

Sustaining citrus production under hill agroecosystem: Everaging microbial solutions

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ABSTRACT

Citrus crop in a hill agroecosystem is the choicest fruit crop, known for its production sustainability. But in recent past, the crop is facing unprecedented decline in its own home-yards. Hill agroecosystem puts forward altogether a different growing scenario negotiating with multiple nutrient constraints in a nature-friendly manner as a result of soil denudation without any back-up support, more so in rainfed scenario, a formidable challenge to cope up, amid current climate conundrum and popular practice of shifting cultivation. Of late, organic farming and natural farming have ignited researchers more intensively than ever before. However, these practices still lack the scientific validity through long term database, thereby finding no big reason to adopt these practices as claimed. Agroecology in hill citrus is indispensable to ensure production and quality sustainability alongside prolonged orchard life. Development of microbial consortium (microbial concoction) exploiting the native and natural microbial synergisms is one of the holistically acclaimed methods of managing multiple soil fertility constraints, a concept dictating another version of natural farming embracing agroecology in functional framework. Retrofitting microbes in nursery citrus plants for nutrient requirement is one of the novel approaches of not only ensuring good health of future citrus industry, but very handy in cutting down the intensity of mortality while planting onto new field. Long term evaluation of microbial consortium and rhizosphere hybridization (introducing the rhizosphere of one crop into the rhizosphere of target crop of citrus to have one rhizosphere of greater microbial diversity as well as microbial functions) in mature citrus orchards showed much better dividends in terms of better soil health indices coupled with environment health and quality production with back-up support of on-farm resource conservation using terrace farming and robust irrigation/fertigation through natural water harvesting. These circular practices would go along way in integrating natural farming with agroecological issues, thereby improving the soil health with superior functional traits in agroecology-driven citriculture. Until such exercises start displaying their visible effects at farm level, agroecology-centric management practices relating organic farming, natural farming and conventional hill farming need serious reframing in light of emerging newer technologies with emphasis on customizing crop nutrient requirement, microbial pyramiding, rhizosphere hybridization, field-based transcriptomic (nutrigenomics) analysis of response of citrus to crop residue-based or microbial inoculants-based bio-inputs to finally arrive at "Crop Expert" for hill agroecosystem.

Keywords: Citrus crops, soil health, agroecology, microbial consortium, natural farming, climate change, rhizosphere hybridization, soil amendments.

INTRODUCTION

In India, major fruit crops, such as mango, banana, citrus, guava and apple, account for more than 72% of the total area under fruit crops; while indigenous (native) fruit crops contribute only 6.56% of the area (0.437 million ha) with quite high productivity (11.47 tons/ha). Citrus is such a fruit crop, which has wide range of soil and climate adaptability (Srivastava, 2010; Srivastava *et al.*, 2025), ranging from coarse textured- to-fine textured soil; acidic soil -to-highly alkaline soils with varying degrees of calcareousness (Sayed *et al.*, 2024); thriving under highly arid climate to humid tropical/humid climate with varying overall

organo-leptic traits and flowering behaviour as well (Srivastava and Singh, 2000; 2001); orchards located at different physiography located from highly flood affected plain-land -to-extreme- hills with altitudes upto 1200-500m above mean sea level (Srivastava and Kohli, 1999), mostly intercropped during pre-bearing growth stage to mono-cultivation in post bearing period (Srivastava *et al.*, 1994; Singh *et al.*, 1999; Srivastava *et al.*, 2007) under rainfed-to-highly mechanized form of irrigation (Shirgure *et al.*, 2001; 2002; 2003; 2004a; 2004b) and sensor-based fertigation (Meshram *et al.*, 2025a; 2025b). These merits of the crop propel citrus as one of highly researched crop world over. However, in spite of these facts, crop displays a

colossal yield disparity between plain land citriculture versus hill citriculture (Srivastava *et al.*, 2007; Srivastava and Singh, 2008b; Huchche *et al.*, 2010), frequently debated on the basis of seedlings origin -to-grafted citrus using a great range of rootstocks, which again has impounding implications on the extent of water and nutrient use (Kohli and Srivastava, 1997; Srivastava and Singh, 2008a; Krug *et al.*, 2025), off course besides the plant health implications on the long run, which we will not debate in this review considering the theme of our discussion.

While comparing the citrus on plain land against under hill agroecosystem, what separates the two forms of citriculture, is the major point of discussion. In hill agroecosystem, an element of natural farming is infused, a kind of regenerative agriculture, considering stronger resilience against abiotic / biotic stress plus climate change-related issues. As broadly perceived, natural farming integrates five major issues viz., soil health care, soil biodiversity, crop biodiversity, production stability, environmental health and water quality. Of these, soil health is the central core of the natural farming (Keditsu and Srivastava, 2014). Soil is an environmental medium, playing crucial role in global C cycle (soil C pool as the second biggest carbon pool), mainly through changes in soil fertility. Soil is, therefore, as not only roots supporting medium supplying the necessary metabolic requirements to above-ground plant canopy, besides strong blanket against climate change problem, a better part of the multiple solution. The elevated CO₂, changes in rainfall pattern and increase in average temperatures brought about by climate change with inflicting over-riding effects on soil fertility changes vis-à-vis crop performance, such complex issue is, therefore still far from realms of any tangible solution from production sustainability point of view. Synergism between the effect of CO₂ and nutrients is stronger under no water limiting conditions (Srivastava, 2015a). However, such short term changes in fertility dynamics do not portray the long term effect either on soil fertility or on production responses, unless supported by defined analogues of soil and climate (Srivastava and Singh, 2008). Different citrus crops sequestering 24 – 109 tons CO₂/ ha display their ability to moderate climate change-related issues on one hand, and elevate the crop fertilising ability for improved plant nutrition, besides water-use-efficiency, on the

other hand (Srivastava and Singh, 2009). Therefore, response of different citrus crops under elevated CO₂ condition is a function of nutrition status of the crop, where soil microbial ecology plays a pivotal role; an issue comprehensively over-looked with disbelief.

Previously, our studies (Srivastava and Singh, 200a; 2004b) demonstrated the maximum nutrient demand at fruit set stage (March-April for winter crop and August-September for summer crop under sub-humid tropical climate of central India). As per crop ontogeny unless there is some mitigation strategy available. Of late, certain citrus growing pockets of central India irrespective of orchard nutrient status (possibility of disturbed K metabolism), exhibited abnormal fruit growth (greater growth along equatorial than radial axis), the exact cause and effect relation still remains to be established (Srivastava, 2011). A large difference in fertility of two sites (Ustorthent versus Haplustert) indicated by a much greater increase in yield response at the low fertility soil site (Ustorthent) than the high fertility soil site (Haplustert), when added nutrient augmented to the same optimal fertility. But, with climate change, such responses will be caused by nutrient limitation that can develop in poor fertility sites having shallow rooting depth. The recommended dose of fertilizers (RDF) worked out in 1990 – 91 is no longer effective now (2010 - 2015), due to rise in average temperature by 1.5 – 2.0 °C during fruit set stage, necessitated addition of 25% more K to moderate such temperature stress in citrus (Srivastava and Singh, 2000; 2020). How does RDF behave in the long run in different crops when agroecology is to be integrated with emphasis on microbial turnover of the nutrients (Srivastava *et al.*, 2025). 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 (Srivastava *et al.*, 2022).

There are ample evidences accrued through worldwide research that nutrient-microbe synergy is the launching pad for any citrus crop to mobilize and accumulate the required nutrients as per the metabolic nutrient demand, a pre-requisite to improved input-use-efficiency (Srivastava *et al.*, 2015). 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 (Chiou, 2007) .

A still bigger question emerges, whether rhizosphere competent microbes could collectively contribute toward improved resilience of plant's rhizosphere against potential nutrient mining (Srivastava, 2012). 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. 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 (Klitgard and Serge, 2011). 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 (Srivastava and Singh, 2006). Long term data accrued on response of organic manuring versus inorganic fertilizers demonstrated that important soil quality indices like soil microbial diversity, soil microbial biomass nutrient (C_{mic} , P_{mic} , and N_{mic}) and organic carbon partitioning displayed significant changes, but without much difference in quantum of fruit yield (Huchche *et al.*, 1998). These factual figures put forth a possibility of integrating natural farming issues with conventional agroecology to sustain the production of citrus fruits with global perspective sin mind.

Citrus under hill agroecosystem

Agro-ecosystems, highly site specific are commonly defined as ecological systems modified by human beings to produce food, fibre or other agricultural products as they have evolved along diversified ecosystems. Ecosystem based adaptation is , therefore, considered highly sustainable management from points of view of conservation and restoration of ecosystems , besides bringing cost-effective solution to generate social, economic and environmental co-benefits and resilient to climate change impacts . Hill agroecosystem by and large known as an agro-biodiversity hotspot due to enormous variety of lesser known,

underutilized and valuable ethnomedicinal fruit crops ,either along the arid and semiarid regions (Meena *et al.*, 2022) or north-eastern Himalayas (Mitra and Roy, 2014). These natural fruit crops have significant nutritional and medicinal benefits. However, despite their excellent nutritional composition, hard to find in nature, these fruit trees are not widely cultivated or cared for. Extensive researches have been conducted on bioactive compounds and the health benefits of wild and underutilized citrus fruits (Srivastava and Singh, 2006). Fruit crops extensively grown under himalayan agroecosystems possess the ability to act as antioxidants, anti-microbial, anti-inflammatory agents, anti-allergic agents, anti-spasmodics, chemo-preventive agents, hepato-protective agents, neuro-protective agents, hypolipidemics, hypotensives, anti-aging agents, anti-diabetes agents, anti-osteoporosis agents, DNA damage repair agents (Bora *et al.*, 2024) . However, despite these bioactive compounds and the proven natural antioxidant activities of himalayan wild edible fruits, their detailed phytochemical fingerprints are yet to be sufficiently explored. The systematic studies on such fruit-based agroecosystems are still in an infancy stage.

To improve sustainability of citrus under hill agroecosystem, terrace farming used for centuries is most commonly practiced to facilitate better crop performance. Terrace farming is considered one of the oldest and most successful techniques for conserving soil and water during cultivation on steep slopes. However, there is no concrete information available about the quantitative estimate of area and numbers of farmers, including fruit growers are precisely involved. The remoteness of many terrace farms away from cities, thereby consuming relatively limited fuel, energy and water resulting in a low carbon and environmental footprint. The maintenance of traditional knowledge has been shown to help maintain biodiversity and diverse ecosystem in hill ecosystem. Despite the benefits of terrace farming, there are challenges as well. Only subsets of terrace farms across the globe have shifted from ancient to modern techniques. Zheng *et al.* (2024) while studying the long evolution of soil quality in terraced citrus orchards observed plantation age had strongest effects on soil aggregate stability and nutrient reserve that contributed significantly towards

orchard life. A meta-analysis of 601 runoff and 636 sediment observations involving a diversity of terrace structures confirmed that terracing significantly and positively affected water erosion control. In terms of different terrace structures, bench terraces were superior with respect to runoff and sediment reductions. In addition, a significant positive correlation between slope gradient (3° – 15° and 16° – 35°) and the effect of terracing on water erosion control was observed with the greatest decreases in water erosion occurred at slopes of 26° – 35° and 11° – 15° (Chen *et al.*, 2017). Previously, Zheng *et al.* (2023) reported that large-scale mechanized land preparation during terrace construction can seriously disturb soil layers, leading to fundamental changes in soil profile structure, nutrient status, and overall soil quality, ultimately affecting crop yields. The current literature regarding terrace farming has focused on estimating soil, soil and water conservation, land use dynamics, economic benefits and ecological impacts, and sustainability.

Challenges of plant nutrition vis-à-vis citrus nutrition

Historically, economic development has been faster in those world regions where fertilizer- use and crop yields rose in parallel. Despite bundles of scientific literature have poured in recent past dealing with a detailed description of one or few scientific goals like molecular genetics, plant mineral nutrients related to molecular biology, physiology processes related to plant nutrition, fertiliser application strategies, and beneficial plant–microbe interactions; yet the success of plant nutrition in ensuring the quality production of citrus crops is registered far below efficacy, and more candidly so in climate change scenario (Srivastava, 2015b). In such scenario of climate change, there are multiple expectations (from biochemical bases underlying nutritional processes described down to molecular level, involving differential selectivity of membrane transporters for nutrients or signaling functions of mineral nutrients in plant development to serving large parts of the society from farmers to policy makers, allowing further enhancements in crop yield, and quality while increasing nutrient-efficiency and reducing nutrient losses from crop production systems) from plant nutrition, thereby

aimed to accomplish varied objectives. Hence, the current understanding of plant nutrition is largely focused on monocultural situations, needs to be augmented by the interaction that occur in more complex systems (Srivastava and Singh, 2009). Therefore, mineral nutrients in soils and crops have important and still difficult to predict positive as well as negative interactions with global climate change, although negative impacts of climate change appear to outweigh positive impacts.

Plant nutrition stands on two legs, one leg anchored in plant physiology explaining the fundamental processes underlying and regulating the acquisition, allocation, and utilization of mineral elements in plants; while the other leg is deeply rooted in an applied, problem-oriented context, explaining the use and action of fertilizers and the cycling of mineral elements in agricultural plant production. These dimensions of plant nutrition perch different challenges to different perspectives (Srivastava *et al.*, 2008). It has been previously pointed out the main challenge of plant nutrition as to increase the width of the domain between the access and excess frontiers, rather than to define the crop nutrient response as a single 'economic optimum' points involving two approaches;

i. the technical paradigm of precision farming and the ecological analogue approach based on filter functions and complementarity of components in mixed plant systems. A new paradigm has been proposed for responsible plant nutrition following a food systems and circular economy approach to achieve multiple socioeconomic, environmental, and health objectives involving critical actions such as: i. sustainability-driven nutrient roadmaps, ii. digital crop nutrition solutions, iii. nutritious crops, iv. nutrient recovery and recycling, v. climate-smart fertilizers, and vi. accelerated innovation (Srivastava *et al.*, 2007).

Ironically, plant responses to deficiencies of different nutrients have been studied mainly as a separate event and only a few studies discussed the molecular basis of biological interaction amongst the nutrients, comprehensively overlooking the interaction between macro- and micronutrients at two-by-two or multi-level nutrient interactions involving transcription factor or phosphate starvation

response, the master regulator of multiple nutrients homeostasis using an integrative study of multiple nutrient signaling cross-talks in plants for ensuring better biological significance narrating higher yield- and nutrient-use-efficiency.

The current definitions of essential or beneficial elements for plant growth rely on narrowly defined criteria that do not fully represent a new vision for plant nutrition and often compromise with fertilizer regulation and practice. An effective plant nutrition is supposed to ensure the level of fruit nutrition, much beyond simple minerals and vitamins, more inclined towards freshness (taste quality) as a matter of extended storability (Srivastava *et al.*, 2015). The raised CO₂ concentration in the environment reduced the overall quantity of twenty-five minerals in plants, including calcium, potassium, zinc, sulphur, copper, and iron (8% on average), and also increased the ratio of carbohydrates to minerals in food plants. A new analysis of long-term trends of the mineral content of fruits and vegetables showed all elements except P declined in concentrations between 1940 and 2019, the greatest overall reductions during this 80-year period were Na (52%), Fe (50%), Cu (49%) and Mg (10%). Studies of historical fruit nutrient composition data are inherently limited, most of the other methods focus on single crops of any kind, can include any nutrient of interest and can be carefully controlled. Therefore, the proposed new definition of mineral plant nutrient is: an element which is essential or beneficial for plant growth and development or for the quality attributes of the plant or harvested product, of a given plant species, grown in its natural or cultivated environment (Marschner *et al.*, 2004).

There is a renewed strong emphasis on enlarging the proportion of fruits, vegetables, and grains in the diet, likely to increase the intake of a wide range of minerals and phytonutrients thought to have specific nutritional benefit. However, the effect of climate change on the accumulation of these phytochemicals and their biosynthetic pathways in the light of crop phenology remains poorly understood (Srivastava and Singh, 2004a). The recent developments in metabolomic analysis methods are of great help in obtaining novel insights into qualitative and quantitative changes in the composition of plant phytonutrients under different climate change scenarios in addition to linking carbon sequestration ability of crops with

plant nutrition. All these issues have to be dealt with another fast emerging field known as “Nutriomics” exploiting genomics tools. Do citrus crops respond differently when compared under plain land versus hill agroecosystem at various genomic levels as function of transcriptome, metabolome and proteome, still no prudent answer available to researchers? How are different communication signals involved at molecular, physiological/biochemical and morphological manifestations of nutrients (under both nutrient deficiency as well as nutrient sufficiency levels as a function of phytobiome microbial niches) for a crop like citrus under two contrasting agro-pedological conditions, remains to be decoded.

Integrating natural farming as regenerative approach

Land degradation (96.4 million ha of degraded land accounting to 29.3% of the country's total geographical area of 328.7 million ha) neutrality has been one of the prudent strategies of national agriculture policy where coalition of conventional and traditional farming takes place with singular objectivity of sustainability through regenerative agriculture. In a way, regenerative agriculture (no legal or regulatory definition of term “regenerative agriculture” exists nor has a wide accepted definition emerged in common usage) is firmly rooted to the same basics of modern form of agriculture (using conservation and rehabilitation approach for sustaining the top soil fertility functions, frequently coined as quite opposite to conventional agriculture), addressing core issues like natural resource conservation, soil microbial diversity, resilience against forging climate change, expanding water intake capacity, scavenging soil contaminants, usage of cover crops (field buffers and plant strips on contours) for reduced run-off loss and maintaining the environmental health as well, but it emphasizes more firmly the rejuvenation of depleted land from physical, biological and chemical barriers restricting the targeted optimised crop agronomy and aid further in recuperating the full potential for crop carrying capacity of a given land or land use in a farming system module (Srivastava *et al.*, 2008). Regenerative agriculture recognizes all sustainable practices those affect the natural systems and uses all the management techniques to restore the system towards

improved crop productivity. Despite these accruing benefits, regenerative agriculture is often associated with number of disadvantages like need for new knowledge and skills, excessive weeds infestation and potentially lower crop yields. However, regenerative agriculture is applicable to all types of farms, big, small or organic in nature. The term "Regenerative Agriculture" came into existence by Robert Rodale Institute in 1980s. India made some modest contribution to realise the strength of this form of sustainable agriculture through i. National project on organic farming, ii. Systematic rice intensification and iii. Zero budget natural farming.

Natural farming on the lines of regenerative agriculture also into account towards sequestering atmospheric carbon into the annual/perennial framework of crops as well as soil, so that atmospheric CO₂ offset is exercised through two-way process, offering carbon neutral approach amidst climate change. The importance of regenerative agriculture was prominently emphasized in Intergovernmental Panel on Climate Change enlisting ecological functions in building resilience of agroecosystems as climate –smart regenerative agriculture. On the other hand, no scientifically structured studies have been conducted on water –use-efficiency and water savings in relation to natural or regenerative agriculture with any fruit crop, including citrus (Srivastava and Singh, 2004b). The magnified impact of regenerative agriculture reaching out to reduction in water foot prints of fruit crops including citrus could offer a carbon trading route in international market.

Regenerative agriculture is very often compared with organic agriculture. Both the concepts have some difference with a common goal of outcomes on ecological balance and biological diversity, leading to emergence of another concept called regenerative organic agriculture. The regenerative agriculture (about principles not practices as adaptive management approach supported by soil health principles) is based around observable improvements in ecological and social function of the farm and farming community, while organic agriculture (prescriptive standards for crop production) is more about a set of rules to follow with major emphasis on avoidance of agrochemicals. Interestingly, the technique of cover cropping as a part of regenerative agriculture, the

definition remains murky, and many other beneficial practices are in a grey area covering the legal definitions, certification and clear methods of measurements and monitoring (Srivastava and Singh, 2005). On the other hand, organic farming may not have a specific definition, but certifications at least provide a clear understanding about the required practices to adopt. While comparing regenerative agriculture with organic agriculture, both often connected with natural farming, we comprehensively overlook the harmful effects of organic pesticides, could be even more harmful than synthetic pesticides in organically produced fruits and vegetables as an example as wide spread myth. Are natural pesticides safer than artificial pesticides? The candid answer is, not necessarily in the context of comparing citrus under plain land against hill agroecosystem.

Role of organic manures and composts, biochars and terra- preta, no till and pasture cropping, annual organic cropping, holistic management of grazing, ecological aquaculture, perennial cropping, silvipasture and agroforestry, all aid in developing a sound success of regenerative agriculture vis-à-vis natural farming (Srivastava *et al.*, 2002). Of late, some novelties have emerged suiting to regenerative agriculture, comprising microbial consortium (developing synthetic microbes using synonymous molecules of secondary metabolites secreted by different microbes participating in both plant growth regulation as well as microbial bioagents) exploiting varied microbial niches of phytobiome to develop microbes- mediated crop production system (Srivastava *et al.*, 2022), rhizosphere hybridization (Cheke *et al.*, 2018; Srivastava *et al.*, 2025) for developing more biochemically active rhizosphere through elevated loading of active and novel microbes, on-farm organic module for organic farm waste recycling and exploiting the rhizosphere and endosphere microbial diversity's, in addition to bioprospecting microbiome for soil health-plant health management addressing both soil fertility constraints and plant diseases as a value –chain –management of microbes (Srivastava *et al.*, 2022; 2025). Development of crop-based soil health card addressing biological improvements in soil health in response to regenerative agriculture is another futuristic pivotal agenda (Srivastava and Singh, 2006).

Phytobiome manipulation for soil health - induced production incentives

Plant phytobiome posing microbial diversity through different microbial niches comprising rhizosphere and endosphere offer the best opportunity to develop and upscale the combination of rhizo-competent microbes, popularly called as microbial consortium or microbial concoction (Wu and Srivastava, 2012). 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 Bora, 2023). 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 consortium 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. Many of the past studies provided the basis for classifying microbial consortium as: i. artificial (carrying two or more wild type microbes whose interactions do not typically occur naturally), ii. synthetic (carrying microbes which are modified through manipulations of genetic content) and iii. natural (carrying microbes having much wider applications like bioremediation, wastewater treatment, biogas synthesis etc (Srivastava *et al.*, 2002).

We carried out studies with an aim to develop rhizosphere specific microbial consortium. Growth promoting microbes were isolated from rhizosphere (0-20 cm) for development of microbial consortium through extensive soil sampling (from the rhizosphere of as many as 110 plants) at the experimental site (Lallan Ram *et al.*, 1997; Srivastava *et al.*, 2008; Srivastava, 2015b). The microbial diversity existing within rhizosphere soil was isolated

following standard procedures, and characterized the promising microbes for their nutrient mobilizing capacity through laboratory-based incubation study using the same experimental soil. The efficient microbes viz., *Aspergillus flavus* (MF113270, P- solubilizer), *Bacillus pseudomycoides* (MF113272, K- solubilizer), *Acinetobacter radioresistens* (MF113273, N- solubilizer), *Micrococcus yunnanensis* (MF113274, P- solubilizer) and *Paenibacillus alvei* (MF113275, P- solubilizer) were finally identified. Pure culture of these microbes in value added form was developed in broth, and prepared a mixture called microbial consortium. The compatibility amongst these microbes was tested by thoroughly their population dynamics in consortium mode which showed no antagonism amongst them upto 210-days of laboratory oriented incubation study (Srivastava *et al.*, 2021).

Retrofitting microbial consortium in citrus 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 in primary nursery

A nursery experiment was set up at, Nagpur Experimental Farm of ICAR-CCRI, Nagpur (Maharashtra) to observe the progressive response of different microbes on germination rate of acid lime seeds and subsequent growth. Different treatments consisted of: T₁ (Control), T₂ (Ar, *Acinetobacter radioresistens*, MF113273), T₃ (Ar, *Acinetobacter radioresistens*, MF113273 + My, *Micrococcus yunnanensis* MF113274), T₄ (Ar, *Acinetobacter radioresistens*, MF113273)+ My, *Micrococcus yunnanensis*, MF113274 + Bp, *Bacillus pseudomycoides*, MF113272), T₅ (Ar, *Acinetobacter radioresistens*, MF113273) + My, *Micrococcus yunnanensis*, MF113274 + Bp,

Bacillus pseudomycolides, MF113272) + *Pa*, *Paenibacillus alvei*, MF113275) and T_6 (*Ar*, *Acinetobacter radioresistens*, MF113273) + *My*, *Micrococcus yunnanensis*, MF113274 + *Bp*, *Bacillus pseudomycolides*, 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 one- 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 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 treatment T_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 treatment. Hence, our studies 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. There is every possibility, we can further rationalize the use of function specific microbes as per growth stages of nursery plants. 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.

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

Treatments	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 (<i>Ar</i>)	12.5	18.2	35.6	40.5	40.5	40.5	40.5	40.5	40.5	40.5	1.13
T_3 (<i>Ar+My</i>)	17.3	21.3	46.2	50.2	50.2	50.2	50.2	50.2	50.2	50.2	1.79
T_4 (<i>Ar+My+Bp</i>)	13.2	19.2	62.5	65.5	65.5	65.5	65.5	65.5	65.5	65.5	2.39
T_5 (<i>Ar+My+Bp+Pa</i>)	14.3	20.9	70.3	70.3	70.3	70.3	70.3	70.3	70.3	70.3	2.67
T_6 (<i>Ar+My+Bp+Pa+Af</i>)	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 pseudomycolides* (MF113272), *Paenibacillus alvei* (MF113275) and *Aspergillus flavus* (MF113270) respectively. Note: Seed viability index was calculated at 100 days of germination as Germination percentage \times Average seedling length (mm)/100

Source: Unpublished data

Microbial response of rhizosphere hybridization

Artificially, the rhizosphere can be modified or reconstructed as per the need of

plant metabolism to enhance the physiological efficiency by rhizosphere engineering, rhizosphere hybridization, or by creating artificial environment suitable for the plant growth-promoting microorganisms (PGPMs) to surplus a

protective layer against the pathogenic microbes (Rhizosphere microbial 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. The concept of "rhizosphere hybridization" is therefore, advocated to harness the value-added benefit of nutrient-microbe synergy, besides providing dynamism to microbial consortium suiting to wide range of perennial fruits (Srivastava and Singh, 2006). 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 response in terms of agronomic parameters, changes in soil physical properties, and pool of plant available nutrients (Cheke *et al.*, 2018). 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. In field, the rhizosphere hybridization can be implemented by collecting rhizosphere soil of healthy trees and injected into weaker trees to rationalise distribution of microbes across field/orchard as a part of natural farming with agro-ecology exploited as its best.

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

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 pseudomycoloides* (MF113272), *Paenibacillus alvei* (MF113275) and *Aspergillus flavus* MF113270) respectively.

Source: Unpublished data

With these efforts, we succeeded in answering some popularly raised questions summarised as: i. microbes can replace nutrients requirement of citrus nursery, considering abysmally 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 irrigation (using water extract of healthy rhizosphere either alone or in combination with cow urine, water extract of dung or mixture of water extract of healthy rhizosphere and fish pond water designed to suit natural farming) to evolve a new concept called "biofertilization" for exclusively citrus nursery and

ix. 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). The concepts such as this need to be fitted in a hill agroecosystem under fruit crops -based land uses (Srivastava *et al.*, 2008; 2025).

Conclusion and futuristic viewpoints

While addressing different issues of fruit nutrition, a cultivar evaluated under both intensive farming, organic farming or natural farming system may not perform with similar magnitude of success. The major difference lies with respect to differential soil health indices in a hill agroecosystem. Do we need to breed the fruit crops specifically tailored to such forms of farming (molecular approach to breeding of mineral deficiency resistance and mineral efficiency would facilitate produce nutritionally efficient biotypes in order to maximise the quality production of fruit crops on sustained basis), the answer is wrapped in an enigma for researchers to either refute such hypothesis or accept with sound scientific database proof. Another issue that keeps haunting is the strong necessity of developing on-farm module of natural farming, like organic farming, unless we succeed in these attempts, we will continue using natural farming more like a revitalistic model rather than forward looking agriculture model with more emphasis on genetic, functional and metabolic diversity of soil microorganisms within the rhizosphere of wide range of fruit crops (Srivastav and Singh, 2009). The capacity of soil microbial communities to maintain functional diversity of those critical soil processes could ultimately be more important to ecosystem productivity and stability than mere taxonomic diversity. In this context, it remains to be assessed how nutrient-microbe synergism is associated with productivity of perennial fruit crops.

The success of citrus crop, especially under hill agroecosystem needs to decipher the mechanistic pathways of both negative and positive crops responses to delve upon an effective mitigation technique (Kohli *et al.*, 1998; Srivastava and Singh, 2004a; 2004b; 2008a; 2008b; Srivastava *et al.*, 2014) including with improved irrigation and fertigation strategies (Shirgure *et al.*, 2001a; 2001b; 2001c; 2001d;

2001e; Srivastava *et al.*, 2003). Fruit quality traits involving primary metabolism (flavor and soluble solid/acid ratio defining the external fruit quality...?) are improved coupled with biochemical pathways (accumulation of antioxidants) relating plant defense. Interestingly, the other fruit parameters defining peel/skin color, skin disorder, and nutritional value (nutrient density traits) are invariably negatively affected by climate change to varying proportions. Such kind of responses of climate change infect neutralize the increasing intervention of production technologies, unless a holistic strategic framework is developed involving concepts like biofertigation with subsurface micro irrigation, microbial consortia loaded with multi-utility microbes (including mycorrhizas) up scaled to integrated soil fertility options, rhizosphere hybridization, fruit crops-based integrated land use, biochars-mediated soil fertility regulations (Agegnehu *et al.*, 2017; Mousavi *et al.*, 2022) though field evidences are still limited (Chaplot *et al.*, 2025), citrus under protected system coupled with an eye on reduction in carbon and water foot-prints, plant nutrition mediated plant defense and extended post-harvest life (Tripathi *et al.*, 2022), further backed up by microbial engineered fruit crops loaded with endophytes for additional soil-plant health resilience, dip-digging of climate-smart 4R- nutrient stewardship (Srivastava, 2020), besides carbon trade-offs between carbon sequestration and rural development better placed with fruit crops. Amid these issues, the application of artificial intelligence in form of nutrient modelling for prediction of nutrient constraints (Srivastava, 2011; 2015b; Srivastava *et al.*, 2025) and their tailoring via exploiting the spatial variability in soil fertility for precision management (Srivastava, 2013; Srivastava *et al.*, 2006; Srivastava and Singh, 2016), including the application of citrus-based customized fertilizers (Srivastava and Pandey, 2021) hold new normals of citriculture fitted under hill agroecosystem. Some of the major issues needing a fresh relook to better ward off climate change using hill agroecosystem are further highlighted as below:

i. Most of the fruit crops have C3 photosynthetic pathways, and introduction of C4 pathway in C3 fruit crops including citrus would be quite an accomplishment in offering a climate resilient and nutrient responsive fruit production system.

ii. A stewardship concept involving 4Rx4W (Nutrients x Irrigation water) deserves fresh look for fertigation-driven fruit production system. In this regard, role of growth regulators, anti-transpirants, and orchard floor management is undeniable. Introduction of plant-based biostimulants could also be a very good option.

iii. Microbial assembly disruption due to depletion in soil fertility over a period of time makes such exercise quite revealing about the nutrient load of fruit crops.

iv. Biofortification in citrus crop is often used to define their nutrient supplying capacity, despite they are known for rich source of mineral, vitamins, phytonutrients, and antioxidants, but these are highly questionable in nutrient depleted degraded soils. And, the nutrient density of crops is further reduced due to post-harvest losses and processing losses. But, without any well proven historical data about changes in nutrient density of citrus crops grown on pre-selected sites over a period of time, this issue is interchangeably interpreted using reductionist approaches as industrial biofortification (traits related to consumers acceptance), genetic biofortification (overlooking regional nutritional issues coupled with cultural issues targeting single crop-single nutrient), and agronomic biofortification (looking at frequently occurring deficiencies like Fe, Zn, P ignoring other nutrients). None the less, a bio-fortified crop should also look at consumer related traits and cooking characteristics. Accordingly, a practical meaning of nutrient density of citrus crops should be redefined catering to varied utility purposes.

v. Elevation in nutrient -use- efficiency in citrus crops has been the most intensively researched issue in the past, though more for theoretical implications. In this regard, we need some practical interventions to ensure the better fertilizer use by fruit crops, e.g. combination of growth regulators and nutrients, as foliar spray or a module involving nutrient sources having dynamic nutrient release kinetics with emphasis on reduced carbon and nutrient foot-print.

vii. Current citrus nutrition program is characterized by fertilizer application strategy addressing straight nutrient interaction overlooking the multi-nutrient interactions (e.g. Fe-Zn-N-P or N-S-Ca-Mg-K, level of interaction to be defined depending upon the nature and properties of soil and crop in question as a part

of coordinating nutrient interaction and nutrient stress signaling) need to be understood at physiological and molecular levels and their cross-talks with fruit yield in field and utilize these in developing alternative strategy of optimizing the fertilization options. The important candidate genes for such nutrient interactions and/or metabolic pathways could be of high utility to plant breeders to improve nutrient-use-efficiency and eventually unlocking the fruit yield barriers.

viii. Growing citrus crops under protected system (more popular now with heavy incidence of Huanglongbing, now operational with full artificial intelligence) is considered a kind of abatement to ongoing process of climate change with assured quality production, unless supported with suitable crop management technology. Many of the pandemic diseases, like citrus greening has forced citrus growers follow protected cultivation with great ease of success. Let's learn from such success and be prepared for any such disease urgency forcing a complete overhaul of plant nutritional strategy.

ix. Plant diseases and plant nutrition very often go hand-in-hand (Tripathi *et al.*, 2022), with the result nutrient diagnostics an optimum fertilizer doss coupled with their application schedule earlier developed for disease free fruit crop, may not be exactly so effective with introduction of diseases. We need to develop a dynamic strategy to address two contrastingly different production scenarios comparing ameliorative response versus curative response of crop under hill agroecosystem versus conventional plain land citrus growing, beside catering any adverse response of climate change.

x. Various forms of conventional farming viz., organic farming, natural farming, and regenerative farming, all advocating more rationale use of agroecology, need a more incisive analysis with emphasis on plant nutrition vis-a-vis production sustainability, keeping an eye on silicon nutrition and post-harvest fruit quality.

These are only a few examples of challenges in citrus nutrition in hill agroecosystem emphasizing how plant nutrition needs to integrate over different scientific disciplines to meet the societal requests for productive, sustainable, and environmentally sound fruit nutrition program. The other side of the coin is that plant nutritionists face a

challenging time with great opportunities for novel developments and exciting discoveries pertaining a scientifically sound climate- neutral citrus nutrition program. A gene-edited citrus is near to commercialization against citrus greening (Huanglongbing), let's hope in years to

come, we find some plausible solution to multiple nutrient constraints-low nutrient absorption rate/utilization rate through microbially engineered citrus or gene edited citrus for elevated nutrient-use-efficiency.

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