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# Response of microbial consortium to leaf phenotype of acid lime

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**Abstract**
Citrus is an important crop of world and acid lime hold third position after sweet orange and mandarin. All the citrus crop response to biofertilizers treatments very well. The present study was carried out in ICAR-Central Citrus Research Institute, Nagpur to evaluate different microbial fortification treatments to the rhizosphere of acid lime. Treatments involved rhizosphere soil of acid lime and Nagpur mandarin, microbial consortium (*Bacillus pseudomycoides* Nakamura, *Acinetobacter radioresistens* Nishimura, *Micrococcus yunnanensis* Cohn, *Aspergillus flavus* Link and *Paenibacillus alvei* Cheshire and Cheyne) and freshly prepared vermicompost (nitrogen equivalent basis to RDF) in various combinations. Eight treatments with three replications were set up with Randomized block design. Results revealed that equivalent ratio of RDF in vermicompost form along with microbial consortium significantly increased the leaf length and width. Which shows its ability to photosynthesize more food and ultimately more yield.

**Keywords:** microbial consortium, rhizosphere, soil micro-organism, bio-fertilizers, acid lime

## Introduction

Citrus, an important genus from Rutaceae family (Velasco and Concetta, 2014) [15], is an ancient perennial crop more often cultivated in tropical and sub-tropical parts of the globe (Hvarleva *et al*., 2008) [7]. Acid lime is an important citrus crop in India with more than 25 lakh tonnes of annual production and 2.5 lakh area under cultivation. India is the largest producer of acid lime in the world (Singh, 2010) [9]. It is generally grown under both tropical and subtropical climatic conditions in the plains and up to an elevation of 1200 MSL. Lime is appetizer, stomachic, antiscorbutic, antihelmintic and it checks biliousness (Thirugnanavel *et al*. 2007) [13]. As lime juices have profound amounts of citric acid, comprising 1.44 g/oz (Penniston *et al*., 2008) [10], beverages with citric acid are reported to reduce the content of calcium, and ultimately enhancing an excretion of urinary citrates. Hence, it could be a good dietary supplement for preventing and managing calcium urolithiasis (kidney stone) (Aras *et al*., 2008) [1]. The productivity (8 t ha-1) of acid lime is low in India as compared to the higher productivity (20 t ha-1) in other countries. These production and productivity targets will be achieved through modern intensive horticulture practices and use of most recent technologies, including nutrient management.

The rhizosphere (zone of soil influenced by plant roots and characterized by an important microbiological activity) represents a highly dynamic region governed by a complex mosaic of interactions between plants and microorganisms (Kennedy and De Luna 2004) [8]. Structural properties of rhizosphere depend on the functioning of roots and microflora. Despite extensive experimental evidence of root-mediated changes to the physical, chemical, and biological status of rhizosphere soil, the quantitative significance of these changes for nutrient availability vis-à-vis crop response has not been fully assessed (Darrah 1993) [3]. Microbial structural and functional diversity of soil present within the rhizosphere heavily influence the soil and plant quality, besides ecosystem sustainability (Wu, Srivastava, and Zou 2013) [17]. Many genes involved in key ecological processes such as nitrogen cycling, carbon fixation, phosphorus utilization, metal homeostasis, and resistance were significantly greater in healthy than in disease-infected citrus rhizosphere (Trivedi *et al*. 2011) [14].

Conventional methods of fertilization although helped in improving the fruit quality but continuous fertilization, has failed to sustain the same yield on a long-term basis due to depletion of soil carbon stock and consequently emerged multiple nutrient deficiencies, would be further triggered through changes in microbial communities and activities within the rhizosphere (Patel *et al*., 2009) [9]. Such changes will adversely affect the orchard's productive life in the long run. Integrated nutrient management is a holistic approach which maintains the soil fertility and nutrient supply to an optimum level for sustaining the desired crop productivity (Chundawat, 2001; Deshmukh *et al*., 2018) [2, 4]. Organic manure applied to soil, improve the soil physical properties and add important nutrients to the soil increase the availability and its ultimate absorption by plant. Biofertilizer like *Azospirillum*, *VAM* and *PSB* fix major nutrients in soil. The application organic manure and biofertilizers help in better utilization of added inorganic fertilizers and reduce its application level as well as the deleterious effect of harsh chemical fertilizers use **(**Dheware and Waghmare, 2009) [5]. For this reason, the development of new vigorous plants production techniques, able to be adapted to different pedoclimatic conditions once implanted, promote healthier farming systems, reduce the use of chemical inputs and ensure the profitability of crops and the quality of the environment becomes necessary. It is for this purpose that rhizosphere fortification in favor of citriculture was found important. Keeping in view the present investigation was carried out to testify the effect fortified rhizosphere on flowering behaviour in an established acid lime orchard.

## Materials and Methods
The present experiment was carried out in seven-year-old acid lime cv. NRCC acid lime-7, planted in a spacing of 20 feet x 17.20 feet at experimental farm of ICAR-Central Citrus Research Institute, Nagpur, Maharashtra, India during 2019-20 and 2020-21. For the experiment, forty-eight trees were selected on the basis of uniform vigour and were maintained under uniform cultural practices during the entire course of investigation.

## Rhizosphere soil collection
Some well grown plants of Nagpur mandarin and Acid lime having high vegetative growth, coinciding with the period of leaves completing 6–8 months of new flush growth (spring bloom, August–October) and the zone having maximum concentration of feeder roots at soil depth of 0–20 cm, were selected and irrigated twenty-four hours before its collection to revive the microbial growth in the rhizosphere. The rhizosphere soils (two kilograms for each plant) were collected and isolated in early morning, 1-1.5 m distance from trunk and at a depth of 0-20 cm, as maximum feeder roots and high amount of microbial mass is present in this zone of soil depth. The rhizosphere soils are taken by "V" notch method with the help of spade.

Treatments involved rhizosphere soil of acid lime and Nagpur mandarin, microbial consortium (*Bacillus pseudomycoides* Nakamura, *Acinetobacter radioresistens* Nishimura, *Micrococcus yunnanensis* Cohn, *Aspergillus flavus* Link and *Paenibacillus alvei* Cheshire and Cheyne) and freshly prepared vermicompost (nitrogen equivalent basis to RDF). The details of treatments are $T_1$- Control, $T_2$- $VC_{20}$ (Vermicompost 20kg), $T_3$- $VC_{20}$ + MC (Vermicompost 20 kg + Microbial Consortium), $T_4$- $VC_{10}$ + MC (Vermicompost 10 kg + Microbial Consortium), $T_5$- $VC_{20}$ + RzAL (Vermicompost 20 kg + Rhizosphere soil of Acidlime), $T_6$- $VC_{20}$ + RzNM (Vermicompost 20 kg + Rhizosphere soil of Nagpur mandarin), $T_7$- $VC_{20}$ + RzAL + RzNM (Vermicompost 20 kg + Rhizosphere soil of Acid lime + Rhizosphere soil of Nagpur mandarin), $T_8$- $VC_{10}$ + MC + 2,4- D (Vermicompost 10 kg + Microbial Consortium + 2,4- dichlorophenoxyacetic acid). A sufficient irrigation was given to each plant before the treatment application to active rhizosphere zone of acid lime. Eight treatments with three replications were set up with Randomized block design. Ten leaf sample were collected from each direction and length and breadth were measured by Vernier calliper and expressed as mm.

## Statistical analysis
The statistical analysis was carried out for each observed character under the study using MS-Excel, OPSTAT. The data generated from these investigations were appropriately computed, tabulated and analysed as described by Gomez and Gomez (1983) [6] by applying Randomized Block Design (RBD). The level of significance was tested for different variables at 5 per cent level of significance.

## Results and Discussions
It can be seen from the graph that leaf length and leaf width varied significantly by the treatments. Maximum growth of leaf length was found in $T_3$ (Vermicompost 20 kg + Microbial Consortium) treatments followed by $T_7$ (Vermicompost 20 kg + Rhizosphere soil of Acid lime + Rhizosphere soil of Nagpur mandarin) and $T_8$ (Vermicompost 10 kg + Microbial Consortium + 2,4-dichlorophenoxyacetic acid). However, all these treatments are significantly higher than the control. Similarly, Leaf width also followed a similar pattern like leaf length. In $T_3$ (Vermicompost 20 kg + Microbial Consortium) treatment, width was found to be highest, which is statistically at par with $T_7$ (Vermicompost 20 kg + Rhizosphere soil of Acid lime + Rhizosphere soil of Nagpur mandarin) and $T_8$ (Vermicompost 10 kg + Microbial Consortium + 2,4-dichlorophenoxyacetic acid). All the treatments were significantly superior than control.

Presence of various microorganism in the different treatments played important role in nutrient mobilization. In the applied microbial consortium, Aceinetobacter is known for its nitrogen fixation, Bacillus pseudomycoides is known for potassium mobilization and other microorganism are known to be phosphorous mobilization. These microorganisms may help in mobilization of nutrient to the plant helped to increase the leaf length and leaf width. These results are accordance with Srivastava *et al* (2015) [12].

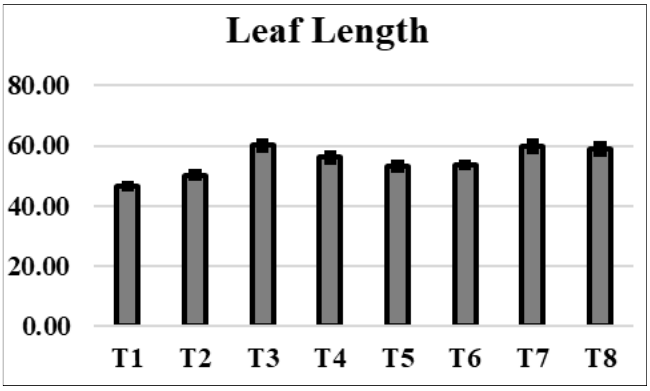


**Fig 1**

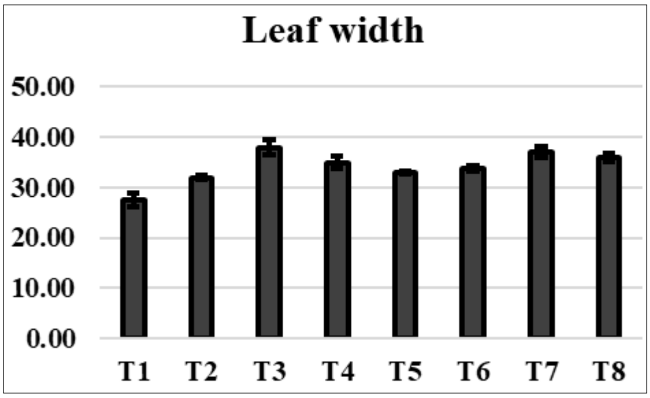


**Fig 2**

## Conclusion
Acid lime rhizosphere response positively to biofertilizers treatments. In the above study, microbial fortification leads to increase the leaf length and leaf width significantly over the control. However, more research should be done to see the efficacy of microbial consortium in various crops.

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