



Agronomic performance of acid lime in response to microbial fortification of rhizosphere

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ABSTRACT

Rhizosphere security has become a pre-requisite to sustain the production by exploiting the microbiome profile of acid lime, fortifying with microbes is one effective way of deciphering the negative interaction between plants and microbes. We conducted an experiment to testify the effect of microbial fortification from different crop rhizosphere in combination with vermicompost on growth and quality of acid lime (cv NRCC Acid Lime-7) at the Experimental Farm of ICAR- Central Citrus Research Institute, Nagpur, Maharashtra. As many eight treatments consisting of rhizosphere soil of acid lime (*Citrus aurantifolia* Swingle), Nagpur Mandarin (*Citrus reticulata* Blanco) and microbial consortium (*Bacillus pseudomycolides*, *Acinetobacter radioresistens*, *Micrococcus yunnanensis*, *Aspergillus flavus* and *Paenibacillus alvei*) along with vermicompost were tested in acid lime during 2018-20. All the vegetative growth parameters, viz. plant height, trunk girth, tree spread and canopy volume were observed significantly higher with treatment T₃ (20 kg vermicompost + 50 ml microbial consortium), well supported by yield attributing physical characters (fruit length, width and weight) and fruit quality parameters (juice content, TSS and acidity). Our studies, hence, suggested a significant interplay of microbial consortium with small starter dose of vermicompost producing both, qualitative as well as quantitative improvements in acid lime cultivation.

Key words: Acid lime, Biometric response, Microbial consortium, Physico-chemical parameters, Rhizosphere fortification

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). Soil microbial diversity within the rhizosphere is highly effective in changing the different rhizosphere properties by changing the soil microbial biomass nutrient, microbial communities and plant available supply of nutrients (Wu *et al.* 2013, Ngullie *et al.* 2015). Microbial fortification aid in increasing the soil organic matter of soils to boost citrus productivity, sequester CO₂, enhance soil microbial growth and activities and improve water capture and retention (Srivastava *et al.* 2017). However, still bigger question emerges, whether rhizosphere competent microbes could collectively contribute towards improved resilience of plant's rhizosphere (Wang *et al.* 2014) and hence better soil healthcare (Srivastava and Singh 2004a, 2004b). A sound understanding of nutrient- microbe synergy could possibly lay a solid foundation in unlocking the productivity potential of perennial fruit crops (Srivastava *et al.* 2014). Continuous fertilization has failed to sustain

the yield expectancy on a long term basis (Srivastava and Singh 2009, Wu and Srivastava 2012), with the result, such changes will adversely dictate on the orchard's productive life in long run (Srivastava *et al.* 2015).

Organic manure applied to soil, improve the soil physical properties and add important nutrients to the soil to raise the soil fertility and facilitate absorption by citrus (Srivastava *et al.* 2019). Biofertilizers like *Azospirillum*, VAM and PSB fix major nutrients in soil. The development of new production techniques, adapted to different pedoclimatic conditions to be able to promote healthier citrus rhizosphere, reduce the use of chemical inputs and ensure the profitability of citrus crop have become so mandatory (Srivastava *et al.* 2002). In this background, studies were carried out to evaluate various methods of rhizosphere fortification for field response of acid lime with regard to yield and fruit quality on an alkaline clay soil.

MATERIALS AND METHODS

Experimental set up: The present study was carried out during 2018–20 in seven-year-old acid lime cv. NRCC acid lime-7, planted with spacing of 6 m × 5 m at Experimental Farm of ICAR-Central Citrus Research Institute, Nagpur, Maharashtra, India. As many, forty-eight trees were selected on the basis of uniform vigour and maintained under

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uniform cultural practice (drip irrigation in alternate day, use of imidachloprid to control psylla and leaf miner, no pruning, weeding of orchard along with plant basin as when observed). The initial soil fertility analysis showed: soil texture (clay 38.8%, silt 23.0% and sand 38.2%) pH 7.7, EC 0.158 dS/m, potassium permanganate (KMnO_4)-N 114.6 mg/kg, Olsen-P 9.3 mg/kg, ammonium acetate (NH_4OAc)-K 114.5 mg/kg, diethylene triamine pentaacetic acid (DTPA)-iron (Fe) 6.95 mg/kg, DTPA-manganese (Mn) 6.11 mg/kg, DTPA-copper (Cu) 1.11 mg/kg and DTPA-zinc (Zn) 0.65 mg/kg. The soil was taxonomically classified as alkaline smectite rich Vertic Ustochrept as per USDA soil taxonomy. The rhizosphere soils (two kilograms for each plant) were collected from well-established and high yielding plants of Nagpur mandarin and acid lime at 1–1.5 m distance from trunk and at a depth of 0–20 cm coinciding with zone of maximum feeder roots and microbial biomass.

Treatments involved rhizosphere soil of acid lime and Nagpur mandarin, microbial consortium (*Bacillus pseudomycoides* Nakamura (MF113272), *Acinetobacter radioresistens* Nishimura (MF113273), *Micrococcus yunnanensis* Cohn (MF113274), *Aspergillus flavus* Link (MF113270) and *Paenibacillus alvei* Cheshire and Cheyne (MF113275)) and freshly prepared vermicompost (nutrient composition: 2.87% N, 0.62% P, 1.77% K, 300 ppm Fe, 121 ppm Mn, 26 ppm Cu and 25 ppm Zn). 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 acid lime), 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). The microbial consortium of 100 ml (with base population of *Micrococcus yunnanensis* Cohn (MF113274) 33×10^7 cfu/ml, *Paenibacillus alvei* Cheshire and Cheyne (MF113275) 14×10^7 cfu/ml, *Aspergillus flavus* Link (MF113270) 32×10^7 cfu/ml, *Acinetobacter radioresistens* Nishimura (MF113273) 10×10^6 cfu/ml, and *Bacillus pseudomycoides* Nakamura (MF113272) 7×10^5 cfu/ml) was applied within the perimeter of trees corroborating with 15 days prior to flowering. A sufficient irrigation was given to each plant before the treatment to active rhizosphere zone of acid lime. Eight treatments with three replications were evaluated under randomized complete block design.

Observation on plant growth, yield and quality: The height and spread (E-W $\times$ N-S) of the trees was measured in meters (m) with the help of graduated flag staff, once before the start of the growing season and application of treatment followed by observation at the termination of growth cycle. Tree height and spread were expressed as tree volume 0.524 HD^2 ; where H and D stand for plant height and tree spread, respectively. The fruit size in terms of length and diameter of ten randomly selected fruits per

replication was recorded with the help of digital Vernier Calliper (Model: CD 6" CS). The fruit shape index was calculated by dividing fruit length by fruit diameter. Fruit quality parameters like juice percentage (calculated by dividing juice weight to fruit weight and multiplying into 100), total soluble solids (measured by hand refractometer) and titratable acidity carried out as per the guideline suggested by Ranganna (2001).

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 analysed as described by Gomez and Gomez (1983) by applying randomized block design (RBD) at 5% level of significance.

RESULTS AND DISCUSSION

Growth response: Different treatments of rhizosphere fortification exerted a significant influence on plant height, trunk girth, tree spread (E-W and N-S) and canopy volume. The plant height, trunk girth, east-west spread, north-south spread and canopy volume under various treatments varied from 3.70–3.90 m, 38.24–40.95 cm, 4.08–4.22 m, 4.26–4.46 m and 29.19–34.36 m^3 , respectively (Table 1). However, cumulative percentage increase in growth parameters like plant height, trunk girth, east-west spread, north-south spread and canopy volume over these two years was observed to be highest with T_3 involving VC_{20} + MC as 13.59%, 8.05%, 17.76%, 18.68% and 52.52%, respectively compare to corresponding value of 8.75%, 3.94%, 15.38%, 15.54% and 36.86% with control treatment. However, T_7 (VC_{20} + RzAL + RzNM) and T_8 (VC_{10} + MC + 2,4-D) were observed statistically at par with T_3 . The increase in trunk diameter was attributed to the stimulatory activity of microflora in the rhizosphere leading to increased nutrient availability, thereby, ensuring vigorous plant growth. Application of vermicompost as source of organic carbon further aided in building of added microbial consortium to multiply fast and support the plant growth by maintaining the nutrient supply rate to plants (Srivastava *et al.* 2015, Hota *et al.* 2020).

Fruit yield response: Various rhizosphere fortification treatments showed a differential response on fruit size, weight and yield parameter (Table 2). The fruit length, width, weight and yield/plant here observed to vary from 42.75–50.46 mm, 41.57–48.52 mm, 37.97–48.53 g and 13.63–22.31 kg, respectively. Out of all the treatments, T_3 (VC_{20} + MC) was observed to be far superior over rest of other treatments including control. The treatments T_7 (VC_{20} + RzAL + RzNM) and T_8 (VC_{10} + MC + 2,4-D) was found to be statistically similar with T_3 except fruit yield parameter, where T_3 was out performed all the other treatments. Fruit shape index showed no significant difference, since it is governed by the genetic factor rather than response with any cultural practice. The increase in vegetative growth is accounted to an increased photosynthesis associated with elevated translocation of nutrients to developing fruits, resulting in increase in fruit dimension and fruit weight. Since the total plant canopy volume increased significantly

Table 1 Effect of various rhizosphere fortification treatments on vegetative growth of acid lime during 2018–20

Treatment	Plant height (m)	Trunk girth (cm)	Tree spread (m)		Canopy volume (m ³)
			E-W (m)	N-S (m)	
T ₁ - Control	3.73 (3.43)	39.30 (37.81)	4.20 (3.64)	4.46 (3.86)	31.52 (23.03)
T ₂ - VC ₂₀	3.76 (3.42)	40.22 (38.39)	4.21 (3.64)	4.43 (3.82)	31.89 (22.86)
T ₃ - VC ₂₀ + MC	3.76 (3.31)	39.45 (36.51)	4.11 (3.49)	4.32 (3.64)	31.13 (20.41)
T ₄ - VC ₁₀ + MC	3.88 (3.48)	39.53 (37.15)	4.22 (3.62)	4.37 (3.73)	33.70 (23.21)
T ₅ - VC ₂₀ + RzAL	3.70 (3.33)	38.66 (36.51)	4.19 (3.60)	4.30 (3.68)	30.31 (21.11)
T ₆ - VC ₂₀ + RzNM	3.66 (3.31)	38.24 (36.29)	4.08 (3.52)	4.26 (3.66)	29.19 (20.59)
T ₇ - VC ₂₀ + RzAL + RzNM	3.90 (3.46)	38.73 (36.01)	4.21 (3.58)	4.41 (3.74)	34.36 (22.87)
T ₈ - VC ₁₀ + MC +2,4-D	3.84 (3.42)	40.95 (38.30)	4.11 (3.51)	4.31 (3.66)	32.41 (21.95)
CD (P=0.05)	0.13 (0.11)	1.34 (1.28)	NS (0.11)	NS (0.15)	2.44 (1.62)

Data in the parentheses indicate initial value before the experiment 2018. VC₂₀- Vermicompost 20 kg, VC₁₀- Vermicompost 10 kg, MC- Microbial consortium, RzAL- Rhizosphere soil of acid lime, RzNM- Rhizosphere soil of Nagpur mandarin, 2,4-D- 2,4-dichlorophenoxyacetic acid.

Table 2 Effect of various rhizosphere fortification treatments on yield and physico-chemical parameters of acid lime during 2018–20

Treatment	Fruit length (mm)	Fruit width (mm)	Fruit shape index	Fruit weight (g)	Yield (kg/ plant)	Juice (%)	TSS (°Brix)	Acidity (%)
T ₁ - Control	42.75	41.57	1.03	37.97	13.63	44.85	8.43	6.10
T ₂ - VC ₂₀	46.22	44.13	1.05	40.57	15.58	42.45	8.61	6.36
T ₃ - VC ₂₀ + MC	50.46	48.52	1.04	48.53	22.31	48.66	9.23	8.18
T ₄ - VC ₁₀ + MC	47.62	45.62	1.04	44.41	18.72	44.39	8.79	7.42
T ₅ - VC ₂₀ + RzAL	46.68	45.21	1.03	43.18	17.17	43.01	8.67	6.95
T ₆ - VC ₂₀ + RzNM	46.08	44.82	1.03	41.66	16.33	43.33	8.58	6.79
T ₇ - VC ₂₀ + RzAL + RzNM	49.00	46.13	1.06	46.00	20.19	48.54	9.00	7.72
T ₈ - VC ₁₀ + MC +2,4-D	49.59	46.94	1.06	46.91	20.17	45.71	8.86	7.87
CD (P=0.05)	1.62	1.96	NS	2.13	1.97	3.32	0.34	0.28

VC₂₀, Vermicompost 20 kg; VC₁₀, Vermicompost 10 kg; MC, Microbial consortium; RzAL, Rhizosphere soil of acid lime; RzNM, Rhizosphere soil of Nagpur mandarin; 2,4-D- 2,4-dichlorophenoxyacetic acid.

over control, thereby, increasing fruit bearing area of treated plant. Increase in total fruiting area along with fruit size and fruit weight led to an increase in fruit yield of acid lime. Earlier studies showed that 75% of RDF + 25% vermicompost + microbial consortium increased the yield and quality of Nagpur mandarin grown on black clay soil of central India (Srivastava *et al.* 2015).

Fruit quality response: The variation in response to qualitative parameters of acid lime fruits in relation to rhizosphere fortification treatments was observed statistically significant (Table 2). Different fruit quality parameters, viz. juice percentage, TSS and acidity were observed favourable with treatment T₃ (VC₂₀ + MC), statistically significant over other treatments like T₇ (VC₂₀ + RzAL + RzNM) and T₈ (VC₁₀ + MC +2,4-D). Increase in fruit size led to an increase juice percentage. Srivastava *et al.* (2019) tested the efficacy of microbial consortium to reduce the recommended fertilizer dose of Nagpur mandarin and observed that microbial consortium along with vermicompost not only reduced the fertilizer dose up to 30–40% but also increased

the qualitative parameters of fruits significantly and added an increase carbon loading of rhizosphere. Microbial consortium containing different plant growth promoting bacteria helped in production of phytohormones, nitrogen fixation and phosphorus mobilization, collectively aided in increasing the fruit quality parameters such as TSS and acidity of fruit (Kundan *et al.* 2015).

Our studies, hence, provided a database proof that microbial fortification of rhizosphere through microbial consortium enhanced the quality attributes of acid lime via better physiological and biochemical preparedness of inoculated without compromising with fruit yield.

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