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Yield and quality improvement in Kinnow mandarin using nano formulations

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ABSTRACT

The present study aimed at evaluating the effects of nano-chitosan, nano-micronutrients, and bio-capsules on the yield and quality attributes of Kinnow mandarin (*Citrus nobilis* × *C. deliciosa*) over two consecutive years: 2021-22 and 2022-23. Based on pooled data, treatment T14–consisting of NPK soil drenching with nano-chitosan at a concentration of 100 ppm, bio-capsules at 500 ppm, and foliar application of nano-micronutrients (Zinc oxide and Iron oxide)–demonstrated superior performance in both yield and qualitative parameters. This treatment resulted in a low fruit drop of 35.76%, a high fruit set of 79.60%, a maximum number of fruits per plant at 893.83, a fruit diameter of 7.33 cm, a fruit weight of 153.97 g, a yield per plant of 88.43 kg/tree, and high qualitative measures such as Total Soluble Solids (TSS) at 11.62%, acidity at 1.09%, ascorbic acid content at 26.69 mg/100 g, juice content at 50.06%, and fruit pulp weight of 50.0 g. By combining bio-capsules, nanoparticles, and nano-chitosan with the recommended dose of fertilizer (NPK), the yield parameters were enhanced; thereby, preserving the qualitative aspects through anti-pathogen effects and nutrient fixation in Kinnow plants.

Keywords: *Citrus nobilis* × *Citrus deliciosa*; fruit set; fruit drop; total soluble solids; acidity

INTRODUCTION

Within the mandarin family, Kinnow, an interspecific hybrid [‘King’ (*Citrus nobilis*) × ‘Willow leaf’ (*Citrus deliciosa*)] imported from California, USA, has revolutionized the citrus industry in India and is now the top choice for consumers seeking fresh fruits. Kinnow mandarins are highly sought after for juice processing. The Kinnow tree exhibits early fruit production, often yielding fruit as early as its third year of growth (Pooja *et al*., 12). However, a successful production system should not only aim to increase yield but also enhance the quality of the fruit produced. Due to its high yield potential, Kinnow is susceptible to fruit drop, attributed to factors such as moisture stress, pathological fruit drop, and nutrient depletion. Integrated nutrient management strategies demonstrate extensive antimicrobial activity against fungal pathogens. However, their effectiveness is hindered by poor solubility due to large particle size. Chitosan nanoparticles offer significant advantages over bulk counterparts due to size-induced alterations in various properties (Joshi *et al*., 7). Scientists at the Indian Council of Agricultural Research (ICAR) have developed a technique to encapsulate bio-fertilizers in tiny capsules, which can be applied to seeds, soil, or growing plants to improve soil nutrients or make them physiologically available (Singh *et al*., 15). Nano-particles of iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn) in mixed foliar sprays offer reduced plant mobility, increased sustainability, and enhanced field productivity. Despite these advancements, the use of bio-capsules, nano-micronutrients , and nano-macronutrients on Kinnow mandarin has not been extensively studied. Considering these factors, our research was planned and executed.

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MATERIALS AND METHODS

The present investigation was carried out during the years 2021-22 and 2022-23 at the Central Research Field, Department of Horticulture at the Naini Agricultural Institute, SHUATS, Prayagraj, Uttar Pradesh. The soil of the experimental farm had a low clay content and a high proportion of sand particles with consistent fertility. With fifteen treatments and three replications of each, the experiment was set up in the form of randomized block design (RBD).

The treatment combinations consisted of alternatives such as the recommended dose of fertilizer (NPK @ 500:250:500 kg/ha), nano-fertilizers (NPS) (FeO @ 100 ppm, ZnO @ 150 ppm), nano-chitosan @ 100 ppm, and bio-capsules with plant growth promoting rhizobacteria (PGPR) (Table 1). The kinnow plants were strategically spaced at a distance of 5 m × 5 m, grafted onto *Citrus karna khatta* rootstock, at the Central Research Field, Department of Horticulture. Every single experiment involved three replications, with two trees designated to each replication. Nitrogen was evenly split into two doses, with the first given in February and the second in April. By fertilizer standards, potassium and

Table 1. Details of Treatment combinations:

Treatment	Treatment combinations
T_0	Control (no treatment)
T_1	N P K (RDF)
T_2	Bio-capsule 500 ppm (Soil drenching)
T_3	Nano-chitosan 100 ppm (Soil drenching)
T_4	Nano-chitosan 100 ppm (Soil drenching) + Bio-capsule 500 ppm
T_5	N P K (RDF) + Bio-capsule 500 ppm (Soil drenching)
T_6	N P K (RDF) + Nano-chitosan 100 ppm (Soil drenching)
T_7	N P K (RDF) + Nano-chitosan 100 ppm (Soil drenching) + Bio-capsule 500 ppm
T_8	N P K (RDF) – foliar application of ZnO and FeO nano-particles (NPs)
T_9	Bio-capsule 500 ppm (Soil drenching) + foliar application of ZnO and FeO nano-particles (NPs)
T_{10}	Nano-chitosan 100 ppm (Soil drenching) + foliar application of ZnO and FeO nano-particles (NPs)
T_{11}	Nano-chitosan 100 ppm (Soil drenching) + Bio-capsule 500 ppm + foliar application of ZnO and FeO nano-particles (NPs)
T_{12}	N P K (RDF) + Bio-capsule 500ppm (Soil drenching) + foliar application of ZnO and FeO nano-particles (NPs)
T_{13}	N P K (RDF) + Nano-chitosan 100 ppm (Soil drenching) + foliar application of ZnO and FeO nano-particles (NPs)
T_{14}	N P K (RDF) + Nano-chitosan 100 ppm (Soil drenching) + Bio-capsule 500ppm + foliar application of ZnO and FeO nano-particles (NPs)

phosphorus were administered concurrently with the initial nitrogen dosage.

The bio-capsules were sourced from M/s Codagu Agritech, located in Kushal Nagar, Karnataka. They are the first authorized startup licensed under the brand name “Powercap”. Each one-gram capsule is equivalent to 1 kg of PGPR. Nano-chitosan was obtained from the online store IndiaMart, specifically from a company offering reagent-grade and bio-tech-grade powder Chitosan Nano-particles. As it is in powder form, it was converted into a liquid form to achieve the required parts per million (ppm) concentration. The micronutrients were purchased from the online store IndiaMart, supplied by Sanskar International based at Prayagraj.

Yield attributes are observed under various parameters like- fruit set (%,) fruit drop (%), average number of fruits per tree, fruit diameter (cm), average weight of fruit (g) and average yield of fruits per tree (kg/tree). While the data of quality attributes are recorded under ascorbic acid (mg/100 g of edible portion), TSS (^{0}Brix), acidity (%), reducing and non-reducing sugars, juice content, peel thickness in millimetres, fruit firmness and weight of fruit pulp (g). The quality attributes are measured as per the standardized protocol of AOAC (1).

Further, statistical analysis was done to determine the interdependencies between different variables, the collected data were submitted to analysis of variance approach. (Mead,10).

RESULTS AND DISCUSSION

Nano-chitosan, nano-micronutrients and bio-capsules showed significant effects on various yield attributes, as pooled data are depicted in Table 2 and Fig. 1. It was observed that the treatment T_{14} with the combination of N P K (Recommended dose of fertilizer) + Soil drenching of Nano-chitosan 100 ppm + Bio-capsule 500 ppm + foliar application of nano-particles (ZnO and FeO) recorded the maximum fruit set (79.60%). Significantly the minimum fruit set, *i.e.,* 54.24% over all other was observed in T_0. Nano-chitosan and nano-micronutrients help in maintaining chloroplast fine structure and stomatal conductance. The mentioned factors may have significant impact on the changes in protein programming. As a result, this may have helped buds grow and develop, leading to improved flowering. Earlier, it has been noted an increase in fruit set due to these physiological changes (Gong *et al*., 6), while Singh *et al*. (14) noted the same for strawberries.

While minimum fruit drop (%) was observed in treatment T_{14}, *i.e.,* 35.76 % (Table 2). Combining nano-chitosan with nano-micronutrients, it may trigger specific enzymes that impact cell degradation and enhance fruit retention and also suppress the

Table 2. Effect of nano-chitosan, nano-micronutrients and bio-capsules on various yield attributes of Kinnow mandarin (pooled data of both years *i.e.,* 2021-22 and 2022-23).

Treatment	Fruit set (%)	Fruit drop (%)	Av. No. of fruits per tree	Fruit dia. (cm)	Fruit wt. (g)	Av. yield of fruits per tree (kg)
T_0	54.24	42.74	504.32	6.36	150.07	43.34
T_1	67.84	38.17	692.75	6.89	153.25	65.66
T_2	58.46	40.27	565.24	6.65	152.84	51.62
T_3	56.41	40.73	535.54	6.56	152.67	48.47
T_4	60.21	39.91	587.13	6.67	152.89	53.95
T_5	71.18	37.55	741.20	7.00	153.43	71.03
T_6	69.87	37.72	724.17	6.98	153.40	69.20
T_7	73.61	37.36	785.85	7.12	153.60	75.63
T_8	71.58	37.39	748.14	7.02	153.45	71.89
T_9	63.96	39.05	633.57	6.78	153.06	59.12
T_{10}	62.23	39.40	612.61	6.75	153.03	56.82
T_{11}	65.79	38.68	656.94	6.81	153.09	61.68
T_{12}	77.09	36.25	841.87	7.20	153.79	82.56
T_{13}	75.64	36.60	824.50	7.30	153.75	80.38
T_{14}	79.60	35.76	893.83	7.33	153.97	88.43
F-test	S	S	S	S	S	S
SE (m) (±)	0.44	0.03	5.02	0.02	0.02	0.78
CD @ 5%	1.25	0.10	14.21	0.05	0.06	2.20

signaling pathway linked to ethylene production, which stimulates ripening and fruit drop. Liaquat *et al*. (8) reported similar results on mandarin. Similarly, treatment T_{14} recorded the maximum average number of fruits per tree, *i.e.*, 893.83. Significantly the minimum average number of fruits per tree was recorded in T_0 throughout the study period. By using bio-capsules and nano-micronutrients like zinc (Zn) and iron (Fe), there is a possibility of enhancing the production of various plant growth regulators, such as auxin and cytokinin, thereby the improved movement of nutrients from leaves to fruits (Bistgani *et al*., 3). Comparable outcomes were stated by Lustriane *et al*. (9) in banana and by Singh *et al*. (14) in strawberry. Treatment T_{14} showed the maximum fruit diameter, *i.e.*, 7.33 cm and the maximum fruit weight (g) of 153.97 (Table 2). The combination of nano-chitosan with bio-capsules enhances nutrient utilization efficiency, supports vegetative growth, and speeds up the transfer of photo-assimilates from source to sink, ultimately boosting dry matter in fruits that causes an increase in fruit weight and diameter. Singh *et al*. (14) reported the similar findings in strawberry. One must consider the potential impact of ZnO on increasing the weight of fruits and their diameter. In a study, the small size of nano- Zn allows it to penetrate the leaf epidermis more effectively and release Zn^{2+} ions (Bala *et al*., 2).

Likewise, treatment T_{14} observed the maximum average yield of fruits per tree 88.43 kg/tree. (Table 2). When nano-chitosan and nano-micronutrients are combined, they interact in a complex way that influences gene expression, metabolic processes, and various biological reactions in plants. Due to improved nutrient translocation it leads to a higher accumulation of dry biomass. This causes increase in fruit weight, size, and quantity. Similar reports were reported by Shehata *et al*. (13) in cucumber.

Nano-chitosan, nano-micronutrients and bio-capsules also show significant effects on various quality attributes as pooled data of both years are presented in Table 3. According to the results obtained, treatment T_{14} recorded the highest ascorbic acid, *i.e.*, 26.69 mg/100 g of edible portion. This combination can influence gene expression, metabolic processes, and various biological reactions in plants. This ultimately enhances nutrient use efficiency and increases the production of ascorbic acid in plants Verma *et al*. (17). Applying nano-chitosan to the leaves results in a reduction in oxygen concentration, which hinders the function of ascorbic acid oxidase enzymes. The data obtained aligns with

Table 3. Effect of nano-chitosan, nano-micronutrients and bio-capsules on different quality parameters (pooled data of both years *i.e.,* 2021-22 and 2022-23)

Treatment	Ascorbic acid (mg/100 g)	TSS (°Brix)	Acidity (%)	Reducing sugar (%)	Non-reducing sugar (%)	Juice content (%)	Peel thickness (mm)	Fruit firmness (kg/cm²)	Pulp weight pulp per fruit (g)
T_0	24.87	10.46	0.91	1.81	2.53	45.53	3.42	2.49	62.67
T_1	25.91	11.38	1.02	2.30	3.21	47.92	3.59	2.53	66.24
T_2	25.21	10.80	0.99	2.01	2.80	46.19	3.46	2.50	66.1
T_3	25.13	10.33	0.99	1.74	2.43	45.77	3.45	2.50	66.04
T_4	25.32	10.59	0.99	1.88	2.63	46.49	3.49	2.51	66.12
T_5	26.12	11.56	1.06	2.37	3.31	48.44	3.63	2.57	66.3
T_6	26.14	11.00	1.06	2.13	2.97	48.28	3.61	2.54	66.29
T_7	26.22	11.05	1.06	2.16	3.01	48.95	3.66	2.56	66.36
T_8	26.33	11.58	1.06	2.40	3.35	48.59	3.64	2.55	66.31
T_9	25.61	10.85	1.01	2.02	2.82	47.17	3.54	2.52	66.175
T_{10}	25.52	10.62	1.00	1.91	2.67	46.87	3.52	2.51	66.16
T_{11}	25.70	10.65	1.01	1.93	2.69	47.54	3.57	2.53	66.185
T_{12}	26.41	11.62	1.09	2.41	3.36	49.60	3.69	2.57	68.92
T_{13}	26.60	11.17	1.09	2.22	3.09	49.31	3.68	2.56	66.41
T_{14}	26.69	11.23	1.09	2.24	3.13	50.06	3.71	2.58	70.48
F-test	S	S	S	S	S	S	NS	NS	S
SE (m) (±)	0.03	0.02	0.01	0.01	0.02	0.08	0.07	0.06	0.01
CD @ 5%	0.10	0.06	0.04	0.04	0.06	0.24	NS	NS	0.02

the results presented by researcher Xing *et al*. (18) in jujube fruits.

It has been observed that the treatment T_{12} reported the highest TSS (°Brix), *i.e.*, 11.62 and significantly, the lowest TSS, *i.e.*, 10.33 was recorded in T_3 (Table 3). Consequently, there is an accumulation of photosynthates in plants and higher TSS levels in treatment T_{12}. The treatment combination of T_3 may have an inhibitory effect on the genes that control enzymes responsible for converting starch to sugar, resulting in declining of the fruits' total soluble solids (Lustriane *et al*., 9). Also, comparable outcomes were observed on strawberry by Nguyen *et al*. (11).

The treatment T_{14} showed the maximum acidity (%), *i.e.*, 1.09 throughout the study period. Utilizing nano-chitosan results in a reduced gaseous exchange of oxygen and carbon dioxide between fruits and the atmosphere, which results in a reduced respiration rate that restricts enzymatic activities and the process hinders the transformation of organic acids into sugars, resulting in a rise in titrable acidity (Verma *et al*., 17). The presence of Zn and Fe nano-particles at an optimal level may have interacted in a way that resulted in increased production of organic acids instead of sugars, leading to a rise in titrable acidity. Earlier, Singh *et al*. (14) also found comparable outcomes on strawberry.

Further, treatment T_{12} recorded the maximum reducing sugars 2.41%. Similarly, the maximum non-reducing sugar (%) 3.36 was noted in treatment T_{12} (Table 3). Using nano-chitosan resulted in a reduction in fruit respiration, leading to the inhibition of various enzymatic activities. As a result, there was a significant decrease in non-reducing sugar production. Singh *et al*. (15) found comparable results in Kinnow mandarin. ZnO and FeO nano-particle substances enabled the rapid delivery of essential nutrients to the root system. With abundant nutrients available, photosynthetic efficiency in leaves improves, and hence sugar and starch are synthesized within the chloroplasts in the leaves Dey *et al*. (4).

Uniformly, treatment T_{14} observed the maximum juice content (%), *i.e.*, 50.06. Nano-chitosan could possibly affect sugar metabolism, leading to an improvement in dry matter. Earlier, Nguyen *et al*. (11) and Singh *et al*. (15) reported comparable findings on Kinnow mandarin. These studies have shown that ZnO nano-particles can improve photosynthetic efficiency in plants, resulting in increased plant metabolism, dry matter, and juice content.

However, treatment T_{14} was recorded with the maximum peel thickness (mm), *i.e.*, 3.71 (Table 3). The experimental results indicate that the treatment T_{14} shows an insignificant effect on peel thickness. Fruit peel thickness is determined by innate genetic variables that interact with the environment. Relatable results were reported by Lustriane *et al*. (9) on banana.

Nevertheless, treatment T_{14} gave the maximum fruit firmness, *i.e.*, 2.58 kg/cm^2 (Table 3). The inhibitory effect of the coating on moisture loss and the delayed breakdown of insoluble protopectins into soluble protopectins and pectic acid pectin, together with other oxygen-mediated reactions, could elucidate the preservation of fruit firmness (Temiz and Ayhan, 16)

The treatment T_{14} recorded the significant maximum weight of pulp per fruit (g), *i.e.*, 70.48 throughout the study period. Significantly lowest weight of pulp per fruit (g), *i.e.*, 62.67 over all other treatments was recorded in T_0 (control) (Table 3). Nano-chitosan acts as a semi-permeable barrier. This barrier significantly obstructs the exchange of gases and moisture, leading to a reduced rate of physiological processes. The combined application improves plant nutrient use efficiency, resulting in optimal photosynthesis and maximum dry matter accumulation. Gardesh *et al*. (5) also reported comparable findings.

In conclusion, the usage of Chitosan-based NPK fertilizer by seed priming, foliar or soil application, increased agricultural plant growth and yield. Using a specific amount of nano-fertilizers and biofertilizers leads to enhanced soil fertility and had a distinctive synergy with positive connections when solely utilizing biofertilizer or nano-particles. Throughout the experiment, the treatment T_{14} was resulted to be the most effective in enhancing plant growth and soil health when compared to the other treatments.

AUTHORS' CONTRIBUTION

Conceptualization of research (SKE, SM); Designing of the experiments (SKE,SM,VB,AVJ); Contribution to experimental materials (VB, SET); Analysis of data and interpretation (SKE, SM, VB, SET); Preparation of the manuscript (SKE, SM); Review and editing (SM,AVJ, SET).

DECLARATION

The authors declare that there is no conflict of interest.

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