Indian Journal of Agricultural Sciences **93** (4): 392–396, April 2023/Article
https://doi.org/10.56093/ijas.v93i4.134247

Effect of different biofertilizer strains on soil, leaf and yield parameters of Kinnow mandarin

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Received: 12 March 2023; Accepted: 31 March 2023

ABSTRACT

Present field study was carried out to investigate the effects of biofertilizer strains on soil, leaf and yield parameters of Kinnow mandarin (*C. nobilis* × *C. deliciosa*) during 2018–19 and 2019–20 at the experimental orchard of Department of Horticulture, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana. The study revealed that soil, leaf nutrient status and yield parameters were significantly affected with the application of biofertilizers. There was synergistic influence of *Azotobacter* and PSB strains. The magnitude of increase with the application of different bio-strains was more pronounced when applied in combination with lower dose of fertilizers (75% RDF) than their combination with higher dose (100% RDF). 100% RDF + *Azotobacter chroococcum* Mac 27 + *Pseudomonas* P 36 (T_7) was found most effective in enhancing soil macro-micro nutrients, leaf nutrient status and yield parameters. Soil available N and P increased significantly in 0–15 cm soil layer during both years but in 15–30 cm soil layer, the enhancement was observed only during second year of investigation, however Fe, Zn and Mn showed significant improvement only in 0–15 cm soil layer during both years. Leaf N content increased in September, however no significant effect was observed in March values. Leaf N content showed fast increment as compared to leaf P content.

Keywords: *Azotobacter*, Kinnow, Macro-micro nutrients, Microbial count, *Pseudomonas*

Kinnow mandarin (*C. nobilis* × *C. deliciosa*) is the most important citrus crop of the regions of Haryana and Punjab adjacent to Rajasthan where the prevailing agro-climatic conditions favour cultivation of this crop. It is very efficiently cultivated in Haryana under arid to semi-arid soil conditions. In Haryana, citrus crops occupy 23.3 thousand hectares (ha) area with yield of 517828 million tonnes (MT) and productivity of 22.21 MT/ha (Anonymous 2021). Due to the excessive use of chemical fertilizers and pesticides, the soil health in terms of physical, biological and chemical terms is apparently deteriorating in the whole tract of this high productivity zone and hence, there is utmost need of integrating the organic options with inorganic measures. Overuse of chemical fertilizers not only depletes soil nutrients but also reduces the yield and poisons the whole ecosystem (Li *et al.* 2020). Secondly, excessive use of chemicals is proving detrimental to the health and very existence of soil flora and fauna in terms of soil microorganisms, earthworms etc. and this has become an area of interest for scientists, farmers and whole agriculture sector to find soil and environment friendly substitutes and alternatives to chemical farming without having negative effect on production. Besides this, another vital research area is to find crop specific strains in long duration fruit crops like Kinnow which can establish themselves in particular agro-climatic and soil conditions.

Substitute of even a smaller fraction of chemical fertilizers with these strains may be helpful in improving soil, human and plant health along with a promising short term financial support to the farming community. The information regarding this aspect in Kinnow mandarin is hardly traceable in the available literature and thus the present investigation was carried out with the objective to extrapolate strain specific effect of biofertilizers on soil, leaf and yield in Kinnow.

MATERIALS AND METHODS

The present study was carried out at experimental orchard of the Department of Horticulture, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana during 2018–19 and 2019–20 on 10 years old kinnow plants budded on rough lemon rootstock and planted at 6 m × 6 m. The experiment comprised of 9 treatments, viz. T_1, 75% RDF + *Azotobacter chroococcum* Mac 27;

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Table 1 Initial status of soil

Soil properties	Depth (cm)	
	0–15	15–30
*p*H (1:2)	7.70	7.90
EC (dS/m)	0.28	0.30
Available Nitrogen (kg/ha)	124.8	120.5
Available Phosphorus (kg/ha)	11.60	11.27
Available Potassium (kg/ha)	340.1	331.6
DTPA-extractable Iron (ppm)	6.65	6.58
DTPA-extractable Manganese (ppm)	6.59	6.41
DTPA-extractable Zinc (ppm)	2.10	1.99
DTPA-extractable Copper (ppm)	0.41	0.38

T_2, 75% RDF + *Azotobacter chroococcum* HT 54; T_3, 75% RDF + *Azotobacter chroococcum* Mac 27 + *Pseudomonas* P 36; T_4, 75% RDF + *Azotobacter chroococcum* HT 54 + Pseudomonas P 36; T_5, 100% RDF + *Azotobacter chroococcum* Mac 27; T_6, 100% RDF + *Azotobacter chroococcum* HT 54; T_7, 100% RDF + *Azotobacter chroococcum* Mac 27 + *Pseudomonas* P 36; T_8, 100% RDF + *Azotobacter chroococcum* HT 54 + *Pseudomonas* P 36 and; T_9, Control (RDF). The 50 ml biofertilizer culture (each of *Azotobacter* and PSB with 10^7 cells/ml) was diluted with water to make final volume to 5 litres. Bio-fertilizers were applied in a ring, 75 cm away from the trunk of kinnow at a depth of 20–30 cm during the 3rd week of March. Initial status of soil was recorded by taking soil samples from 0–15 and 15–30 cm depth (Table 1).

Leaf nitrogen content was estimated as per standard procedure (Jackson 1967), leaf phosphorus content was estimated by Vanado-molybdo phosphoric acid yellow color method as described by Jackson (1967) and leaf potassium content was estimated from digested extract using Flame photometer as suggested by Piper (1966) and expressed in per cent. The alkaline permanganate method prescribed by Subbiah and Asija (1956) was used for the estimation of available nitrogen in soil. For the estimation of available phosphorus, Olsen's method (Olsen *et al.* 1954) was used. Available potassium was estimated by neutral normal NH_4OAC solution using flame photometer (Hanway and Heidal 1952). Available micronutrients (Fe, Mn, Zn and Cu) were estimated in DTPA solution using atomic adsorption spectrophotometer as suggested by Lindsay and Norvell (1978). Yield was calculated by multiplying total number of fruits/tree with average fruit weight and average has been expressed in kilograms per tree (kg/tree). The data during 2018–19 and 2019–20 was analyzed in Randomized Block Design (RBD) and mean values are presented.

RESULTS AND DISCUSSION

Rhizosphere application of *Azotobacter* and *Pseudomonas* strains significantly affected leaf nutrient status (Table 2). Maximum leaf N content (2.41% and 2.44% during September 2018–19 and 2019–20, respectively) was observed with T_7 closely followed by T_5 and T_8. The effect of *Azotobacter chroococcum* Mac 27 was found more pronounced than *Azotobacter chroococcum* HT 54. T_7 resulted in an increment ranging from 8.5–9.91% in leaf N content over control. All the 100% RDF + biofertilizer treatments (T_5, T_6, T_7 and T_8) were found superior to that of control in increasing leaf N content. No significant effect was exploited in March leaf N content. However, a temporal gap was observed in leaf P content increment as compared to leaf N content. Delayed and significant improvement in leaf P content was reflected in second half of 2019–20, whereas, in case of leaf N content improvements were fetched in quite short time frame. Maximum leaf P content (0.19%) was found with T_7 closely followed by T_8. The increment over control was 26.67%, whereas in case of leaf N the enhancement was restricted to quite lower range. The effect of *Pseudomonas* P 36 might be considered highly effective and significant but more time dependent. Most interesting fact uncovered was that T_3 was successfully not only found able to substitute 25% RDF, but was found significantly superior to control in enhancing leaf N and P content. Moreover, with surplus 25% RDF, i.e. T_7, about 5 and 11% increase in leaf N and P content respectively, was observed over T_3, the impact on P content again being higher. Improved leaf nutritional status with increased RDF might be due to more availability of food source for micro-organisms. Leaf nutrient content directly reflects nutritional status of plant and soil and significantly affects fruit growth and development stages. Difference in March and September values quantifies source-sink relationship and could be considered important for future research endeavours as earlier studies were focused either on pathways of nutrient supply chain or more fundamental approaches to detect nutritional deficiency or status. Leaf K content was not significantly affected.

The increased status of nutrients in the plant may be due to the increased availability of N and P in soil which might be ascribed to biofertilizers inoculation that helped the plants to increase the dehydrogenase, alkaline phosphatase, nitrogenase and hydrolysis enzymatic activities mainly due to increase in the rhizosphere microbial population as a consequence of the inoculation treatments (Aseri and Tarafdar 2006). Phosphatases in the soil environment are considered to play a major role in the mineralization process of organic phosphorus by catalyzing hydrolytic cleavage of inorganic phosphate groups from organic phosphorus compounds (Dick 1980). The dehydrogenase enzyme catalyses the reduction of several compounds having triple bonds (Powar and Daginwala 2004). The pectinolytic enzymes soften the middle lamella of the cell wall and increase the mineral absorption resulting into increase in leaf nitrogen content. The increased leaf nutrient status may also be attributed to the fact that biofertilizers in combination with FYM might have improved the physical condition of soil, root development and more soil moisture retention which resulted in increased uptake of water and nutrients (Morselli *et al.* 2004). The more availability of phosphorus

Table 2 Impact of soil application of *Azotobacter* and *Pseudomonas* strains on leaf nutrient content and soil macro nutrients

Treatment	Leaf N content (%)				Leaf P content (%)				Soil available N (kg/ha)				Soil available P (kg/ha)			
	2018–19		2019–20		2018–19		2019–20		2018–19		2019–20		2018–19		2019–20	
	March	September	March	September	March	September	March	September	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm
T_1	1.24	2.29	1.24	2.30	0.08	0.14	0.09	0.15	128.6	125.1	129.2	125.6	11.78	11.49	11.91	11.68
T_2	1.21	2.24	1.22	2.24	0.09	0.14	0.09	0.15	128.2	124.7	128.9	125.0	11.70	11.33	11.89	11.56
T_3	1.24	2.30	1.24	2.32	0.09	0.15	0.11	0.17	129.7	125.6	129.5	126.0	12.36	12.21	13.05	12.23
T_4	1.22	2.25	1.23	2.26	0.09	0.15	0.12	0.17	128.9	125.4	129.3	125.6	12.05	11.68	12.91	12.21
T_5	1.30	2.39	1.32	2.41	0.09	0.14	0.09	0.15	131.0	129.4	132.0	129.7	11.78	11.91	12.33	12.10
T_6	1.26	2.37	1.27	2.38	0.09	0.14	0.09	0.15	130.2	128.1	131.2	128.9	11.67	11.21	12.05	11.89
T_7	1.31	2.41	1.33	2.44	0.09	0.16	0.12	0.19	131.3	129.4	132.2	129.9	13.91	12.67	14.55	13.87
T_8	1.28	2.38	1.29	2.40	0.09	0.16	0.12	0.18	130.5	128.7	131.5	129.2	13.78	12.35	14.21	13.35
T_9	1.20	2.22	1.21	2.22	0.08	0.14	0.09	0.15	123.7	120.5	124.4	120.3	11.70	11.49	11.70	11.59
CD (P=0.05)	NS	0.04	NS	0.04	NS	NS	NS	0.02	2.10	NS	2.50	3.10	1.44	NS	1.49	1.45

Treatment details are given in Materials and Methods.

Table 3 Impact of *Azotobacter* and *Pseudomonas* strains on yield and soil micronutrient status

Treatment	Yield (kg/tree)		Available Zn (ppm)				Available Fe (ppm)				Available Mn (ppm)			
	2018–19	2019–20	2018–19		2019–20		2018–19		2019–20		2018–19		2019–20	
			0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm	0–15 cm	15–30 cm
T_1	76.0	79.7	2.07	1.97	2.09	1.98	6.69	6.60	6.70	6.61	6.85	6.70	6.84	6.69
T_2	74.8	78.5	2.10	1.98	2.11	2.00	6.72	6.63	6.73	6.65	6.83	6.67	6.82	6.66
T_3	78.6	81.8	2.12	2.04	2.13	2.07	6.83	6.68	6.86	6.69	7.04	6.72	7.05	6.73
T_4	77.5	81.2	2.15	2.05	2.15	2.04	6.85	6.67	6.85	6.69	7.04	6.73	7.06	6.72
T_5	81.2	83.3	2.10	1.98	2.11	1.99	6.70	6.61	6.69	6.59	6.81	6.66	6.82	6.65
T_6	79.7	82.6	2.11	1.99	2.11	1.97	6.73	6.64	6.71	6.60	6.80	6.64	6.80	6.64
T_7	82.9	85.9	2.12	2.04	2.14	2.02	6.85	6.70	6.86	6.70	7.07	6.75	7.08	6.72
T_8	81.8	84.6	2.15	2.05	2.15	2.04	6.88	6.71	6.87	6.72	7.08	6.74	7.07	6.74
T_9	72.3	75.6	2.07	1.96	2.06	1.97	6.70	6.62	6.69	6.63	6.80	6.65	6.81	6.66
CD (P=0.05)	4.2	4.0	0.04	NS	0.03	NS	0.12	NS	0.14	NS	0.22	NS	0.21	NS

Treatment details are given in Materials and Methods.

content in kinnow leaves might be attributed to the fact that PSB acts as chelating agent and forms stable compounds with iron and aluminium, thereby releasing phosphorus to soil making it available for uptake by plants (Gogoi *et al. 2004*). Sau *et al.* (2017) reported that available N, P, K content in leaves were found to increase with the application of *Azotobacter chroococcum* + *Azospirillum brasilense* + AM (*Glomus mosseae*) + Panchagavya 3% as compared to control plants due to the role of microorganisms in soil which improves the nutrient availability from source to sink, i.e. from soil to plants. The increased leaf mineral content might be due to the fact that the microorganisms increased the solubilization, root surface to volume and permeation of hyphal-pads beyond the explore zone of root hairs. Further, with the application of bio-fertilizers, the soil microbial population increased and led to improved soil health and consequently increased the growth and productivity.

Soil available N was significantly enhanced by rhizosphere application of different biofertilizer strains. Available nitrogen decreased with depth and higher values were obtained in 0–15 cm soil layer as compared to 15–30 cm layer. During first year of investigation, i.e. 2018–19, soil available nitrogen was not found significant in 15–30 cm soil layer, however, different biofertilizer strains/treatments were found effective in increasing nitrogen in 0–15 cm layer. Maximum soil available nitrogen (131.3 kg/ha) was registered with T_7 closely followed by T_5 and T_8. All biofertilizer treatments were found statistically superior to that of control in increasing soil available nitrogen. Minimum soil available nitrogen (123.7 kg/ha) was recorded with T_9. During second year of investigation, i.e. 2019–20, soil available nitrogen was found to be significantly affected in both soil layers (0–15 cm and 15–30 cm). Maximum soil nitrogen values (132.2 kg/ha and 129.9 kg/ha, in 0–15 cm and 15–30 cm, respectively) were observed with T_7. Higher values were obtained during second year of investigation, i.e. 2019–20 over 2018–19. Effect of *Azotobacter chroococcum* Mac 27 was found somewhat pronounced than HT 54 strain. Similar trend was observed with soil available P (Table 2). Maximum soil available P (13.91 kg/ha) was recorded with T_7 followed by T_8. *Pseudomonas* P 36 showed synergistic relationship with *Azotobacter chroococcum* Mac 27 at both RDF levels. Only T_7 and T_8 proved superior to control in enhancing available P. During 2018–19, T_7 showed an increase of 18.89% over control and 12.54% over T_3 in upper layer, i.e. same treatment with 25% reduced RDF. This signifies the role of strain rather than RDF dependence. However, *Pseudomonas* P 36 effectiveness in T_7 over control dropped from 18.89–10.27% in 15–30 cm soil layer. Soil available K was not significantly affected by different treatments.

The micro-nutrients concentration was also significantly influenced in terms of zinc, iron and manganese except copper in 0–15 cm soil depth (Table 3). More increase in 0–15 cm soil layer in terms of macro- and micro-nutrients as compared to 15–30 cm layer might be due to the enhanced activity of microorganisms in upper layer of soil owing to biofertilizer application in the soil. Maximum zinc, iron and manganese content were recorded with T_8 but significantly at par with T_7. The increased soil macro-micro nutrient status might be due to the fact that the microorganisms increased the solubilization, and permeation of hyphal-pads beyond the explore zone of root hairs. Further, with the application of bio-fertilizers, the soil microbial population increased and led to improved chemical status of soil. The increased soil nutrient status may also be attributed to the fact that biofertilizers in combination with FYM improved the physical condition of soil, rhizosphere environment and more soil moisture retention. Ambient results were earlier reported by Medhi *et al.* (2007), Pasrischa *et al.* (2002) in mandarin; Hoda *et al.* (2013) in Valencia orange; Marathe and Bharambe (2007) in sweet orange; Sau *et al.* (2017), Dutta and Kundu (2012) in mango. Soil *p*H and EC were not affected significantly by various biofertilizer applications.

All the biofertilizer treatments brought either significantly or marginally superior fruit yield over control (RDF) during both the years (Table 3). The highest yield of 82.9 and 85.9 kg/tree was recorded in T_7 during 2018–19 and 2019–20, respectively, which was statistically at par with T_5, T_6 and T_8. The lowest yield of 72.3 and 75.6 kg/tree during the respective years was registered in control (T_9). When 75% RDF was applied in combination with either of the *Azotobacter* strains + *Pseudomonas* strain P 36, there was significant increase in fruit yield over control. These results indicate the synergistic impact of both the *Azotobacter* and *Pseudomonas* strains. It also reflects that with the application of *Azotobacter* and *Pseudomonas*, 25% of chemical fertilizers may be reduced without sacrificing fruit yield.

Enhanced fruit yield in all the biofertilizer treatments over control might be due to accumulation of more food material in the trees and lead to an efficient utilization of the same for development of fruit under the influence of biofertilizers, NPK and FYM. Moreover, these microbes are known for their anti-pathogen characters and role in improving plant immunity. Nitrogen is responsible for increasing the efficiency of metabolic processes of the tree and thus encourages the growth of the plant and other parts of the plant including fruit (Godage *et al.* 2013 and Sourabh *et al.* 2018). The nitrogen fixers and phosphorus solubilizers increase the availability of nitrogen and phosphorus by increasing their translocation from roots and leaves to fruit (Singh and Singh 2009). The present findings are in close conformity with the findings of Mishra and Tripathi (2011), Tripathi *et al.* (2014), Kumar and Tripathi (2020) in strawberry; Marathe and Bharambe (2007) in sweet orange; Athani *et al.* (2007), Ram *et al.* (2007), Godage *et al.* (2013) in guava; Patel and Naik (2010) and Meena *et al.* (2019) in sapota.

One of the most important problems of small and marginal fruit growers of north-western India is the increasing cost of cultivation and injudicious use of pesticides and chemical fertilizers which, in turn, increases financial liability and contribute towards deterioration of soil health, fruit quality and decrease/stagnation of fruit yield of Kinnow mandarin. The present study infers that a combination of soil applied biofertilizers and chemical fertilizers helps in enhancing fruit yield of Kinnow besides improving chemical and biological status of soil. Application of *Azotobacter chroococcum* Mac 27 + *Pseudomonas* P 36 in combination with 100% recommended dose of fertilizers (T_7) gave the highest fruit yield and contributed in improving chemical and biological health of soil and nutritional status of canopy.

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