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Conjoint effect of organic formulations and biofertilizers on soil health and fruit quality of citrus (Citrus reticulata Blanco)

Jesna Jomon
Lovely Professional University
Parikshit Verma
Lovely Professional University
Suman Bodh
sumanbodh122@gmail.com
Lovely Professional University
Praveen Verma
Lovely Professional University

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Abstract

The experiment was conducted to investigate the influence of organic manure and biofertilizers on citrus. Trees were treated with different amounts of Azospirillum, including organic manures like jeevamrit, ghanjeevamrit, and FYM. The experiment was laid out in a Randomized Block Design with seven treatments and three replications to see their effects on the soil properties, tree growth, and fruit quality. Among 7 treatments, T_7 containing FYM (30 kg) + Azospirillum (300 g) showed the maximum increase in plant height, plant spread, and trunk girth. In T_3, which contains jeevamrit (2.5 L) + Azospirillum (300 g), it gives the maximum increase in fruit weight, fruit size, number of fruits and fruit yield. T_3 gave the maximum efficiency in terms of biochemical analysis and the maximum efficiency in soil pH, soil moisture, soil organic carbon, and EC. The maximum increase in availability of soil nitrogen, phosphorus and potassium was in treatment T_7. The results indicate that the integration of jeevamrit with Azospirillum is a potential method to improve the quality of fruits, fruit production and the health of the soil as a sustainable option for kinnow mandarin cultivation.

Introduction

Citrus (*Citrus reticulata* Blanco) species originate from Southeast Asia, and particular in the India-China centre (Goldschmidt and Koch 2017). More production of crops has been achieved through the use of inorganic fertilizers. Increased use of chemical fertilizers can decrease soil fertility and it will be impact for soil parameters. Excessive use of chemical fertilizers also affects crop quality and ecological environment degradation (Wan et al., 2021). Continuous use of chemical fertilizers shows negative impacts on the environment, including movement of nitrates to underground water phosphorus and nitrogen runoff (Adesemoye and Klopper, 2009). Chemical fertilizers play a major role in enhancing crop yield. More use of biofertilizers reduce soil organic matter (SOM) and decrease quality of soil and leads to soil pollution, water and even air pollution. The excessive application of chemical fertilizers can result in the modification of soil pH, causing a reduction of organic matter in the soil, humus, micro-organisms, plant growth, and even being a reason for greenhouse gas emissions (Pahalvi et al., 2021). Biofertilizers play a major role in increasing root biomass and root area. The application of organic manures such as farmyard manure, vermicompost, forest litter and biofertilizers including Azotobacter, Phosphate Solubilizing Bacteria decrease the expenses of cultivation (Prasad et al., 2016). Organic fertilization is important in increasing soil fertility and sustainable development of agroecosystems. Also, the research showed that it improves soil physical properties including bulk density (Chatzistathis et al., 2021).

The application of biofertilizers can enhance the growth of plants by providing nutrients to them and even helps to maintain productivity of environment.These functions are the fixation of nutrients in the rhizosphere, growth regulation production, soil stabilization, biological control, biodegradation, nutrient cycling, and mycorrhiza association (Cruz et al., 2008). Application of the biofertilizer with the organic manure is helpful for improving the quality, yield and growth of the fruit crops (Katiyar et al., 2012). The various microorganisms employed as biofertilizers for the various purposes include the nitrogen-fixing microorganisms such as Azotobacter, Azospirillum, Rhizobium, and Frankia, phosphorus solubilizing

microorganisms such as Pseudomonas and Bacillus, phosphorus mobilization, as well as arbuscular mycorrhiza and Plant Growth Promoting Rhizobacteria (PGPR). This will help in increasing soil nutrient availability, productivity of trees, quality of fruits, rhizospheric microbial population and reduce damage by pathogens (Abobatta and El-Azazy 2020).

Biofertilizers like Azospirillum, Azotobacter, and PSB together with organic manures (FYM, vermicompost, jeevamrit, ghanjeevamrit) have been proved beneficial for horticultural crops (Bora et al., 2016). Application of primary nutrients forms the natural farming method: jeevamrit (microbe culture in the soil) and ghanjeevamrit is dried jeevamrit. The impact of organic amendments improves the health of the soil and increases nutrient and water use efficiency (Shyam et al., 2023). The current experiment was carried out to investigate the effect of biofertilizers and organic manure on growth, yield and fruit quality of citrus and to assess the effect of different organic manures and biofertilizers on soil health in a citrus orchard.

Materials and Methods

Site, Climate and Weather

Lovely Professional University in Phagwara in Jalandhar region of Punjab state (geographical coordinates of the site are 31.2381210°N latitude, 75.7012624°E longitude) was the site of the field experiment, which took place between 2024 and 2025. Summers with temperatures of 44°C in May and June, and winters are 3–5°C in December and January. The majority of yearly rainfall (700–800 mm) during southwest monsoon from July to September. The physico-chemical properties of the soil in the experimental field were evaluated.

Experimental Design and Treatments

The experimental material consists of 21 trees of Citrus (*Citrus reticulata* Blanco) with 7 treatments and 3 replications each. The treatment combinations are given as follows:(T1); control, (T2);Jeevamrit(2.5l)+*Azospirillum*(200g),(T3);Jeevamrit(2.5l)+*Azospirillum*(300g), (T4);Ghanjeevamrit(2.5kg)+*Azospirillum*(200g),(T5);Ghanjeevamrit(2.5kg)+*Azospirillum*(300g), (T6);FYM(30kg)+*Azospirillum*(200g),(T7); FYM(30kg)+*Azospirillum* (300g). Jeevamrit and Ghanjeevamrit are biofertilizers used in farming to enrich soil health and microbial activity. Acts as a potent bio-stimulant for the soil and introduces numerous beneficial bacteria and enzymes.

Preparation of Manures

The ingredients for the preparation of jeevamrit include fresh cow dung (2.5 kg), cow urine (2.5 L), gram flour (500 g), jaggery (500 g), orchard soil (250 g), water (50 L). Mix the contents using a wooden stick. The mixture should be mixed twice in a day morning and evening, for aerobic microbial action. Cover the

container lightly with a cloth. Ferment the mixture for 5–7 days. After the fermentation process is completed, the jeevamrit becomes ready for use. The ingredients for ghanjeevamrit are fresh cow dung (50 kg), cow urine (1.5 L), gram flour (1 kg), jaggery (1 kg), orchard soil (250 g). Mix all the contents by using hand or with a stick. Dry the mixture for 2–3 days and stored till the period of application.

Time of application

Organic manures like jeevamrit, ghanjeevamrit and FYM were applied in January according to the recommendations and biofertilizer *Azospirillum* is applied in February.

Soil Parameters

Soil samples were taken from different locations of the experimental plot. After being air- dried, the samples passed through a metallic sieve. The pH, electrical conductivity (EC) and organic carbon of the soil were measured as per the method (Jackson 1973). Soil moisture was determined by the gravimetric method (Black 1965). The available nitrogen (kg ha^{-1}) in soil was estimated by alkaline potassium permanganate method as given by Subbiah and Asija, (1956). Available phosphorus (kg ha^{-1}) was determined using Olsen's method with a spectrophotometer at 660 nm. Ammonium acetate was used to extract available soil potassium (kg ha^{-1}) determined by flame photometer (Merwin and Peech,1951).

Tree and Fruit Parameters

The tree height was measured by using a pre-marked bamboo pole of length 15 m from the ground to the top of the tree. Tree canopy spread was measured by placing a bamboo stick horizontally with respect to the tree in the east-west and north-south directions. Trunk girth was measured by using measuring tape by leaving 15cm above the ground level. Fruit size is measured using a digital vernier calliper; the length and width of the fruit are expressed in centimetres (cm). Fruit weight (g) is measured using an electronic top pan by randomly selecting four fruits from each treatment and replication. The total number of fruits is calculated by counting fruits when harvesting. Fruit yield is calculated by considering the number of fruits harvested per plant and the average weight of the fruit to calculate the yield per plant in kilograms (kg).

Biochemical Parameters

Total soluble solids are measured using an Erma hand refractometer expressed as °Brix. Titrable acidity is determined by titrating the sample against sodium hydroxide (NaOH). The titration end point was light pink colour. Firmness is measured using a penetrometer by randomly selecting five fruits. Total sugar content (%) was estimated by the Lane and Eynon method (Ranganna, 2010). The process was adopted up to the point when the filtrate was obtained. Fehling's A and Fehling's B solutions were also used in this process. The concentration of ascorbic acid was determined through the method of 2,6-

dichlorophenol indophenol (DCPIP) visual titration method (Ranganna, 2010). The dye was standardized by the use of standard ascorbic acid and 3% metaphosphoric acid. Juice samples were collected from fresh fruits and mixed with 3% metaphosphoric acid. This mixture was then filtered, and a portion of the mixture was titrated with the dye to the point when there was a persistent light pink colour after 15 seconds.

Statistical Analysis

Statistical analysis was performed using the MS Excel program. The data were analyzed according to the procedure analysis of Randomized Block Design (RBD) suggested by Wilk (1955). The values were compared using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Soil Parameters

The observations of soil pH, EC, soil organic carbon and soil moisture as influenced by organic manure and biofertilizer application are presented in Table 1.

Table 1
Influence of organic manure and biofertilizer on soil pH, EC, soil organic carbon and soil moisture.

Treatments	Soil pH	Soil EC (dsm^{-1})	Soil organic Carbon (%)	Soil Moisture (%)
T_1: Control	8.11^{a}	0.34^{a}	0.75^{g}	6.60^{d}
T_2: Jeevamrit (2.5l) + *Azospirillum* (200g)	7.79^{bc}	0.28^{bc}	0.93^{f}	6.93^{cd}
T_3: Jeevamrit (2.5l) + *Azospirillum* (300g)	7.15^{f}	0.24^{c}	1.14^{e}	7.12^{c}
T_4: Ghanjeevamrit (2.5kg) + *Azospirillum* (200g)	7.95^{ab}	0.31^{ab}	1.29^{d}	8.05^{b}
T_5: Ghanjeevamrit (2.5kg) + *Azospirillum* (300g)	7.47^{de}	0.27^{bc}	1.47^{c}	8.25^{b}
T_6: FYM (30kg) + *Azospirillum* (200g)	7.63^{cd}	0.28^{bc}	1.65^{b}	9.82^{a}
T_7: FYM (30 kg) + *Azospirillum* (300g)	7.31^{ef}	0.26^{bc}	1.85^{a}	10.12^{a}
$CD_{(0.05)}$	0.22	0.04	0.02	0.34
SE(m)	0.07	0.01	0.009	0.11

A decrease in pH values was recorded in T_3, signifying the acidification of soils, which can be attributed to an increase in the activities of microbes and organic acid formation (Table 1). This has been proven to

occur according to findings from the works of Mukherjee *et al*. (1991), Thakur (2020), and Saharan et al. (2023). The lowest EC value was observed in T_3, signifying that the use of organic fertilizers is beneficial in regulating ionic concentration and decreasing the EC values. Likewise, Singh (2019), Thakur (2020), and Thakur (2018) stated that organic fertilizer application helped improve soil biological activities, nutrient cycling, and decreased the EC values. The rise in organic carbon content is a result of the input of organic residues along with the growth in microbial biomass. This is reliable with observations from Choudhary et al. (2022), Thakur (2018), Thakur (2020), and Sharma et al. (2020), where there was an increase in the organic carbon content of soils that had FYM and Jeevamrit added to them. The highest moisture content was observed in T_7 treatment. This observation is confirmed by studies conducted by Sharma et al. (2020) and Saharan et al. (2023).

Table 2
Influence of organic manure and biofertilizer on soil nitrogen, soil phosphorus and soil potassium (kg ha^{-1}).

Treatments	**Available soil N (kg ha^{-1})**	**Available soil P (kg ha^{-1})**	**Available soil K (kg ha^{-1})**
T_1: Control	242.39^{g}	19.37^{g}	226.48^{g}
T_2: Jeevamrit (2.5l) + *Azospirillum* (200g)	275.24^{d}	20.32^{f}	232.76^{f}
T_3: Jeevamrit (2.5l) + *Azospirillum* (300g)	282.25^{c}	21.31^{e}	239.05^{e}
T_4: Ghanjeevamrit (2.5kg) + *Azospirillum* (200g)	260.30^{f}	22.19^{d}	245.34^{d}
T_5: Ghanjeevamrit (2.5kg) + *Azospirillum* (300g)	267.77^{e}	23.19^{c}	251.62^{c}
T_6: FYM (30kg) + *Azospirillum* (200g)	290.25^{b}	24.11^{b}	257.96^{b}
T_7: FYM (30 kg) + *Azospirillum* (300g)	294.80^{a}	24.45^{a}	261.78^{a}
$CD_{(0.05)}$	0.36	0.14	1.02
SE(m)	0.11	0.04	0.32

The highest level of nitrogen availability was found in T_7 treatment (Table 2), suggesting that a combination of FYM and biofertilizer has the potential to greatly improve nitrogen mineralization. Such increases in nitrogen availability through the combined effect of both organic materials and microbial inoculation have been found by Dutta et al. (2011), Thakur (2018) and Choudhary et al. (2022). Available soil phosphorus also increased in organic treatments. The increase can be attributed to the microbial dissolution of bound phosphorus due to organic acids, as reported in previous studies by Garhwal et al. (2014) and Sharma et al. (2020). In the case of available potassium, T_7 had the highest percentage

compared to others. The increase in potassium can be linked to the mineralization and fixation of the element reduced by microbes, as seen in findings reported by Shyam (2019), Saharan et al. (2023) and Choudhary et al. (2022).

Tree and Fruit Parameters

Table 3
Influence of organic manure and biofertilizer on tree height, tree spread and trunk girth.

Treatments	Plant height (%)	Plant spread		Trunk girth (%)
		EW (%)	NS (%)	
T_1: Control	4.39^{e}	6.36^{d}	6.41^{e}	1.87^{d}
T_2: Jeevamrit (2.5l) + *Azospirillum* (200g)	4.89^{de}	7.32^{c}	7.54^{de}	2.40^{cd}
T_3: Jeevamrit (2.5l) + *Azospirillum* (300g)	5.38^{cde}	7.79^{c}	8.11^{cd}	2.81^{c}
T_4: Ghanjeevamrit (2.5kg) + *Azospirillum* (200g)	6.03^{cd}	8.98^{b}	9.02^{bc}	3.44^{b}
T_5: Ghanjeevamrit (2.5kg) + *Azospirillum* (300g)	6.63^{bc}	9.19^{b}	9.36^{ab}	3.78^{b}
T_6: FYM (30kg) + *Azospirillum* (200g)	8.02^{ab}	10.10^{a}	10.30^{a}	4.31^{a}
T_7: FYM (30 kg) + *Azospirillum* (300g)	8.93^{a}	10.23^{a}	10.53^{a}	4.59^{a}
CD $_{(0.05)}$	1.47	0.84	1.17	0.52
SE(m)	0.47	0.27	0.37	0.16

The maximum increase in the height of the plants was recorded in T_7 treatment (Table 3), indicating the synergetic role of FYM and *Azospirillum* in stimulating vertical growth through increased nutrient supply and uptake. Similarly, there was an increase in spread of the plants in both EW and NS directions with treatment T_7. This might be due to increased nutrient availability and higher soil microbial activity, thus producing a healthy canopy. The results are in accordance with the findings by Meena (2016) on sapota and Gain et al. (2019) on sweet orange. The raise in the girth of the trunk in treatment T_7 is due to the effect of FYM and *Azospirillum* in increasing the structural vigour and health of the kinnow trees. This is evident from the results presented by Garhwal et al. (2014), who noticed that the use of FYM and Masta (2022), in which the use of FYM maximize the girth of the trunk and tree height of apricot trees.

Table 4
Influence of organic manure and biofertilizer on fruit weight, fruit size, number of fruits and fruit yield.

Treatments	Fruit weight (g)	Fruit size(cm)		No. of fruits	Fruit yield (kg)
		Fruit length	Fruit width		
T_1: Control	131.25^{g}	5.11^{e}	6.15^{g}	370.33^{g}	48.60^{f}
T_2: Jeevamrit (2.5l) + *Azospirillum* (200g)	141.37^{e}	5.37^{cd}	6.62^{d}	379.60^{e}	53.67^{d}
T_3: Jeevamrit (2.5l) + *Azospirillum* (300g)	161.57^{a}	5.87^{a}	6.96^{a}	472.09^{a}	76.27^{a}
T_4: Ghanjeevamrit (2.5kg) + *Azospirillum* (200g)	135.27^{f}	5.32^{d}	6.43^{e}	374.22^{f}	50.61^{e}
T_5: Ghanjeevamrit (2.5kg) + *Azospirillum* (300g)	158.97^{b}	5.53^{b}	6.84^{b}	435.94^{c}	69.30^{b}
T_6: FYM (30kg) + *Azospirillum* (200g)	143.73^{d}	5.35^{d}	6.34^{f}	401.82^{d}	57.74^{c}
T_7: FYM (30 kg) + *Azospirillum* (300g)	152.27^{c}	5.42^{c}	6.73^{c}	453.43^{b}	69.04^{b}
CD $_{(0.05)}$	1.60	0.02	0.01	1.89	0.62
SE(m)	0.51	0.008	0.004	0.61	0.20

Treatment combinations used: T_3 was the best treatment (Table 4). The weight of fruits, the size of the fruits, the number of fruits per plant, and fruit yield per plant more in treatment 3. T_1 gave the lowest values for all the measured parameters at all stages. T_3 treatment was far better than the control in terms of the number of fruits and their yield. The present study has once again confirmed the efficacy of the use of liquid organic manure (Jeevamrit) and bio-fertilizer (*Azospirillum*) for improving fruit yield and quality in Kinnow mandarin.

Biochemical Parameters

Table 5
Influence of organic manure and biofertilizer on TSS, titratable acidity and TSS: acid.

Treatments	**TSS (°Brix)**	**Titratable acidity (%)**	**TSS/ acid**
T_1: Control	9.84^{f}	0.89^{a}	11.02^{g}
T_2: Jeevamrit (2.5l) + *Azospirillum* (200g)	10.33^{e}	0.88^{a}	11.74^{e}
T_3: Jeevamrit (2.5l) + *Azospirillum* (300g)	11.59^{a}	0.81^{b}	14.19^{a}
T_4: Ghanjeevamrit (2.5kg) + *Azospirillum* (200g)	9.97^{f}	0.88^{a}	11.27^{f}
T_5: Ghanjeevamrit (2.5kg) + *Azospirillum* (300g)	11.22^{c}	0.84^{ab}	13.24^{c}
T_6: FYM (30kg) + *Azospirillum* (200g)	10.70^{d}	0.86^{ab}	12.39^{d}
T_7: FYM (30 kg) + *Azospirillum* (300g)	11.43^{b}	0.84^{ab}	13.56^{b}
CD (0.05)	0.02	0.01	0.21
SE(m)	0.009	0.005	0.06

T_3 proved to be the most efficient treatment, having high TSS, low Titratable acidity, and the best TSS/acid ratio among other treatments (Table 5). Regarding the acidity parameter, the minimum amount of titratable acidity was noted in treatment T_3, then T_5 and T_6. Reduction in fruit acidity influenced by microorganism activity and balanced nutrient supply. Expanding in the TSS/acid ratio indicates a good sugar to acid relationship, which is favorable for sweet fruits. The observed results are consistent with the results obtained by Verma *et al*. (2021) and Jhade et al. (2020), they studied the importance of organic and biofertilizers on strawberries and papaya fruits.

Table 6
Influence of organic manure and biofertilizer on total sugar, ascorbic acid and firmness.

Treatments	Total sugar (%)	Ascorbic acid (mg/100ml)	Firmness (kg/cm²)
T1: Control	8.32^{g}	24.41^{g}	2.27^{g}
T2: Jeevamrit (2.5l) + *Azospirillum* (200g)	8.57^{e}	27.97^{e}	2.46^{e}
T3: Jeevamrit (2.5l) + *Azospirillum* (300g)	8.93^{a}	34.29^{a}	3.05^{a}
T4:Ghanjeevamrit(2.5kg)+*Azospirillum* (200g)	8.45^{f}	26.88^{f}	2.38^{f}
T5: Ghanjeevamrit (2.5kg)+*Azospirillum* (300g)	8.74^{c}	30.66^{c}	2.73^{c}
T6: FYM (30kg) + *Azospirillum* (200g)	8.65^{d}	29.73^{d}	2.63^{d}
T7: FYM (30 kg) + *Azospirillum* (300g)	8.83^{b}	32.16^{b}	2.83^{b}
CD (0.05)	0.03	0.10	0.03
SE(m)	0.01	0.03	0.01

T_3 always delivered the most favourable outcomes, which confirmed the potential of this approach in enhancing the quality and texture of fruits (Table 6). The maximum total sugar content was found in T_3, much greater than in all the other treatments. The increase in total sugar content in T_3 can be attributed to the increased metabolic activity due to the application of Jeevamrit and *Azospirillum*, which could have improved nutrient availability and root growth, leading to an increase in photosynthate production and carbohydrate accumulation in the fruit. It is supported by the studies of Choudhary et al. (2022) and Verma et al. (2024), which indicated that nutrient management systems improved sugar content in fruits like pomegranate and strawberry. Firmness is a very important parameter for shelf-life and handling properties of fruits, as it is one of the primary indicators of the maturity of fruits. In the present study, T_3 delivered the highest value of firmness. The increase in the levels of ascorbic acid under organic application, especially in T_3 treatment involving Jeevamrit and *Azospirillum*, can be attributed to the increased metabolism in the plants as a result of nutrient availability and stimulation of biosynthesis processes leading to vitamin C production. This studies by Ravi *et al*. (2023) and Verma *et al*. (2021), where they recorded an enhance in the vitamin C content in citrus due to the application of biofertlizers.

Conclusion

In conclusion, the combination of T7 treatment FYM (30kg) + *Azospirillum* (300g) gave maximum vegetative growth. The combination T3 treatment jeevamrit (2.5l) +*Azospirillum* (300g) shows remarkable performance in various aspects of biochemical parameters. The treatments T3 and T7 also

show an increase in soil health. This study shows that the application of organic formulations and biofertilizers can enhance the vegetative growth, soil health and biochemical characters of the plant.

Declarations

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CRediT authorship contribution statement

SB conceived, designed the experiment; PV performed the experiment on field, JJ wrote the manuscript; PV edited the manuscript; All the authors have read and reviewed the manuscript.

Disclosure statement

The authors report there are no financial or non-financial competing interests to declare.

Data availability statement

The study's supporting data can be obtained from the corresponding author upon a reasonable request.

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Conflict of Interest

Authors declare no conflict of interests

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