) and diameter (8.73 mm) of sprout, number of leaves (14.61), leaf area (17.00 sq cm), N, P and K content in leaves and roots of Kinnow budlings. The highest N, P and K (1.51, 0.16 and 1.05 % respectively) in roots of budded plants was recorded in soil + vermicompost with consortium of AMF inoculation. The present study established the positive role of organic growing media and potent isolates of AMF on seedling growth and nutrient and bud take of rough lemon seedlings. Soil + vermicompost combined with consortium of AMF was found to be the most promising for raising quality nursery and maximum bud take of Kinnow on rough lemon.

***Key words*:** *Citrus jambhiri*, Kinnow, organic media, AMF, seedlings, bud take.

INTRODUCTION

Kinnow is the most cultivated fruit crop and rough lemon (*Citrus jambhiri* L.) is the most promising rootstock used commercially for Kinnow in central humid region of Punjab, as it provides deep root system with vigorous growth, large fruit size, precocious bearing, tolerance to drought, salinity, citrus tristeza virus, exocortis and xyloporosis viroid. The nursery raised in open field conditions is often found infected with nematodes, foot rot and noxious weeds. Containerized nursery offers the possible solution to these problems to a great extent. After all this is taken care of, the containerized nursery growing media has to be selected and managed efficiently as it directly affects the growth and development of root and shoot system of the nursery plants. Soils of Punjab generally have high pH, as a result the availability of nutrients such as phosphorus, zinc, iron and manganese to the plant gets reduced greatly, leading to poor growth of rough lemon seedlings which take more time to attain buddable size. Mineral fertilizers generally applied to replenish the nutrient needs of the nursery result in increased production cost and adverse impact on environment. The growing media of poor nutritional status, poor porosity and water holding capacity can be favourably altered with different organic materials. The organic nursery growing media maintain soil nutrient status through recycled waste material and offer a possible solution to minimize the use of chemical fertilizer. These promotegrowth of nursery plants by providing plant macro and micro nutrients and humic substances and improving the microbial biomass in plant rhizosphere.

Vermicompost has been quite effective alternative containerized organic growing media component in combination with soil for efficient raising of horticultural nursery plants. Vermicompost being porous, supplies concurrently sufficient levels of water and oxygen to the roots, stores nutrients efficiently for continued slow release and balances the biological, physical and chemical requirements of the plant for excellent homogenous growth of nursery plants (Atefe *et al.,* 1) and contributes positively in improving soil health (Goswami *et al.,* 5). Farmyard manure has been used as organic growing media since time immemorial because of its cheapness, easy availability, richness of nutrients, porosity and is used in nursery fruit plants as well as full grown orchards. Enrichment of organic growing media with bioinoculants is highly

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favoured for quick availability of nutrients in fruit nursery. The arbuscular mycorrhizal fungi (AMF) potentially forms the mutualistic symbiosis with the roots thus improving the nutrient uptake (Bona *et al*., 2). To develop efficient AM fungal inoculation system combined with organic nursery media in containerized nursery system, the present study has been chalked out keeping in view the potential of organic growing media Soil + Vermicompost and Soil + FYM in combination with potent isolates of AMF for strategizing the better establishment and growth performance of rough lemon seedlings and bud take of Kinnow nursery.

MATERIALS AND METHODS

The present experiment was carried out during the years 2018 and 2019 in the citrus nursery, at Department of Fruit Science, Punjab Agricultural University, Ludhiana, India. Geographical the location of experimental site lies at 29.3° N latitude and 76.5° E longitude at an altitude of 270 m above mean sea level. Three different organic growing media, viz. soil + FYM (2:1 v/v) and soil + vermicompost (2:1 v/v) were tested and soil as control. The organic growing media were pre-solarized by spreading in 30 cm thick layer on concrete floor and covered with transparent polythene sheet for 21 days to eliminate soil borne pathogens. The solarized media was then filled in 12" × 7" UV stabilized black polythene bags having 100 micron thickness.The cultures of different AMF bio-inoculants alone or in combinations (*Glomus mossae*, *Gigaspora* and *Acaulospora sp*. and consortium of AMF) were inoculated in these polythene bags at a depth of 10 cm @ 5 g/bag. Seeds extracted from rough lemon fruits from healthy single mother tree and sown in pre-inoculated bags in mid-September and placed in glasshouse for guarding the emerging seedlings from severe winter months. Due to nucellar polyembryony, more than one emerging seedlings were thinned out to keep one seedling per bag and shifted to a 50 % screen net house (40 mesh size) in first week of October.

A total of twelve treatment combinations were evaluated in the present study, viz., soil (control), soil +*G. mossae*, soil +*Gigaspora* and *Acaulospora sp*., soil + consortium of AMF, soil + FYM (2:1) (standard check), soil + FYM +*G. mossae*, soil + FYM +*Gigaspora* and *Acaulospora sp*., soil + FYM + consortium of AMF, Soil + vermicompost(2:1), Soil + vermicompost + *G. mossae*, Soil + vermicompost + *Gigaspora* and *Acaulospora sp*. and Soil + vermicompost + consortium of AMF. Each treatment was replicated thrice with 100 seedlings per replication. During the period of investigation, all the recommended cultural operations were uniformly followed in all the treatments.

The data on growth attributes was recorded periodically in rough lemon seedlings from germination till budding. The data on seed germination (%) was recorded after a month of sowing. The plant samples were prepared by washing thoroughly with running tap water and 0.1 N HCl. The root and shoot length (cm) was measured from soil surface to tip with a meter scale. Stem and tap root diameter of seedlings (mm) was measured with Vernier Calipers. The fully developed green leaves of seedlings were counted before budding starting from lower portion of the stem and the average was worked out for each date per replication for each treatment. The number of feeder roots per plant was counted and averages were worked out for each replication in the treatment. Plant biomass (%) was recorded by taking the fresh weight of the plant, keeping the same for oven drying at 65°C for 72 hours and calculating the per cent dry weight over fresh weight. On attaining the buddable stage (stem diameter ≥ 6.5 mm), inverted T budding was performed at the height of 22.5 cm above ground level on rough lemon seedlings using the bud wood procured from the virus free foundation block of Kinnow, maintained in College orchard in a stainless steel screen house of 400 µm mesh size.The budding success was determined after 60 days of budding by calculating the number of sprouted buds and expressed as percentage. Sprout length (cm) was measured with scale and sprout diameter (mm) of Kinnow mandarin on rough lemon was measured in millimeter with Vernier Calipers at a height of 22.5 cm above the ground level. Number of leaves of Kinnow plants on rough lemon was counted. Leaf area of Kinnow leaves was calculated using graph paper by marking the boundary of leaf and counting the squares inside the boundary. More than half squares were counted as one, while less than half squares were not counted.

For leaf sampling, third, fourth and fifth leaf per seedling were collected from the kinnow budling 60 days after budding from the terminal end at random. These were then pooled together and a composite sample was prepared. Cleaning, drying, grading and storage of samples were carried out according to the procedures followed by Srivastava (13). First the leaves were thoroughly washed in ordinary water, then with 0.1 N HCL solution and finally, sequential rinsing was done in two sets of distilled water to remove traces of acid, if any. The samples were dried in hot air oven at 65°C for 48 hours and ground finely to store in butter paper bags for analysis. Before analysis, the leaves were again oven dried for an hour to remove the moisture, if any. Total nitrogen in leaves was estimated by Kjeldahl's method by using K_2SO_4:

$CuSO_4$ (8:1) as catalyst mixture. The phosphorus was estimated by Vanodo Molybdate phosphoric yellow colour method and potassium was estimated by flame photometer method (Usha *et al.,* 14). Root samples were prepared in similar way as leaves and estimation of available N, P and K was done as per the described procedures.

The periodical microbial count in all organic growing media was noted for bacteria, actinomycetes and fungi at 90 days interval till budding. 10 g of each growing media was taken and 90 ml was added to make a dilution of 1/10 (10^{-1}). 1 ml of suspension was taken out of it and put into 9 ml water blank to get a dilution of 10^{-2}. This step was repeated until final dilution for bacteria (10^{-6}), actinomycetes (10^{-5}) and fungi (10^{-4}) was achieved. One ml aliquot was taken from each final dilution and transferred in separate petri plates. 12 ml of microbe specific media was poured in each of the plates and these plates were moved to allow the soil inoculum to get evenly distributed throughout the medium. Nutrient agar, potato dextrose agar and actinomycetes isolation agar were used for recording the bacterial, fungal and actinomycetes count, respectively. The poured media was allowed to solidify and incubated at 28°C for 1 week. At the end, average colony count was calculated as CFU (colony forming unit) per gram of soil by using following formula.

Population per gram of wet soil = X

Population per gram of dry soil = X×100/90

The pH of the growing media/constituent was determined as per the procedures suggested by Jackson (6), EC as suggested by Richards (10), OC, available soil nitrogen, phosphorus by and potash by Sharma *et al* (12). Soil and FYM had pH, EC, OC, N, P and K concentrations of 7.9, 0.13 dsm^{-1}, 0.25 %, 138.21 kg/ha, 24.2 kg/ha, 165.4 kg/ha and 5.33, 0.47 dsm^{-1}, 0.84 %, 0.52 %, 0.38 % and 0.26 %, respectively. The same constituents for vermicompost had values of 5.83, 0.49 dsm^{-1}, 0.49 %, 0.46 %, 0.33 % and 0.22 %.

The data was analyzed using Randomized Block Design with computer software SAS 9.3 and the mean comparison (LSD) at the 5% level of probability was worked out for knowing the treatment effects, where found significant.

RESULTS AND DISCUSSION

The data on seed germination and growth attributes of rough lemon seedlings are depicted in Table 1. Seed germination data in rough lemon revealed significant impact of organic growing media, AMF individually and in combination, registering maximum seed germination % in soil + vermicompost supplemented with consortium of AMF. Among all growing media tested, treatment combination of soil and vermicompost showed the highest mean seedling height (59.88 cm), diameter (6.41 mm), number of leaves (83.46) and plant biomass (56.35%), followed by soil and FYM, indicating about 69.34, 27.18,54.30 and 10.12 % improvement over control. The AMF consortium enumerated the highest mean seedling height (55.02 cm), diameter (6.15 mm), number of leaves (81.53) and plant biomass (56.10 %). The combinational effect of growing media and AMF showed the maximum plant height (68.00 cm), diameter (6.81 mm), number of leaves (94.23) and plant biomass (58.82 %) in soil + vermicompost + consortium of AMF (Table 2). The minimum shoot growth was observed for un-inoculated soil. Amongst the organic media, the maximum tap root length (41.38 cm), diameter (6.44 mm) and number of secondary roots (44.80) were registered for soil + vermicompost, followed by soil + FYM. Consortium of AMF recorded the maximum tap root length (38.05 cm), diameter (6.39 mm) and number of secondary roots (42.15) when compared with other bioinoculants. The combination of soil and vermicompost when enriched with consortium yielded the highest tap root length, diameter and number of secondary roots as compared to uninoculated soil, resulting in the enhancement of around 131.48, 62.19 and 96 % over uninoculated soil.

The physical and chemical properties of the rooting medium containing vermicompost improved the soil porosity and aeration leading to better root penetration (Knapp *et al.,*7). The AMF inoculation contributes positively towards beneficial synthesis of the hormones such as auxins, cytokinin and gibberellins leading to increased cell multiplication and cell division as observed by Damar *et al*. (3) in pomegranate. The significant enhancement in plant growth attributes with vermicompost and AMF might be due to modulation in soil structure, increased availability of nutrients and water retention capacity of the media, increment in microbial and enzymatic activities, increased mobilization and absorption of nutrients (Edwards *et al*., 4).

The microbial count (bacteria, actinomycetes and fungi) were significantly highest in soil + vermicompost with consortium of AMF fortification (Table 3, 4 and 5, respectively). The enhancement in microbial activity was observed from day 0 to 90, 180, 270 and 360, soil + vermicompost registering the maximum microbial count in all the intervals. At 360 days of inoculation, the highest average bacterial, actinomycetes and fungal count was obtained in soil + vermicompost (52.50 CFU/g × 10^6, 68.25 CFU/g × 10^5 and 49.50 CFU/g × 10^4, respectively), followed by soil + FYM media.

Table 1. Effect of organic growing media and AMF on seed germination and growth attributes of rough lemon seedlings.

Treatments	Rooting Media			
	Soil	Soil + FYM	Soil + Vermicompost	Mean
Seed germination (%)				
No bio inoculant	70.02^{h}	89.71^{d}	87.85^{e}	82.53 ± 3.15^{d}
Glomus mossae	81.11^{g}	92.58^{c}	91.39^{cd}	88.36 ± 1.84^{c}
Gigaspora and *Acaulospora*	82.72^{fg}	97^{ab}	95.27^{ab}	91.66 ± 2.27^{b}
Consortium of AMF	84.07^{f}	97.91^{a}	97.48^{a}	93.15 ± 2.29^{a}
Mean	79.48 ± 1.69^{c}	94.30 ± 1.04^{a}	93.00 ± 1.14^{b}	
LSD (p≤0.05)	Media (M) = 0.91 Treatment (T) = 1.05 M × T = 1.82			
Seedling height (cm)				
No bio inoculant	29.20^{f}	40.13^{d}	41.19^{d}	36.84 ± 1.96^{c}
Glomus mossae	38.02^{de}	54.03^{c}	65.23^{a}	52.43 ± 3.99^{b}
Gigaspora and *Acaulospora*	35.23^{e}	55.32^{bc}	65.10^{a}	51.88 ± 4.44^{b}
Consortium of AM fungi	39.00^{d}	58.06^{b}	68.00^{a}	55.02 ± 4.30^{a}
Mean	35.36 ± 1.20^{c}	51.89 ± 2.15	59.88 ± 3.32^{a}	
LSD (p≤0.05)	Media (M) = 1.92 Treatment (T) = 1.66 M × T = 3.32			
Seedling diameter (mm)				
No bio inoculant	4.91^{e}	5.61^{d}	6.00^{cd}	5.51 ± 0.18^{b}
Glomus mossae	4.99^{e}	6.24^{bc}	6.45^{abc}	5.89 ± 0.24^{a}
Gigaspora and *Acaulospora*	5.13^{e}	6.27^{bc}	6.38^{abc}	5.93 ± 0.22^{a}
Consortium of AM fungi	5.11^{e}	6.54^{ab}	6.81^{a}	6.15 ± 0.28^{a}
Mean	5.04 ± 0.06^{c}	6.17 ± 0.13^{b}	6.41 ± 0.11^{a}	
LSD (p≤0.05)	Media (M) = 0.23 Treatment (T) = 0.27 M × T = 0.47			
Number of leaves				
No bio inoculant	49.12^{g}	58.96^{cd}	66.69^{c}	58.26 ± 2.80^{c}
Glomus mossae	54.21^{de}	79.23^{b}	85.65^{ab}	73.03 ± 5.02^{b}
Gigaspora and *Acaulospora*	55.36^{de}	85.62^{ab}	87.28^{ab}	76.09 ± 5.42^{b}
Consortium of AM fungi	57.65^{de}	92.71^{a}	94.23^{a}	81.53 ± 6.20^{a}
Mean	54.09 ± 1.32^{c}	79.13 ± 4.03^{a}	83.46 ± 3.40^{a}	
LSD (p≤0.05)	Media (M) = 4.33 Treatment (T) = 5.00 M × T = 8.67			

Means with same letter are not significantly different at P≤0.05

It is evident from the results that plant growth promoting rhizobacteria act as a helping tool for AMF for proper establishment and efficient functioning of symbiosis resulting in better mobilization and absorption of macro and micronutrients (Reddy ***et al.,*** 9). These PGPRs may colonize the plant rhizosphere, root surface or superficial intercellular spaces. The results are in line with Ortas and Ustuner (8) who registered increment in growth and nutrient levels in sour orange with AMF inoculation and improved microbial colonization.

As evident from Table 6, with respect to growing media, the budding success recorded was maximum (84.52%) in seedlings raised in soil + vermicompostas compared to soil + FYM (81.61%) depicting an increment to the tune of approx. 20.90 and 16.74 % over soil. The highest budding success (88.73%) was observed in soil + vermicompost inoculated with consortium of AMF, recording an enhancement of 3.81 over soil + vermicompost + *Gigaspora* **and** *Acaulospora* and 28.19 % over control, respectively. Increased budding success with soil + vermicompost

Table 2. Effect of organic growing media and AMF on plaant biomass and root attributes of rough lemon seedlings.

Treatments	Rooting Media			
	Soil	Soil + FYM	Soil + Vermicompost	Mean
Plant biomass (%)				
No bio inoculant	47.28^{c}	54.13	54.99	52.13 ± 1.47^{a}
Glomus mossae	52.10^{bc}	55.47	56.48	54.68 ± 1.08^{ab}
Gigaspora and *Acaulospora*	52.25^{b}	55.88	55.12	54.42 ± 1.01^{ab}
Consortium of AMF	53.04^{b}	56.45	58.82	56.10 ± 1.21^{a}
Mean	51.17 ± 0.96^{b}	55.48 ± 0.78^{a}	56.35 ± 0.78^{a}	
LSD (p≤0.05)	Media (M) = 2.48 Treatment (T) = 2.86 M × T = 4.96			
Root length (cm)				
No bio inoculant	19.82^{f}	32.24^{d}	35.33^{cd}	29.13 ± 2.41^{c}
Glomus mossae	24.85^{e}	39.35^{b}	40.41^{b}	34.87 ± 2.57^{b}
Gigaspora and *Acaulospora*	23.74^{e}	38.23^{bc}	43.89^{a}	35.29 ± 3.05^{b}
Consortium of AM fungi	24.63^{e}	43.66^{a}	45.88^{a}	38.06 ± 3.42^{a}
Mean	23.26 ± 0.68^{c}	38.37 ± 1.32^{b}	41.38 ± 1.32^{a}	
LSD (p≤0.05)	Media (M) = 1.55 Treatment (T) = 1.79 M × T = 3.10			
Root diameter (mm)				
No bio inoculant	4.39^{e}	5.56^{cd}	5.71^{c}	5.22 ± 0.22^{c}
Glomus mossae	5.23^{d}	6.25^{b}	6.36^{b}	5.95 ± 0.19^{b}
Gigaspora and *Acaulospora*	5.44^{cd}	6.33^{b}	6.55^{b}	6.11 ± 0.18^{b}
Consortium of AM fungi	5.60^{cd}	6.45^{b}	7.12^{a}	6.39 ± 0.23^{a}
Mean	5.17 ± 0.15 ^{c}	6.15 ± 0.12 ^{b}	6.44^{a} ± 0.16	
LSD (p≤0.05)	Media (M) = 0.22 Treatment (T) = 0.23 M × T= 0.38			
Number of secondary roots				
No bio inoculant	25.52^{f}	35.20^{d}	40.08^{c}	33.60 ± 2.23^{c}
Glomus mossae	29.12^{ef}	42.96^{bc}	43.21^{bc}	38.43 ± 2.43^{b}
Gigaspora and *Acaulospora*	28.52^{ef}	43.85^{bc}	45.89^{b}	39.42 ± 2.83^{b}
Consortium of AM fungi	30.96^{e}	45.48^{b}	50.02^{a}	42.15 ± 2.98^{a}
Mean	28.53 ± 0.73^{b}	41.87 ± 1.36^{b}	44.80 ± 1.29^{a}	
LSD (p≤0.05)	Media (M) = 2.06 Treatment (T) = 2.38 M × T = 4.11			

Means with same letter are not significantly different at P≤0.05

along with consortium could be ascribed to the fact that AMF aided in better nutrient absorption particularly phosphorus in citrus hence increasing photosynthetic rate, which might be an indirect effect of RuBP carboxylase activity, leading to early bud sprouting and enhanced growth in inoculated seedlings (Usha *et al.,*14).

The combination of soil + vermicompost displayed highest mean sprout length (17.08 cm), leaf area (16.03 cm^2), number of leaves (11.78) and budling diameter (8.40mm) in Kinnow mandarin, while, among bioinoculants, consortium of AMF exhibited greatest mean sprout length (16.76 cm), leaf area (14.11 cm^2), number of leaves (10.47) and budling diameter (7.94 mm) followed by *Gigaspora* **and** *Acaulospora*. Soil + vermicompost supplemented with consortium offered maximum sprout length (20.04 cm), leaf area (17.00 cm^2), number of leaves (14.61) and budling diameter (8.73 mm) in Kinnow budlings.

The boosted growth of Kinnow mandarin budlings in terms of budding success, sprout length, number of leaves, leaf area and budling diameter were shown in soil + vermicompost. Vermicompost provides steady moisture to the budlings consistently which facilitates their higher photosynthesis and higher growth (Atefe *et al*.,1).

Table 3. Effect of growing media and bioinoculants on bacterial count (CFU/g × 10^6) at 0, 90, 180, 270 and 360 days after inoculation.

Treatments	Rooting Media			
	Soil	Soil + FYM	Soil + Vermicompost	Mean
0 Day				
No bio inoculant	9.00^{i}	21.00^{g}	32.00^{d}	20.67 ± 3.33^{d}
Glomus mossae	16.00^{h}	25.50^{f}	31.50^{d}	24.33 ± 2.27^{c}
Gigaspora and *Acaulospora*	24.00^{f}	24.00^{f}	38.00^{b}	31.00 ± 2.05^{b}
Consortium of AMF	28.00^{e}	34.00^{c}	45.00^{a}	35.67 ± 2.52^{a}
Mean	19.25 ± 2.21^{c}	27.88 ± 1.53^{b}	36.63 ± 1.68^{a}	
LSD (p≤0.05)	Media (M) = 0.95 Treatment (T) = 1.09 M × T = 1.89			
90 Days				
No bio inoculant	15.50^{g}	25.50^{e}	34.50^{c}	25.17 ± 2.75^{d}
Glomus mossae	22.50^{f}	29.50^{d}	35.00^{c}	29.00 ± 1.83^{c}
Gigaspora and *Acaulospora*	27.00^{e}	33.00^{c}	42.00^{b}	34.00 ± 2.20^{b}
Consortium of AM fungi	30.50^{d}	40.50^{b}	48.00^{a}	39.67 ± 2.56^{a}
Mean	23.88 ± 1.70^{c}	32.13 ± 1.69^{b}	39.88 ± 1.71^{a}	
LSD (p≤0.05)	Media (M) = 1.12 Treatment (T) = 1.29 M × T = 2			
180 DAYS				
No bio inoculant	19.50^{j}	27.50^{i}	36.50^{de}	27.83 ± 2.46^{d}
Glomus mossae	27.00^{i}	35.00^{ef}	38.00^{d}	33.33 ± 1.67^{c}
Gigaspora and *Acaulospora*	29.50^{h}	34.00^{f}	48.00^{b}	37.17 ± 2.80^{b}
Consortium of AM fungi	32.00^{g}	42.50^{c}	51.00^{a}	41.83 ± 2.77^{a}
Mean	27.00 ± 1.42^{c}	34.75 ± 1.62^{b}	43.38 ± 1.91^{a}	
LSD (p≤0.05)	Media (M) = 0.97 Treatment (T) = 1.12 M × T = 1.93			
270 Days				
No bio inoculant	23.00^{j}	33.00^{gh}	42.00^{d}	32.67 ± 2.76^{d}
Glomus mossae	29.50^{i}	39.50^{ef}	41.50^{de}	36.83 ± 1.88^{c}
Gigaspora and *Acaulospora*	31.00^{hi}	38.50^{f}	52.00^{b}	40.50 ± 3.09^{b}
Consortium of AM fungi	34.00^{g}	48.00^{c}	58.00^{a}	46.67 ± 3.50^{a}
Mean	29.38 ± 1.23^{c}	39.75 ± 1.64^{b}	48.38 ± 2.13^{a}	
LSD (p≤0.05)	Media (M) = 1.08 Treatment (T) = 1.25 M × T = 2.17			
360 Days				
No bio inoculant	25.50^{g}	35.50^{f}	45.50^{de}	35.50 ± 2.91^{d}
Glomus mossae	36.50^{f}	43.50^{e}	43.00^{e}	41.00 ± 1.21^{c}
Gigaspora and *Acaulospora*	36.00^{f}	47.00^{d}	56.00^{b}	46.33 ± 2.93^{b}
Consortium of AM fungi	44.50^{e}	52.50^{c}	65.50^{a}	54.17 ± 3.11^{a}
Mean	35.63 ± 2.06^{c}	44.63 ± 1.90^{b}	52.50 ± 2.74^{a}	
LSD (p≤0.05)	Media (M) = 1.46 Treatment (T) = 1.69 M × T = 2.93			

Means with same letter are not significantly different at P≤0.05

The nitrogen, phosphorus and potassium content was registered to be maximum (Table 7) in leaves of Kinnow budlings raised in soil + vermicompost media (2.18, 0.12 and 1.36 %), consortium of AMF (1.99, 0.12 and 1.23 %) individually as well as when grouped together (2.22, 0.13 and 1.52 %). The roots of plants raised in soil + vermicompost had significantly enhanced nitrogen concentration,

Table 4. Effect of growing media and bioinoculants on actinomycetes count (CFU/g × 10^5) at 0, 90, 180, 270 and 360 days after inoculation.

Treatments	Rooting Media			
	Soil	Soil + FYM	Soil + Vermicompost	Mean
0 Day				
No bio inoculant	21.00^{h}	34.00^{f}	44.50^{cd}	33.17 ± 3.41^{d}
Glomus mossae	28.00^{g}	38.50^{e}	44.00^{d}	36.83 ± 2.37^{c}
Gigaspora and *Acaulospora*	36.00^{f}	44.50^{cd}	50.50^{b}	43.67 ± 2.14^{b}
Consortium of AMF	40.00^{e}	46.50^{c}	57.50^{a}	48.00 ± 2.59^{a}
Mean	31.25 ± 2.22^{c}	40.88 ± 1.53^{b}	49.13 ± 1.70^{a}	
LSD (p≤0.05)	Media (M) = 1.22 Treatment (T) = 1.40 M × T = 2.43			
90 Days				
No bio inoculant	27.00^{i}	37.50^{g}	46.50^{cd}	37.00 ± 2.84^{d}
Glomus mossae	34.00^{h}	41.50^{ef}	48.00^{c}	41.17 ± 2.06^{c}
Gigaspora and *Acaulospora*	39.00^{fg}	45.00^{d}	53.50^{b}	45.83 ± 2.15^{b}
Consortium of AM fungi	42.00^{e}	52.50^{b}	59.50^{a}	51.33 ± 3.46^{a}
Mean	35.50 ± 1.74^{c}	44.13 ± 1.70^{b}	51.88 ± 1.60^{a}	
LSD (p≤0.05)	Media (M) = 1.34 Treatment (T) = 1.55 M × T = 2.69			
180 Days				
No bio inoculant	33.00^{h}	41.50^{g}	50.00^{cd}	41.50 ± 2.49^{d}
Glomus mossae	40.50^{g}	48.50^{cde}	51.50^{c}	46.83 ± 1.72^{c}
Gigaspora and *Acaulospora*	43.50^{fg}	48.00^{de}	61.50^{a}	51.00 ± 2.76^{b}
Consortium of AM fungi	45.50^{ef}	56.50^{b}	64.50^{a}	55.50 ± 2.82^{a}
Mean	40.63 ± 1.48^{c}	48.63 ± 1.67^{b}	56.88 ± 1.95a	
LSD (p≤0.05)	Media (M) = 1.59 Treatment (T) = 1.83 M × T = 3.17			
270 Days				
No bio inoculant	35.50^{h}	45.50^{fg}	55.00^{de}	45.33 ± 2.85^{d}
Glomus mossae	42.50^{g}	52.00^{e}	58.00^{cd}	50.83 ± 2.32^{c}
Gigaspora and *Acaulospora*	45.00^{fg}	52.00^{e}	64.50^{b}	53.83 ± 2.91^{b}
Consortium of AM fungi	47.00^{f}	60.50^{c}	70.50^{a}	59.33 ± 3.46^{a}
Mean	42.50 ± 1.36^{c}	52.50 ± 1.68^{b}	62.00 ± 1.89^{a}	
LSD (p≤0.05)	Media (M) = 1.69 Treatment (T) = 1.95 M × T = 3.37			
360 Days				
No bio inoculant	41.00^{f}	51.00^{e}	59.00^{d}	50.33 ± 2.65^{d}
Glomus mossae	52.00^{e}	59.00^{d}	61.00^{d}	57.33 ± 1.49^{c}
Gigaspora and *Acaulospora*	51.50^{e}	62.50^{d}	72.00^{b}	62.00 ± 3.03^{b}
Consortium of AM fungi	60.50^{d}	68.00^{c}	81.00^{a}	69.83 ± 3.08^{a}
Mean	51.25 ± 2.13^{c}	60.13 ± 1.93^{b}	68.25 ± 2.74^{a}	
LSD (p≤0.05)	Media (M) = 1.88 Treatment (T) = 2.17 M × T = 3.76			

Means with same letter are not significantly different at P≤0.05

recording 14.29 and 67.44% improvement over soil + FYM and soil, respectively. The maximum root nitrogen was witnessed in consortium of AMF, trailed by *Gigaspora* and *Acaulospora*. Overall, soil + vermicompost, supplemented with consortium of AMF registered highest root nitrogen content (1.51%). Similar results were obtained for phosphorus and potassium.

Table 5. Effect of growing media and bioinoculants on fungal count (CFU/g × 10^4) at 0, 90, 180, 270 and 360 days after inoculation.

Treatments	Rooting Media			
	Soil	Soil + FYM	Soil + Vermicompost	Mean
0 Day				
No bio inoculant	6.50^{j}	18.50^{h}	29.50^{cd}	18.17 ± 3.32^{d}
Glomus mossae	13.00^{i}	23.00^{f}	28.50^{d}	21.50 ± 2.28^{c}
Gigaspora and *Acaulospora*	21.00^{g}	28.50^{d}	35.00^{b}	28.17 ± 2.04^{b}
Consortium of AMF	25.50^{e}	31.00^{c}	42.00^{a}	32.83 ± 2.45^{a}
Mean	16.50 ± 2.20^{c}	25.25 ±1.48^{b}	33.75± 1.65^{a}	
LSD (p≤0.05)	Media (M) = 0.90 Treatment (T) = 1.04 M × T = 1.81			
90 Days				
No bio inoculant	11.50^{h}	22.00^{f}	30.50^{cd}	21.33 ± 2.75^{d}
Glomus mossae	18.50^{g}	25.50^{e}	31.50^{c}	25.17 ± 1.90^{c}
Gigaspora and *Acaulospora*	23.00^{f}	29.00^{d}	38.00^{b}	30.00 ± 2.20^{b}
Consortium of AM fungi	26.50^{e}	36.50^{b}	44.00^{a}	35.67 ± 2.56^{a}
Mean	19.88 ± 1.70^{c}	28.25 ± 1.64^{b}	36.00 ± 1.67^{a}	
LSD (p≤0.05)	Media (M) = 0.97 Treatment (T) = 1.12 M × T = 1.94			
180 Days				
No bio inoculant	18.00^{j}	26.00^{hi}	35.00^{de}	26.33 ± 2.47^{d}
Glomus mossae	25.50^{i}	33.50^{ef}	36.50^{d}	31.83 ± 1.67^{c}
Gigaspora and *Acaulospora*	28.00^{h}	32.50^{fg}	46.50^{b}	35.67 ± 2.81^{b}
Consortium of AM fungi	30.50^{g}	41.00^{c}	49.50^{a}	40.33^{a} ± 2.78^{a}
Mean	25.50 ± 1.43^{c}	33.25 ± 1.63^{b}	41.88 ± 1.92^{a}	
LSD (p≤0.05)	Media (M) = 1.11 Treatment (T) = 1.28 M × T = 2.21			
270 days				
No bio inoculant	21.00^{h}	31.00^{gh}	39.50^{de}	30.50 ± 2.39^{d}
Glomus mossae	27.50^{g}	37.50^{ef}	40.00^{d}	35.00 ± 1.94^{c}
Gigaspora and *Acaulospora*	29.00^{g}	36.50^{f}	50.00^{b}	38.50 ± 3.10^{b}
Consortium of AM fungi	32.00^{f}	46.00^{c}	56.00^{a}	44.67 ± 3.51^{a}
Mean	27.38 ± 1.23^{c}	37.75 ± 1.65^{b}	46.38 ± 2.13^{a}	
LSD (p≤0.05)	Media (M) = 1.17 Treatment (T) = 1.35 M × T = 2.33			
360 Days				
No bio inoculant	22.50^{h}	32.50	42.50	32.50 ± 2.90^{d}
Glomus mossae	33.50^{g}	40.50	40.00	38.00 ± 1.19^{c}
Gigaspora and *Acaulospora*	33.00^{g}	44.00	53.00	43.33 ± 2.92^{b}
Consortium of AM fungi	41.50^{ef}	49.50	62.50	51.17 ± 3.10^{a}
Mean	32.63 ± 2.05^{c}	41.63 ± 1.89^{b}	49.50 ± 2.73^{a}	
LSD (p≤0.05)	Media (M) = 1.30 Treatment (T) = 1.50 M × T = 2.60			

Means with same letter are not significantly different at P≤0.05

The outcomes evidently depict that vermicompost displayed hormone-like activity and boosted the root as well as plant growth and development. The inferences drawn clearly suggest vermicompost as a better growth medium for plant development and establishment. The enhanced activities of dehydrogenases, alkaline phosphatases and nitrogenases in rhizosphere, extension of fungal

Table 6. Effect of organic growing media and AMF on budded plant attributes (60 days after budding).

T-Budding success (%)				
	Rooting Media			
Treatments	Soil	Soil + FYM	Soil + Vermicompost	Mean
No bio inoculant	63.72^{g}	75.67^{de}	79.54^{cd}	72.98 ± 2.49^{c}
Glomus mossae	70.31^{f}	83.21^{bc}	84.33^{ab}	79.28 ± 2.39^{b}
Gigaspora and *Acaulospora*	71.55^{ef}	82.69^{bc}	85.47^{ab}	79.90 ± 2.27^{ab}
Consortium of AMF	74.04^{ef}	84.88^{ab}	88.73^{a}	82.55 ± 2.35^{a}
Mean	69.91 ± 1.29^{c}	81.61 ± 1.27^{b}	84.52 ± 1.23^{a}	
LSD (p≤0.05)	Media (M) = 2.36 Treatment (T) = 2.73 M × T = 4.73			
	Sprout length			
No bio inoculant	10.33^{h}	13.10^{fg}	15.37^{e}	12.67 ± 0.63^{d}
Glomus mossae	12.63^{g}	14.13^{e}	16.36^{c}	14.37 ± 0.56^{c}
Gigaspora and *Acaulospora*	12.43^{g}	15.04^{d}	17.33^{b}	14.93 ± 0.72^{b}
Consortium of AM fungi	13.97^{ef}	16.26^{c}	20.04^{a}	16.76 ± 0.90^{a}
Mean	12.34 ± 0.40^{c}	14.63 ± 0.37^{b}	17.08 ± 0.61^{a}	
LSD (p≤0.05)	Media (M) = 0.44 Treatment (T) = 0.51 M × T = 0.88			
	Number of leaves			
No bio inoculant	5.37^{h}	7.10^{e}	9.33^{d}	7.27 ± 0.58^{d}
Glomus mossae	6.00^{gh}	9.69^{d}	12.16^{b}	9.28 ± 0.90^{b}
Gigaspora and *Acaulospora*	5.65^{gh}	9.36^{d}	11.01^{c}	8.67 ± 0.79^{c}
Consortium of AM fungi	6.13^{g}	10.67^{c}	14.61^{a}	10.47 ± 1.23^{a}
Mean	5.79 ± 0.10^{c}	9.21 ± 0.40^{b}	11.78 ± 0.58^{a}	
LSD (p≤0.05)	Media (M) = 0.30 Treatment (T) = 0.35 M × T = 0.6			
	Leaf area			
No bio inoculant	9.67^{f}	11.61^{d}	16.63^{b}	11.97 ± 0.73^{c}
Glomus mossae	10.67^{e}	13.10^{c}	16.31^{a}	13.36 ± 0.83^{b}
Gigaspora and *Acaulospora*	11.17^{de}	13.67^{c}	16.19^{a}	13.68 ± 0.74^{ab}
Consortium of AM fungi	11.50^{de}	13.83^{bc}	17.00^{a}	14.11 ± 0.81^{a}
Mean	10.75 ± 0.23^{c}	13.05 ± 0.29^{b}	16.03 ± 0.30^{a}	
LSD (p≤0.05)	Media (M) = 0.43 Treatment (T) = 0.50 M × T = 0.87			
	Shoot diameter			
No bio inoculant	6.63^{e}	7.01^{e}	7.76^{d}	7.14 ± 0.18^{b}
Glomus mossae	6.74^{e}	7.80^{d}	8.43^{abc}	7.65 ± 0.26^{a}
Gigaspora and *Acaulospora*	6.67^{e}	8.15^{cd}	8.68^{ab}	7.83 ± 0.31^{a}
Consortium of AM fungi	6.90^{e}	8.18^{bcd}	8.73^{a}	7.94 ± 0.28^{a}
Mean	6.73 ± 0.07^{c}	7.79 ± 0.16^{b}	8.40 ± 0.14^{a}	
LSD (p≤0.05)	Media (M) = 0.26 Treatment (T) = 0.29 M × T = 0.51			

Means with same letter are not significantly different at P≤0.05

hyphae from the root surface into the soil might be chiefly responsible for these results, in turn, increasing the surface area and ultimately the nutrient uptake (Schnepf *et al.,*11). The AMF inoculated trifoliate orange seedlings depicted higher activities of functional, active as well as total hyphae under drought stress environments, stimulating enhanced absorption of phosphorus (Wu *et al.,*15).

The rhizosphere alteration by organic manure supplemented with AMF isolates aids in enhancing

Table 7. Effect of organic growing media and AMF on leaf and root N, P and K (60 days after budding).

Treatments	Rooting Media			
	Soil	Soil + FYM	Soil + Vermicompost	Mean
		Root N (%)		
No bio inoculant	0.56^{i}	1.2^{e}	1.35^{cd}	1.04 ± 0.12^{d}
Glomus mossae	0.79^{h}	1.20^{e}	1.41^{bc}	1.13 ± 0.09^{c}
Gigaspora **and** *Acaulospora*	1.00^{g}	1.28^{d}	1.47^{ab}	1.25 ± 0.06^{b}
Consortium of AMF	1.08^{f}	1.08^{c}	1.36^{a}	1.32 ± 0.07^{a}
Mean	1.59 ± 0.06^{c}	1.19 **± 0.02**^{b}	1.40 **± 0.02**^{a}	
LSD (p≤0.05)	Media (M) = 0.04 Treatment (T) = 0.04 M × T = 0.08			
		Root P (%)		
No bio inoculant	0.04^{h}	0.11^{ef}	0.12^{ef}	0.09 ± 0.01^{c}
Glomus mossae	0.1^{g}	0.12^{de}	0.15^{b}	0.12 ± 0.01^{b}
Gigaspora **and** *Acaulospora*	0.11^{f}	0.13^{c}	0.13^{cd}	0.12 ± 0.003^{b}
Consortium of AM fungi	0.12^{ef}	0.13^{cd}	0.16^{a}	0.14 ± 0.01^{a}
Mean	0.09 ± 0.01^{c}	0.12^{b}	0.14 **± 0.01**^{a}	
LSD (p≤0.05)	Media (M) = 0.01 Treatment (T) = 0.01 M × T = 0.01			
		Root K (%)		
No bio inoculant	0.52^{h}	0.63^{f}	0.85^{c}	0.67 ± 0.05^{c}
Glomus mossae	0.54^{gh}	0.77^{de}	0.9^{b}	0.74 ± 0.05^{b}
Gigaspora **and** *Acaulospora*	0.55^{gh}	0.74^{e}	0.91^{b}	0.73 ± 0.05^{b}
Consortium of AM fungi	0.57^{g}	0.8^{d}	1.05^{a}	0.81 ± 0.07^{a}
Mean	0.55 ± 0.01^{c}	0.74 **± 0.02**^{b}	0.93 **± 0.01**^{a}	
LSD (p≤0.05)	Media (M) = 0.02 Treatment (T) = 0.02 M × T = 0.04			
		Leaf N (%)		
No bio inoculant	1.28^{f}	2.01^{c}	2.13^{abc}	1.61 ± 0.13^{c}
Glomus mossae	1.37^{ef}	2.11^{abc}	2.16^{ab}	1.91 ± 0.12^{b}
Gigaspora **and** *Acaulospora*	1.41^{e}	2.08^{bc}	2.21^{a}	2.17 ± 0.10^{b}
Consortium of AM fungi	1.58^{d}	2.16^{ab}	2.22^{a}	2.49 ± 0.12^{a}
Mean	1.41 ± 0.03^{c}	2.09 **± 0.02**^{b}	2.18 **± 0.02**^{a}	
LSD (p≤0.05)	Media (M) = 0.06 Treatment (T) = 0.07 M × T = 0.12			
		Leaf P (%)		
No bio inoculant	0.07^{f}	0.10^{d}	0.11^{bc}	7.14 ± 0.003^{c}
Glomus mossae	0.09^{e}	0.11^{cd}	0.12^{b}	7.65 ± 0.004^{b}
Gigaspora **and** *Acaulospora*	0.10^{de}	0.11^{d}	0.12^{b}	7.83 ± 0.004^{b}
Consortium of AM fungi	0.11^{cd}	0.11^{b}	0.13^{a}	7.94 ± 0.01^{a}
Mean	0.09^{c}	0.11^{b}	0.12^{a}	
LSD (p≤0.05)	Media (M) = 0.004 Treatment (T) = 0.01 M × T = 0.01			
		Leaf K (%)		
No bio inoculant	0.64^{i}	1.15^{f}	1.19^{ef}	0.99 ± 0.09^{c}
Glomus mossae	0.75^{h}	1.24^{de}	1.38^{b}	1.12 ± 0.09^{b}
Gigaspora **and** *Acaulospora*	0.78^{h}	1.28^{cd}	1.34^{bc}	1.13 ± 0.09^{b}
Consortium of AM fungi	0.87^{g}	1.31^{bcd}	1.52^{a}	1.23 ± 0.09^{a}
Mean	0.76 ± 0.02^{c}	1.25 **± 0.02**^{b}	1.36 **± 0.03**^{a}	
LSD (p≤0.05)	Media (M) = 0.03 Treatment (T) = 0.04 M × T = 0.07			

Means with same letter are not significantly different at P≤0.05

the availability and acquirement of nutrients by rough lemon seedlings. The organic media soil + vermicompost (2:1) gave rise to augmentation of seedling growth as well as budding success of rough lemon seedlings. The maximum seedling growth and development was testified with consortium of AMF. The soil + vermicompost (2:1) growing medium enriched with consortium of AMF yielded the finest quality seedlings registering maximum height, diameter, leaf number, plant biomass, number of secondary roots, tap root length and diameter and microbial count. The bud take, sprout growth, leaf and root nutrient content recorded in Kinnow budlings was the maximum after two months of budding in the same organic media AMF combination. The current study validated that the amelioration of organic media with isolates of AMF substantiated the maximum significance for establishing, development and bud take of rough lemon nursery plants and is the worthwhile orchard enterprise for quality nursery production.

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