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Retrofitting microbes for inorganic nutrients in citrus nursery: new perspectives

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ABSTRACT

Nutritional health of citrus nursery is the foundation of quality production of mature citrus trees coupled with extended productive life. Retrofitting microbes for nutrient requirement is one of the novel approaches of not only ensuring good health of citrus nursery but cutting down the intensity of mortality during planting into new citrus field. We attempted to tailor the nutrient requirement of citrus nursery through the microbial consortium (*Aspergillus flavus,* MF113270; *Bacillus pseudomycoides,* MF113272; *Acinetobacter radioresistens,* MF113273; *Micrococcus yunnanensis,* MF113274; and *Paenibacillus alvei,* MF113275) developed through extensive isolation, characterization and value addition of different microbial inoculants. The progressive microbial response studies showed that the magnitude of response with microbial consortium outweighed the response of individual microbes with regard to large number parameters, comprising germination percentage, vigour index soil microbial population, changes in available pool of nutrients and leaf nutrient composition, without any additional supplement of inorganic fertilizers. Introducing further the mycorrhizal inoculants, biochars, microbes from rhizospheres of other fruit crops would facilitate towards much better rhizosphere resilience to be fitted in substrate for pre-evaluation of citrus, that could be easily extended to even grown-up orchards as well. With these efforts, we succeeded in retrofitting microbes in place of nutrients to be added from outside sources, synonymous to organic citrus nursery.

Key words: Biochars, Citrus, Plant growth, Leaf nutrients, Microbes, Microbial consortium, Nutrients, Rhizosphere manipulation, Seed vigour, Shelf-life, Soil fertility,

Recognition of the importance of soil microorganisms has led to an increased and thoroughly renewed interest in measuring the quantum of nutrients held in their biomass (Joseph *et al*., 2015). An increase in the microbial biomass often goes along with increased nutrient immobilization. Plant growth promoting microorganisms play an important role exerting various mechanisms such as biological nitrogen fixation, growth hormone production, phosphate solubilisation siderophore production, hydrolytic enzymes production, antagonistic activity, individually or collectively leading to improved nutrient use efficiency (Srivastava *et al*., 2015; Srivastava and Bora, 2023). These metabolites can be either overproduced or combined with appropriate biocontrol strains to obtain new formulations for their more effective applications. Studies have demonstrated that *Azotobacter* inoculation alone can substitute up to 50% nitrogen requirement of banana and 25% phosphorus requirement of papaya (Keditsu and Srivastava, 2014).

Microbes have also been reported to substantially improve nutrient acquisition capacity of host plant, and fruit yield in addition to enriching the rhizosphere biologically in a much activated form (Srivastava *et al*., 2022). Mineral fertilizers on the other hand have limited direct effects, but their application can enhance soil biological activity via increases in system productivity, crop residue return, and soil organic matter (Kohli *et al*., 1998; Malhotra and Srivastava, 2015). Another important indirect effect especially of nitrogen fertilization is the soil acidification, with considerable negative effects on soil organisms (Ngullie *et al*., 2015 ; Srivastava *et al*., 2008).

There are ample evidences accrued through worldwide research that nutrient-microbe synergy is the launching pad for any fruit crop to mobilize and accumulate the required nutrients as per the metabolic nutrient demand, a pre-requisite to improved nutrient-use-efficiency (Srivastava *et al*., 2015) . While reviewing molecular responses of pants to nutrient stresses, many genes play a central role in the acquisition and distribution of nutrients, including many protein-coding genes as well as microRNAs (miR395, miR398, miR397, and miR408) reported that higher tolerance to nutrient deficiency could be explained by better activation of their antioxidant system (Islam *et al*., 2022: Srivastava *et al*., 2022). However, for the other genotypes, tetraploidization did not induce greater tolerance to nutrient deficiency. Rengel *et al*. (1996) observed that the total number of bacterial colony-forming units increased in the rhizosphere of Zn-efficient genotypes of wheat under Zn-deficiency and in Mn-

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efficient genotypes under conditions of Mn-deficiency. In contrast, a Zn-deficiency treatment acted synergistically with the number of fluorescent *Pseudomonas* in the rhizospheres. Fruit crops have displayed an excellent synergy with a variety of microbes, which could play an important role in improving the use efficiency of applied nutrients ((Srivastava, 2013a ; 2013b ;Joseph *et al*., 2015).

A still bigger question emerges, whether rhizosphere competent microbes could collectively contribute toward improved resilience of plant's rhizosphere against potential nutrient mining. And if those microbes are so successful in promoting growth response, addition of starter nutrients in such combination may further magnify the magnitude of response called nutrient-microbe synergy (Srivastava and Ngullie, 2009 ; Srivastava *et al*., 2015) . Our earlier studies have shown that rhizosphere effective microbes have the tendency to play multiple roles to overcome various biotic and abiotic stresses while interacting with an environment (Dzuvichu *et al*., 2023) . Rhizosphere modification through roots by soil microorganisms exudation is an important attribute that regulates not only the availability of nutrients in the soil but also their acquisition by plants (Hota *et al*., 2020). A number of studies have suggested that whole range of microorganisms have helped to alleviate different abiotic stresses in citrus crop and aid in improving the use efficiency of applied nutrients. Ironically, no citrus crop systematic efforts in the past have been made to tailor the nutrient requirement of nursery plants through microbial interventions (Srivastava and Sharma, 2025). Since healthy nursery plants are pre-requisite to robust citrus industry of future , such issues are mandatory to be addressed.

Microbial Consortium, a Novel Concept

The most common objective of developing microbial consortium is to capitalize on both the capabilities of individual microbes and their interactions to create useful systems in tune with enhanced productivity, and soil health improvements through efficient metabolic functionality (Srivastava and Wu , 2012). Two major underlying principles are applied in the whole process of development of microbial consortium. The first one is resource ratio theory which uses both qualitatively and quantitatively to assess the outcomes between component microorganisms competing for shared limiting resources. This permits coexistence of multiple microbes or the competitive exclusion of all but a single microbe. And the second principle theory relevant to microbial consortium is maximum power principle initially proposed and later modified at various levels, is value for analyzing consortial interactions.

It also dictates that biological systems that maximize fitness by maximizing power, is analogous to metabolic rate or the capacity to capture and utilize energy (Kohi et al., 1998; Srivastava *et al*., 2002). The microbial consortium is classified as artificial (carrying two or more wild type microbes whose interactions do not typically occur naturally), synthetic (carrying microbes which are modified through manipulations of genetic content), and natural (carrying microbes having much wider applications like bioremediation, wastewater treatment, biogas synthesis etc.).

In the past, a number of studies have suggested the co-inoculation of different microbes as microbial consortium (referred as group of diverse microorganisms having ability to act together in a community and capitalize both the capabilities of individual microbes and their interactions with enhanced response on productivity and soil health improvements through efficient metabolic functionality) is more effective than single inoculation summarized as: *A. brasilense – P. striata/B. polymyxa, A. lipoferem – Agrobacterium radiobacter/A. lipoferem-Arthrobacter mysorens* (Srivastava *et al*., 2012) , *A. brasilense – Rhizobium, A. brasilense – A. chroococcum – Klebsiella pneumoniae – R. meliloti, A. brasilense – R. leguminosarum,* and A. brasilense/Streptomyces mutabilis – A. chroococcum.

Microbes involving AM fungi and bacteria have also been suggested for improvement in both yield and quality. These include: *A. brasilense – G. fasciculatum* in wheat, strawberry (Amor *et al*., 2008) and *A. brasilense – Pantoea dispersa* in sweet pepper, and *A. chroococcum – G. mosseae* in pomegranate (Aseri *et al*., 2008). Later, we at ICAR-Central Citrus Research institute at put forward a microbial consortium, *Aspergillus flavus* MF113270, *Bacillus pseudomycoides* MF113272, *Acinetobacter radioresistens* MF113273, *Micrococcus yunnanensis* MF113274, and *Paenibacillus alvei* MF113275 developed through isolation, characterization, and evaluation of effective microbes from citrus rhizospheres (Srivastava *et al*., 2019 : Srivastava and Sharma, 2025).

Response of Soil Microbial Inoculation in Acid Lime Nursery

The microbial response study was carried out over the acid lime seedlings at pre-evaluation stage (primary and secondary stages of nursery management) after its morphological and biochemical identification. In the experiment, the progressive response of multiple microbes of the microbial consortium was tested without addition of any inorganic fertilizers through soil inoculation, different microbes were inoculated into the soil (growing medium) on a month-old seedlings of acid lime.

Response of acid lime through soil inoculation: A nursery experiment was set up at CCRI Experimental Farm, Nagpur, to observe the progressive response of different microbes on germination rate of acid lime seeds and subsequent growth. Different treatments consisted of:T_1 (Control), T_2(*Ar, Acinetobacter radioresistens,* MF113273*),* T_3(*Ar, Acinetobacter radioresistens,* MF113273 + *My, Micrococcus yunnanensis* MF113274) , T_4(*Ar, Acinetobacter radioresistens,* MF113273) + *My, Micrococcus yunnanensis,* MF113274 + *Bp, Bacillus pseudomycoides,* MF113272),T_5 (*Ar, Acinetobacter radioresistens,* MF113273)+ *My, Micrococcus yunnanensis,* MF113274 +*Bp, Bacillus pseudomycoides,* MF113272) + *Pa, Paenibacillus alvei,* MF113275) and T_6 (*Ar, Acinetobacter radioresistens,* MF113273)+ *My, Micrococcus yunnanensis,* MF113274 + *Bp, Bacillus pseudomycoides,* MF113272) + *Pa, Paenibacillus alvei,* MF113275) +*Af, Aspergillus flavus,* MF113270) and replicated four times in a CRD experimental design. Microbial treatment as per treatment was applied to the soil over a month-old acid lime seedlings (100 mL) and after 8- days another 100 ml microbial treatment was applied as per the treatment. Response of these microbes was evaluated for changes in germination rate at every 10- days' interval (till 100 days), changes in available nutrient status of soil, leaf nutrient status and microbial status to quantify the magnitude of response with various treatments.

The microbes were observed inflicting response on both seed germination and seed viability index through synthesized microbial metabolites (Srivastava , 2012) . The significant response reported over the germination of acid lime seedlings at the various days of observation. The germination rate was reported as high as 79.8 % with treatmentT_6 at 100-days of observation with seed viability index of 3.20 followed by the treatment T_4, T_5, T_3, T_2 and T_1 respectively in a decreasing order (Table 1). The maximum rate of seed germination was reported within 30-days of observation amongst all the treatments. The seed germination percentage of the treatments T_4 and T_5 was on par with each other depicting the relatively similar response on the growth and development of the growing seedlings in response to added microbes.

Growth response in secondary nursery: Different growth parameters (Shoot parameters viz., shoot length, shoot weight, number of leaves, girth and plant and root parameters viz., root length and root weight) were recorded following the transfer of seedlings from primary nursery to secondary nursery. These growth parameters were significantly affected by treatments (Table 2). The shoot parameters observed higher with the treatment T_6 followed by the treatment T_5, T_4, T_3, T_2 and then control in a decreasing order. The shoot length of the treatments T_4, T_5 and T_6 was on par with each other However, root length and root weight was almost statistically on par with all the treatments, except control, indicating an active response on the root density of the seedlings under the respective treatments (Figs. 1 and 2).

Soil fertility changes: The soil properties like pH and EC were not influenced by any of the microbial inoculation treatments. While organic carbon showed some distinctive changes, which increased from a minimum of 0.10 g kg^{-1} with treatment T_1 to maximum of 0.50 g kg^{-1} with treatment T_6 within120 days of experiment. However, with available soil nutrients the macronutrients like $KMnO_4$-N, Olsen-P, NH_4OAc-K as well as micronutrients like DTPA-Fe, DTPA-Mn, DTPA-Cu and DTPA-Zn reported significant changes suggesting the fact that these microbes bring about the changes in available pool of micronutrients as well as their secondary function. As compared to the control or treatment T_1, maximum increase in the $KMnO_4$-N was observed with treatment T_4, Olsen – P with treatment

Table 1: Changes in germination percentage of acid lime seeds in response to different treatments involving various microbial inoculants

Treatment	Changes in germination percentage (days)										Seed viability index
	10	20	30	40	50	60	70	80	90	100	
T_1 (Control)	15.3	20.1	32.5	39.4	39.4	39.4	39.4	39.4	39.4	39.4	1.05
T_2 (*Ar*)	12.5	18.2	35.6	40.5	40.5	40.5	40.5	40.5	40.5	40.5	1.13
T_3 (*Ar+My*)	17.3	21.3	46.2	50.2	50.2	50.2	50.2	50.2	50.2	50.2	1.79
T_4 (Ar+*My* +*Bp*)	13.2	19.2	62.5	65.5	65.5	65.5	65.5	65.5	65.5	65.5	2.39
T_5 (*Ar+My +Bp+Pa*)	14.3	20.9	70.3	70.3	70.3	70.3	70.3	70.3	70.3	70.3	2.67
T_6(*Ar+My+Bp+Pa +Af*)	15.7	23.7	79.8	79.8	79.8	79.8	79.8	79.8	79.8	79.8	3.20
CD(*P=0.05*)	NS	1.8	2.8	6.1	5.3	5.4	5.9	5.8	6.1	6.4	-

Ar,My, Bp, Pa, Af, stand for *Acinetobacter radioresistens* (MF113273), *Micrococcus yunnanensis* (MF113274) *Bacillus pseudomycoides* (MF113272), *Paenibacillus alvei* (MF113275) and *Aspergillus flavus* (MF113270), respectively.

Note: Seed viability index was calculated at 100 days of germination as Germination percentage x average seedling length (mm)/100

Table 2: Growth response of acid lime seedlings in response to different microbial inoculations (period: 120- days period)

Treatments	Shoot parameters			Root parameters		
	Shoot length(cm)	Shoot weight(g)	No. of leaves/ plant	Girth (mm)	Root length (cm)	Root Weight (g)
T_1 (Control)	16.9	1.70	17	1.60	9.8	0.36
T_2 (*Ar*)	17.5	2.23	22	1.79	10.6	0.42
T_3 (*Ar+My*)	18.9	2.90	26	2.30	16.9	0.53
T_4 (Ar*+My +Bp*)	21.0	3.60	32	2.92	17.0	0.75
T_5 (*Ar+My +Bp+Pa*)	21.8	3.09	30	2.80	16.0	0.66
T_6 (*Ac+Pf +Bm+*Pa+*Af*)	22.7	3.72	34	2.75	17.5	0.79
CD(*P=0.05*)	0.40	0.23	03	0.10	0.72	0.04

Ar,My, Bp, Pa, Af, stand for *Acinetobacter radioresistens* (MF113273), *Micrococcus yunnanensis* (MF113274) *Bacillus pseudomycoides* (MF113272), *Paenibacillus alvei* (MF113275) and *Aspergillus flavus* (MF113270), respectively.

Fig 1: Response of microbial consortium in acid lime (clone NRCC-AL-8) on a commercial-scale use

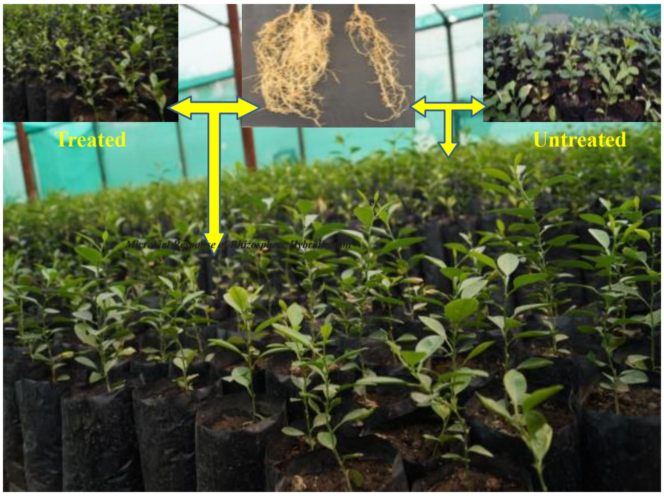

Fig 2: Response of microbial consortium in acid lime (clone NRCC-AL-7) on a commercial-scale use

T_4 and T_6, NH_4OAc-K with treatment T_6, DTPA-Fe with treatment T_5, DTPA-Mn with treatment T_5, DTPA- Cu with treatment T_5 and T_6 and DTPA-Zn with treatment T_6.

Changes in soil microbial population: Changes in soil microbial population (Bacterial count as well as fungal count) were observed to be significantly affected by different microbial treatments. Among all the treatments maximum bacterial count observed with the treatment T_6 as 73 x 10^3cfu g^{-1} followed by treatments T_4 and T_3, both as 69 x 10^3cfu g^{-1} and 67 x 10^3 cfu g^{-1} with treatment T_2 in decreasing order. Similar response reported with fungal count changes in response to the given treatments. The treatment T_4 recorded fungal count as 36 x 10^3 cfu g^{-1} while the treatments T_2, T_5 and T_6 were statistically on par with each other except control with fungal count as 26 x 10^3 cfu g^{-1} soil (Table 3).

Population dynamics of component microbes: All the component microbes of added consortium showed pragmatic changes in their population over the period of 120 days of observation (Table 4). Highest population of the fungus *Aspergillus flavus* reported with treatment T_6 as34 x 10^3cfu g^{-1}soil. The similar response of increase in population of the fungus *Aspergillus flavus* recorded from initial population of 10x 10^3 cfu g^{-1} to 32 x 10^3 cfu g^{-1}soils with treatment T_4 and T_5. The *Bacillus pseudomycoides* population was also differentially similar with all the other treatments; however highest population of *Bacillus mycoides* reported with the treatment T_6 as 46 x 10^3cfu g^{-1} soil. Maximum population of *Paenibacillus polymyxa* reported with the treatment T_4 as 52 x 10^3cfu g^{-1}soil, *Acinetobacter radioresistens* from initial value of 14 x 10^3cfu g^{-1}soil to 50x 10^3cfu g^{-1} soil with treatment T_5 while the population of *Micrococcus yunnanensis* reported shifts from initial value of 25 x 10^3cfu g^{-1}soil to 55x 10^3cfu g^{-1}soil with treatment T_4 after 120 -days of inoculation.

Leaf nutrient composition: Concentration of micronutrients in leaves showed responses of varying proportion. The leaf micronutrients like Fe was reported maximum with treatment T_6 (90.2 ppm) followed by treatment T_4 (88.9 ppm). The concentration of Mn assessed out under the leaves of different treatments was relatively on par with all the treatments. However highest Mn concentration was reported with treatment T_4

(43.2 ppm). The zinc concentrations were differentially affected by the treatments. Among all the treatments zinc concentration were as high as 17.5 ppm with treatment T_5 followed by the treatment T_6 (13.9 ppm) while the treatments T_1, T_2, T_3, T_4 recorded 6.3 ppm, 7.2 ppm, 7.9 ppm and 12.8 ppm respectively.

Microbes-fertilizer interaction response

Microbes and chemical fertilizers often do not match for nutrient-use-efficiency. It is still debatable to arrive at conclusive opinion that both can go hand-in-hand, either in citrus nursery or even grown-up orhards. With this objective, five microbes of microbial consortium were tested *in-vitro* for their colony growth against varying concentration of conventionally used inorganic fertilizers. Five micro-organisms (*Aspergillus flavus* MF113270, *Bacillus pseudomycoides* MF113272, *Acinetobacter radioresistens* MF113273, *Micrococcus yunnanensis* MF113274, and *Paenibacillus alvei* MF113275) were further evaluated *in-vitro* against commercially used fertilizers (Urea, potassium dihydrogen phosphate, muriate of potash, iron sulphate, zinc sulphate, manganese sulphate and borax) to study their colony growth response against these fertilizers at six concentrations viz., 0, 100 ppm, 200 ppm, 400 ppm, 800 ppm and 1600 ppm (Table 5)

Response of *Micrococcus yunnanensis*: Maximum colony growth of microbe was observed covering 30.5 mm area at concentration of 200 ppm urea (N-source), thereafter with higher urea concentrations upto 1600 ppm, there was a concurrent reduction in colony growth upto 16.0 mm. While, the same magnitude of response was observed with 200 ppm potassium dihydrogen phosphate (P-source). The colony growth of *Micrococcus yunnanensis* was observed in an area of 21.3 mm followed by reduction in growth upto 18.5mm 1600 ppm concentration. On the other hand, *Micrococcus yunnanensis* responded more favourably with muriat of potash (K-source) upto 800 ppm concentration, displayed increase in colony growth from 12.6 mm at zero concentration to 25.6 mm at 800 ppm but significantly reduced to 22.3 mm at 1600 ppm concentration.

Micronutrients fertilizer sources showed that a microbe triggering the growth response possess some ability to exploit the nutrient source towards its metabolic activity. Ferrous sulphate was observed to increase the colony growth of *Micrococcus yunnanensis* from 14.6 mm at zero concentration to 21.5 mm at 100 ppm, but thereafter subsequent increase in concentration upto 1600 ppm induced consistent reduction in colony growth upto 16.2 mm. The same was response with respect to different concentrations of zinc sulphate (Zn-source), inducing colony growth as 24.0 mm at 100 ppm concentration, significantly higher as 16.3 mm at zero concentration, but higher concentrations continued to inhibit the colony growth upto 1600 ppm.

Response of *Paenibacillus alvei:* Maximum colony growth was observed covering 32.5mm area at concentration of 1600 ppm urea (N-source), increase in colony growth of *Paenibacillus alvei* was observed from 100 ppm onwards. Similarly, the same response was observed with 200 ppm potassium dihydroge phosphate (P-source). The colony growth of *Paenibacillus alvei* was observed maximum in the area of 30 mm at concentration of 1600 ppm. On the other hand, colony growth of *Paenibacillus alvei* was maximum at 100 ppm in an area of 22 mm, but significantly reduced to 200 mm at 800 ppm concentration. Ferrous sulphate was observed to show the maximum colony growth with an area of 25 mm at 1600 ppm concentration.

Similarly, concurrent increase was observed from 18 mm at zero concentration to 28 mm at 1600 ppm concentration of zinc sulphate (Zn-source). In manganese sulphate colony growth in area of 27 mm at concentration of 1600 ppm was observed maximum. The colony growth was more favourable at the concentration of 100 ppm with 24 mm area, but the higher concentration inhibited the colony growth upto 800 ppm. These observations lend steong support in favour of nutrient-microbe synergy (Srivastava *et al.*, 2002; 2008; Srivastava and Malhotra, 2017)).

Response of *Azotobacter chroococcum* : The maximum colony growth was observed covering 12.5 mm area at the concentration of 100 ppm, thereafter followed by the reduced in the response from 200 ppm to 1600 ppm in urea (N-source). In response against potassium dihydrogen phosphate, maximum colony growth was 16.6 mm at concentration of 400 ppm, but it was followed by concurrent decrease at 1600 ppm concentration with 10.3 mm. The response with respect to different concentrations of muriat of potash (K-source) including colony growth as 13.3 at zero concentration, but higher concentration inhibited the colony growth upto 1600 ppm. Similar response was shown by ferrous sulphate, where the maximum colony growth was 15.3 mm at zero concentration followed by concurrent decrease upto 800 ppm and no growth response at 1600 ppm concentration.

Zinc sulphate also showed the same response where the maximum response was observed at zero concentration with 15.6 mm area followed by lateral decrease till 11.3 mm at 400 ppm concentration and no response in 800 and 1600 ppm concentration. Similarly, no growth response was observed in 800 and 1600 ppm concentration of manganese sulphate. The maximum growth response was shown at zero concentration with 13.3 mm area. The last response was observed in borax, where there was no growth response at 400, 800 and 1600 ppm concentration. It showed maximum growth area of 13.6 mm at the zero concentration. These observations strongly suggest that an efficient microbe has some affinity with exogenous nutrient source, where the higher concentrations continued to inhibit the colony growth (Srivastava and Singh, 2008b; 2009b) .

Response of *Aspergillus flavus:* Highest colony growth of microbe was seen covering 41.3 mm at concentration of 1600 ppm of urea (Table 5). Further maximum growth was observed in potassium dihydrogen phosphate treatment of 1600 ppm concentration with the colony growth of 40.3 mm. While, same magnitude of response was observed with 1600 ppm muriate of potash (K-source). Escalating response was again observed in treatment with ferrous sulphate

with 39.0 mm area at the concentration of 1600 ppm. Zinc sulphate showed increasing growth from 27.6 mm of area of zero concentration to 38.6 mm of 1600 ppm concentration. Manganese sulphate also showed mounting response with the area of 40.0 mm at the concentration of 1600 ppm. No response against any of the concentration was noted against borax, it showed the colony growth of 28.3 mm at zero concentration). These observations established the fact that microbial consortium would serve a strong nutrient sink in field. Based on these observations, we put forward another concept, on these observations, we put forward another concept, on these observations, we put forward another concept, these observations, we put forward another concept, known as "rhizosphere hybridization" (Srivastava *et al.*, 2015), in addition to better nutrient-use-efficiency (Srivastava and Singh , 2009a ; 2009b). According to this concept , we can introduce the rhizo-microbiome of any healthy crop into the rhizosphere of target crop to have more microbiome of much wider functional diversity coupled with better disease suppressiveness (Srivastava *et al.*, 2007).

Microbiome manipulation for rhizosphere engineering

Artificially, the rhizosphere can be modified or reconstruct as per the need the of plant to enhance the physiological efficiency by rhizosphere engineering, rhizosphere hybridization (Cheke *et al.*, 2018; Hota *et al.*, 2020; Srivastava *et al.*, 2025), creating an artificial environment suitable for the plant growth-promoting microorganisms (PGPMs) to surplus a protective layer against the pathogenic microbes (Rhizosphere fortification), or by various agronomic practices. Rhizosphere hybridization is new concept to modify the rhizosphere ecology to create an optimum environment for PGPMs to show the positive effect of plant agronomy (Srivastava *et al.*, 2022). The concept of "rhizosphere hybridization" is therefore, advocated to harness the value-added benefit of nutrient-microbe synergy, besides

Table 3: Changes in soil microbial count in response to different treatments in primary nursery (period: 120 days)

Treatment	Changes in Bacterial count over time (x 10^3 cfu g^{-1} soil)						
	10	20 days	40 days	60 days	80 days	100 days	120 days
T_1 (Control)	31	35	39	41	50	56	60
T_2 (*Ar*)	35	38	42	48	53	59	67
T_3 (*Ar+My*)	30	32	37	40	56	65	69
T_4 (Ar+*My* +*Bp*)	39	38	40	57	60	65	69
T_5 (*Ar+My+Bp+Pa*)	42	45	45	52	57	61	65
T_6(*Ar+My+Bp+Pa +Af*)	37	39	40	47	62	70	73
CD(*P=0.05*)	03	02	02	04	03	03	06
Treatments	Changes in Fungal count over time (x 10^3 cfu g^{-1} soil)						
T_1 (Control)	12	12	14	17	20	22	26
T_2 (*Ar*)	15	16	21	24	27	31	34
T_3 (*Ar+My*)	10	10	13	20	22	26	30
T_4 (Ar+*My* +*Bp*)	19	17	23	25	29	34	36
T_5 (*Ar+My+Bp+Pa*)	12	15	20	29	32	37	34
T_6(*Ar+My+Bp+Pa +Af*)	14	17	19	24	30	31	35
CD(*P=0.05*)	03	02	04	04	06	0.8	07

Ar,My, Bp, Pa, Af, stand for *Acinetobacter radioresistens* (MF113273), *Micrococcus yunnanensis* (MF113274) *Bacillus pseudomycoides* (MF113272), *Paenibacillus alvei* (MF113275) *and Aspergillus flavus* (MF113270) respectively.

Source : Srivastava and Hu (2019)

Table 4: Changes in individual microbial count in response to different treatments (period: 120 days) in primary nursery.

Treatment	Changes in population of different microbes						
	Initial	20 days	40 days	60 days	80 days	100 days	120 days
	Changes in *Aspergillus flavus* population (x 10^3 cfu g^{-1} soil)						
T_1 (Control)	12	15	17	19	17	21	23
T_2 (*Ar*)	17	13	15	17	25	26	27
T_3 (*Ar+My*)	15	10	10	09	19	25	29
T_4 (Ar+*My +Bp*)	10	17	19	21	26	30	32
T_5 (*Ar+My+Bp+Pa*)	12	16	16	25	30	32	32
T_6(*Ar+My+Bp+Pa +Af*)	17	15	17	20	29	30	34
CD(*P=0.05*)	02	02	02	03	04	04	04
	Changes in *Bacillus pseudomycoides* population (x 10^3 cfu g^{-1} soil)						
T_1 (Control)	09	12	15	21	28	32	32
T_2 (*Ar*)	12	16	19	25	25	35	37
T_3 (*Ar+My*)	10	13	17	31	39	42	43
T_4 (Ar+*My +Bp*)	17	19	21	28	32	40	45
T_5 (*Ar+My+Bp+Pa*)	21	25	25	27	35	39	42
T_6(*Ar+My+Bp+Pa +Af*)	20	22	24	30	37	45	46
CD(*P=0.05*)	02	02	03	03	03	02	04
	Changes in *Paenibacillus alvei* population (x 10^3 cfu g^{-1} soil)						
T_1 (Control)	11	15	17	25	30	38	37
T_2 (*Ar*)	14	17	20	22	32	42	45
T_3 (*Ar+My*)	21	24	21	28	35	39	42
T_4 (Ar+*My +Bp*)	20	22	23	32	40	47	52
T_5 (*Ar+My+Bp+Pa*)	19	23	25	29	39	43	45
T_6(*Ar+My+Bp+Pa +Af*)	17	20	27	32	41	48	50
CD(*P=0.05*)	02	02	02	03	02	03	04
	Changes in *Acinetobacter radioresistens* population (x 10^3 cfu g^{-1} soil)						
T_1 (Control)	05	07	09	15	19	23	25
T_2 (*Ar*)	17	15	17	21	25	27	30
T_3 (*Ar+My*)	12	14	16	28	32	39	43
T_4 (Ar+*My +Bp*)	10	18	21	31	39	45	49
T_5 (*Ar+My+Bp+Pa*)	14	17	19	26	35	46	50
T_6(*Ar+My+Bp+Pa +Af*)	09	12	18	27	37	35	35
CD(*P=0.05*)	02	03	03	04	04	03	05
	Changes in *Micrococcus yunnanensis* population (x 10^3 cfu g^{-1} soil)						
T_1 (Control)	18	18	20	15	19	37	40
T_2 (*Ar*)	20	22	25	21	25	39	43
T_3 (*Ar+My*)	27	20	23	28	32	40	42
T_4 (Ar+*My +Bp*)	25	30	32	31	39	51	55
T_5 (*Ar+My+Bp+Pa*)	19	21	27	34	35	42	44
T_6(*Ar+My+Bp+Pa +Af*)	20	18	21	29	37	39	42
CD(*P=0.05*)	02	03	03	04	04	03	03

Ar,My, Bp, Pa, Af, stand for *Acinetobacter radioresistens* (MF113273), *Micrococcus yunnanensis* (MF113274) *Bacillus pseudomycoides* (MF113272), *Paenibacillus alvei* (MF113275) *and Aspergillus flavus* (MF113270) respectively.
Source: Srivastava and Hu (2019)

providing dynamism to microbial consortium suiting to wide range of perennial fruits .

Our studies on response of different treatments involving rhizosphere soil of three perennial trees viz., *Ficus racemosa* L. (Umber tree), *Ficus benghalensis* L. (Banyan tree), and *Ficus religiosa* L. (Pipal tree) along with rhizosphere soil of healthy and highly productive sweet orange trees in sweet orange buddlings showed differential

Table 5: Fertilizer interaction response (measured by colony growth in mm) under controlled conditions

Fertilizer type	Concentration (ppm)						CD (P=0.05)
	Control	100	200	400	800	1600	
Micrococcus yunnanensis							
Urea	14.0	20.5	30.5	21.6	22.0	16.0	1.41
KH_2PO_4	13.3	16.6	21.3	18.5	18.3	18.5	1.64
MOP	12.6	21.0	22.3	23.3	25.6	22.3	0.84
$FeSO_4$	14.6	21.5	20.3	16.0	16.0	16.2	0.94
$ZnSO_4$	16.3	24.0	19.0	20.6	19.0	17.5	1.10
Paenibacillus alvei							
Urea	20	28	29.5	31	32	32.5	0.50
KH_2PO_4	21	23	24	26.5	25	30	1.20
MoP	19	22	21	21	20	21	1.40
$FeSO_4$	19	21	21	22	23	25	1.50
$ZnSO_4$	18	20	22	24	26	28	1.30
$MnSO_4$	18	21	21	25	24	27	2.10
Borax	20	24	22	22	20	21	1.80
Acinetobacter radioresistens							
Urea	12	12.5	10.6	11.0	10.0	10.3	NS
KH_2PO_4	11.6	12.3	11.3	16.6	16.0	10.3	1.1
MoP	13.3	11.0	11.3	11.0	-	-	0.70
$FeSO_4$	15.3	13.6	13.3	12.3	10.6	-	1.10
$ZnSO_4$	15.6	13.3	11.0	11.3	-	-	1.14
$MnSO_4$	13.3	11.3	11.3	11.6	-	-	NS
Borax	13.6	12.6	11.6	-	-	-	NS
Aspergillus flavus							
Urea	34.3	36.0	36.3	38.0	38.3	41.3	1.10
KH_2PO_4	26.3	27.6	30.0	33.6	34.6	40.3	0.80
MoP	28.3	28.6	29.6	34.3	37.6	40.3	1.04
$FeSO_4$	32.3	32.6	34.3	34.6	35.3	39.0	1.20
$ZnSO_4$	27.6	28.6	34.0	28.6	25.3	38.6	0.80
$MnSO_4$	28.3	30.6	33.0	35.3	38.3	40.0	1.10
Borax	28.3	-	-	-	-	-	

These fertilizers are commonly used in citrus fertilization programme

Source: Srivastava *et al.* (2015)

response in terms of agronomic parameters, changes in soil physical properties, and pool of plant available nutrients. However, hybridized rhizosphere of sweet orange and *Ficus racemosa* L. out-smarted the response over other rhizosphere hybridization treatments. These studies lend some support to the fact that inoculation of soil or crops with rhizospheric or endophytic microbes, respectively, can enhance the micronutrient contents in various plant tissues including roots, leaves, and fruits (Cheke *et al.*, 2018).

Conclusion remarks and way forward

These studies hence established that microbial consortium can be effectively retrofitted replacing conventionally used chemical fertilizers in nursery, considering very low nutrient requirement of such juvenile citrus plants (Kohli *et al.*, 1998; Srivastava , 2023; 2025). There is every possibility, we can further rationalize the use of function specific microbes as per growth stages of nursery plants with use of biochars (Agegnehu *et al.*, 2017; Mousavi *et al.*, 2023), mycorrhizal-mediated microbial consortium (Wu *et al.*, 2013; 2017), crop phenology-based fertigation (Shirgure *et al.*, 2001; Srivastava *et al.*, 2003) , speciality fertilizers (Srivastava and Pandey , 2021), orchard efficiency (Srivastava *et al.*, 2008a) , regular flowering (Srivastava *et al.*, 2000) and site-specific nutrient management (Srivastava *et al.*, 2006). However, no distinction in morphological or physiological growth behavior exists in nursery plants, right from growth in primary nursery to secondary nursery. And, morphologically, it is very difficult to identify such shifts in growth stages (Srivastava and Singh 2008a; 2008b).

Our studies also establish and advocate following other impotent issues : i. microbes can replace nutrients requirement of citrus nursery, considering absymally low nutrient requirement of nursery plants ii. microbial consortium is a far better choice than individual microbe(s); iii. liquid formulation of microbes is better than substrate-based inoculants, either individual microbe or consortium of microbes; iv. the quantity of microbial broth needs to be standardized for containerized citrus nursery versus field nursery; v. inoculation of citrus nursery plants with microbial consortium needs to be standardized depending upon substrates used (solarized soil versus soilless medium) ; vi. the treatment of microbial consortium (5ml/plant) reduced the rate of mortality of citrus nursery plants to bare minimum, once transplanted in new orchard site. This is an excellent piece of information, otherwise orchardists are fed up with high rate of mortality of citrus nursery plants; vii. treatment with microbial consortium provided an additional plant immune on account of biopriming effect of microbes, which eventually aided in far better withdrawal of nutrients from soil and ensured better plant health in ultimate terms; viii.the treatment with microbial inoculants individually or as microbial consortium has a strong promise to be integrated with fertigation to evolve a new concept called "biofertigation" for exclusively citrus nursery and ix. the use of microbial inoculants can be tailored in citrus nursery, depending upon contrasting growth stages (initiation, establishment and growth stages, though these stages are poorly differentiated and quite inter-changeable). With these concluding remarks, microbe s-mediate organic citrus nursery would pave away forward to more sustainable citrus industry.

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