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Shashi Kant Ekka
Research Scholar, Department of Horticulture, Fruit science, SHUATS, Prayagraj, Uttar Pradesh, India

Saket Mishra
Assistant Professor, Department of Horticulture, Fruit science, SHUATS, Prayagraj, Uttar Pradesh, India

Abhimanyu Patel
Guest Teacher, Floriculture and landscaping College of Horticulture and Research Station Kunkuri Jashpur MGUVV, Raipur CG. 492001 India

Corresponding Author:
Shashi Kant Ekka
Research Scholar, Department of Horticulture, Fruit science, SHUATS, Prayagraj, Uttar Pradesh, India

Effect of nano chitosan, nano micronutrients and bio capsules on growth and flowering attributes of Kinnow Mandarin (*Citrus reticulata*)

Shashi Kant Ekka, Saket Mishra and Abhimanyu Patel

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Abstract
An experiment was conducted at the Department of Horticulture, Central Research Field at the Naini Agricultural Institute, SHUATS, Prayagraj ,Uttar Pradesh. During the year 2021-22 and 2022-23 to study the effect of Nano chitosan, Nano micronutrients and Bio capsules on Growth and flowering attributes of Kinnow mandarin and Soil physic-chemical characteristics of the soil. In the experiment, 14 treatment combination were taken along with control. And it was set up according to Randomized block design (RBD) with three replications of each treatment. Among different treatment combinations, it was recorded that T_{14} with the combination of [N P K (as recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles (ZnO+FeO)] emerged as most effective in promoting highest plant height, plant spread and stem girth of the plant. Days required for flowering was shortest while Number of flowers per tree-was highest for T_{14}. The soil NPK content increased drastically for T_{14} applied plots while no significant effect were observed in soil pH, organic Carbon % and Electrical conductivity by the application of Nano chitosan, Nano particles (ZnO+FeO) and Bio capsules. So, Nano-Chitosan, nano fertilizers along with biofertilizers application can enhance plant growth and flowering attributes of kinnow mandarin along with sustaining nutrient levels in the soil.

Keywords: Bio capsules, *Citrus reticulata*, FeO, Flowering, Growth, Kinnow Mandarin, Nano chitosan, Nano micronutrients, ZnO

Introduction

The Kinnow mandarin fruit is a hybrid that emerged from the crossbreeding of two different citrus cultivars: 'king' (*Citrus nobilis*) and 'willow leaf' (*Citrus deliciosa*). H.B. Frost is credited with the development of the technology after conducting research at the University of California Citrus Experimentation Station in 1915 (Hui *et al*., 2006) [13]. The hybrid cultivar was officially released for commercial cultivation in 1935. Dr. J.C Bakshi introduced Kinnow in India in 1954 at the Punjab Agriculture University Regional Research Station in Abohar (Hui *et al*., 2006) [13]. Ever since its introduction, Kinnow has received considerable praise from farmers and the public, establishing itself as a popular citrus fruit in India, especially in Uttar Pradesh.

Within India, the mandarin crop covers a land area of 4, 69,000 hectares, yielding a production output of 62, 64,620 metric tonnes in the 2018-19 period (Anonymous, 2019) [6]. Among citrus species, mandarins are considered the second most important group of citrus plants worldwide. Remarkably, they demonstrate the highest level of climatic adaptability among all cultivated citrus varieties. The cultivation of Kinnow mandarin is mainly focused in the north-western region of India, particularly in Punjab, Haryana, Himachal Pradesh, western Rajasthan, and Uttar Pradesh. The Kinnow mandarin is well-known for its superior freshness, high vitamin C content, reliable processing quality, appealing flavour, and minimal levels of saturated fat, cholesterol, and sodium. The fruit displays a medium globose to oblate shape, distinguished by a golden orange outer layer. It exhibits a subtle level of acidity, enhanced by a delicate interplay of sugar and acid. The fruit ripens during the months of January and February, producing an average of 50 kilograms or more per tree.

The citrus plant demands larger amounts of macro-nutrients, particularly nitrogen, phosphorus, and potassium, in comparison to other nutrients.

The macro-nutrients are essential for influencing the fruit yield and quality of the citrus plant (Albrigo, 2002) [5]. The export of citrus fruits has been significantly affected by a variety of factors, such as the build-up of harmful substances like nitrate or nitrite, phosphates, sulphate, and other chemicals in the fruit tissue. This build-up stems from the overuse of chemical fertilization techniques (Montasser *et al*., 2003) [24]. Hence, the use of bio-fertilizers in agricultural practices helps minimize the negative effects of chemical fertilizers, resulting in the cultivation of top-quality crops (El-Khawaga, 2007) [9]. ICAR has established the technology to encapsulate bio-fertilizers in miniature container called as capsules. This technique can eradicates the requirement for farmers to manually transport the packs containing bio-fertilizers. The composition comprises of carrier medium abundant of viable microbes. Application of these capsules to seeds, soil, or any plant species, can improve the availability of nutrients, making them biologically accessible for plants.

Encapsulating fertilizer within nano particles could potentially improve nutrient uptake efficiency. As per Shojaei *et al*. (2019) [34], nano fertilizers have been recognized as a potential solution for tackling prolonged eutrophication (overly enriched in nutrients) problems and improving nutrient utilization competence to combat deficiencies in macro and micronutrients both.

Utilizing nano chitosan in agriculture through nanotechnology is a captivating and interesting area of study. Extensive investigation has been conducted on the antifungal and antimicrobial properties of chitosan. Using nano chitosan formulations can enhance the effectiveness of chitosan in protecting fruits from various diseases and pathogens. Research has been conducted on the potential of chitosan to improve nutrient absorption in plants. Nano chitosan formulations have the potential to enhance nutrient transportation to plant cells. Chitosan demonstrates a broad spectrum of antimicrobial activity against fungal pathogens.

Micronutrients play a crucial role in fruit production, being essential for various physiological processes in plants. It is important to recognize the importance of micronutrients such as ZnO and FeO, which are needed in smaller amounts. Nano-micronutrient fertilizers represent a cutting-edge solution for efficiently handling nutrients in the agricultural sector. The fertilizers have been designed using nanotechnology to enhance nutrient transportation to plants. Zinc oxide nanoparticles, or ZnO-NPs, have been recognized as a safe material that can enhance seed germination and support plant growth. The absorption, movement, and buildup of ZnO-NPs by plants depend on the unique properties of the nanoparticles and the physiological traits of the host plant.

This experiment aims to investigate the impact of advanced agricultural technologies such as Nano chitosan, Nano particles (ZnO+FeO), and Bio capsules on improving the performance of Kinnow mandarin orchards. The experiment's results will offer valuable insights into the potential applications of advanced agricultural technologies in citrus cultivation. Understanding the impact of Nano chitosan, Nano particles (ZnO+FeO), and Bio capsules on the growth, yield attributes, and quality parameters of Kinnow mandarin could precede for sustainable and effective practices in citrus farming.

Materials and Methods

The experiment was conducted at the Central Research Field, Department of Horticulture specifically located within the Naini Agricultural Institute, SHUATS in Prayagraj. The experimental field is located on the left side of Allahabad-Rewa Road, in close proximity to the Yamuna River, at a distance of approximately 8 km from Allahabad city. The experimental site is located at latitude of 25.57° N and a longitude of 81.51° E. The experiment was set up in randomized block design (RBD) with total of fifteen treatments and three replications of each. The treatment combinations consist of various treatment combinations which included 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 containing plant growth promoting Rhizobacteria (PGPR) (Table 1). The *Citrus reticulata* plants were spaced at a distance of 5m X 5m, grafted onto *Citrus karnakhatta* rootstock, at the Central Research Field, Department of Horticulture, SHUATS. Each treatment consisted of three replications, with two trees assigned to each replication. Nitrogen was administered in two equitably divided portions, the first being in the month of February and the subsequent one in April. Phosphorus and potassium were concurrently administered alongside the initial portion of nitrogen as per recommended dose of fertilizers. The recommended fertilizers, bio capsules and Nano chitosan were applied by soil drenching while Foliar application of nanoparticles (FeO + ZnO) was done by spraying these micro-nutrients on kinnow at both the vegetative and reproductive stages according to various treatment combinations.

Observations Recorded

1. **Plant Height**- The tree's height was measured using a precise measuring tool, a measuring Scale, until reaching the highest point. The measurement obtained was then expressed in meters (m).
2. **Plant Spread**- Measurements were conducted to determine the distance between the points where the branches of the tree had grown in the East-West and North-South directions. The average distance obtained was measured in meters (m).
3. **Stem Girth**- Measuring the stem girth involved using a flexible measuring tape. The plant's stem or trunk was carefully wrapped around with measuring tape at a specific height of about 4 feet (1.2 meters) above the ground to ensure consistency. The measurement of the plant stem girth in centimetres was then recorded accurately.
4. **Number of days needed for flowering**- The time was measured from the start of new growth on twigs to the appearance of the first flower.
5. **Number of flowers per tree**- The number of flowers per branch was counted manually and average number of flowers per tree was calculated.

Measurement of Physicochemical properties of Soil

1. **Available Nitrogen in Soil (Kg/hectare)**
   The quantification of nitrogen availability was determined utilizing the alkaline $KMNO_4$ method, originally proposed by Jackson (1967) [14].
2. **Available Phosphorous in soil (Kg/hectare)**

The determination of the soil's available P status was done as per the protocol described by Muhr *et al.* (1963) [26].

3. **Available potassium in soil (Kg/hectare)**
   The quantification of potassium was conducted following the protocols described by Muhr *et al.* (1963) [26].
4. **Organic carbon (%)**
   The quantification of organic carbon content in soil samples was determined utilizing the methodology established by Walkley and Black (1934) [40].
5. **Soil pH-** The pH value of the soil suspension (1:2:5) was measured using a pH meter.
6. **Electrical conductivity (dS/m)** - The soil's electrical conductivity was analyzed by extracting the supernatant solutions from soil-water suspensions using a 1:2.5 ratio. The measurements were taken using a Systronics Direct Digital Conductivity Meter-304.

Statistical Analysis- The data recorded were subjected to Analysis of variance method to ascertain the interdependencies among various variables (Panse and Sukhatme, 1954) [28].

Table 1: Treatment combinations

S. No.	Treatment	Treatment combinations (%)
1	T_0	Control (Without treatment)
2	T_1	N P K (RDF)
3	T_2	Bio capsule 500 ppm (Soil drenching)
4	T_3	Nano chitosan 100 ppm (Soil drenching)
5	T_4	Nano chitosan 100 ppm (Soil drenching) + Bio capsule 500 ppm
6	T_5	N P K (RDF) + Bio capsule 500 ppm (Soil drenching)
7	T_6	N P K (RDF) + Nano chitosan 100 ppm (Soil drenching)
8	T_7	N P K (RDF) + Nano chitosan 100 ppm (Soil drenching) + Bio capsule 500 ppm
9	T_8	N P K (RDF) – foliar application of ZnO and FeO nano particles (NPs)
10	T_9	Bio capsule 500 ppm (Soil drenching) + foliar application of ZnO and FeO nano particles (NPs)
11	T_{10}	Nano chitosan 100 ppm (Soil drenching) + foliar application of ZnO and FeO nano particles (NPs)
12	T_{11}	Nano chitosan 100 ppm (Soil drenching) + Bio capsule 500 ppm + foliar application of ZnO and FeO nano particles (NPs)
13	T_{12}	N P K (RDF) + Bio capsule 500 ppm (Soil drenching) + foliar application of ZnO and FeO nano particles (NPs)
14	T_{13}	N P K (RDF) + Nano chitosan 100 ppm (Soil drenching) + foliar application of ZnO and FeO nano particles (NPs)
15	T_{14}	N P K (RDF) + Nano chitosan 100 ppm (Soil drenching) + Bio capsule 500 ppm + foliar application of ZnO and FeO nano particles (NPs)

Results and Discussions

Effect of Nano chitosan, Nano micronutrients and Bio-capsules on Growth attributes of Kinnow Mandarin

1. Plant Height

According to observation recorded, it was resulted that the treatment T_{14} [NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles (ZnO+FeO)] shows the maximum plant height [4.22 m (2021-22), 4.30 m (2022-23) and 4.26 m (Pooled)] over all other treatments during both the years of experiment as in pooled analysis (Table 2). The lowest plant height [3.39 m (2021-22), 3.45 (2022-23) m and 3.42m (Pooled)] was recorded in T_0 [Control (without treatment)] during both the years of study as well as pooled analysis. The experimental results indicate that the treatment T_{14} [NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles(ZnO+FeO)] reported highest plant height as compared to other treatments. The combination of Nano chitosan, nano particles, and bio capsules could potentially enhance the absorption of macronutrients from the soil. This could lead to various effects such as activating enzymes, synthesizing proteins, aiding in photosynthesis, regulating osmotic balance, controlling stomatal movement, facilitating energy transfer, transporting nutrients through the phloem, maintaining a balanced ratio of cations and anions, and improving resistance to stress (Abd-Elrahman *et al.*, 2023) [3]. When plants are given the bio capsule, it can enter the rhizosphere and plant roots by releasing metabolites. Some beneficial microbial organisms may have the ability to promote the growth of the plant species mentioned, as shown in the research by Lovaisa *et al.* (2015) [42]. The mentioned microorganisms have shown improved plant nutrition and support in promoting plant growth in various environments, as stated by Venkatraman (2015) [38]. Moreover, the practice of nano chitosan has been exposed to increase the movement of nitrogen to active leaves and boost photosynthesis, ultimately supporting growth and maturation of plant (Mondal *et al.*, 2012) [23]. Chitosan has been reported to enhance auxin biosynthesis pathways through a tryptophan-independent mechanism, potentially leading to increased height of kinnow plants (Uthairatanakij *et al.*, 2007) [37]. Agbodjato *et al.* (2016) [4] reported comparable findings in maize, while Sheoran *et al.* (2021) [32] observed similar results in wheat.

2. Plant spread (m)

During the experiment, it was found that the treatment T_{14} [NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles (ZnO+FeO)] recorded the maximum plant spread [3.8 m (2021-22), 3.87 m (2022-23) and 3.83 m (Pooled)] significantly over all other treatments during both the years of study as well as pooled analysis (Table 2). Significantly lowest plant spread [2.85 m (2021-22), 2.91 m (2022-23) and 2.88 m (Pooled)] m over all other treatments was recorded in T_0 [Control (without treatment)] during both the years of study as well as pooled analysis.

Based on the experimental results, treatment T_{14} showed the highest plant spread compared to the other treatments. The treatment included NPK (Recommended dose of fertilizer), Soil drenching of Nano chitosan 100 ppm, Bio capsule with concentration of 500 ppm, and foliar application of nano particles (ZnO and FeO). It is possible that the increased absorption of nano-chitosan was facilitated by the high specific surface area of ZnO and FeO nano particles, resulting in enhanced absorption of soil-water and nutrients. This, in turn, may have contributed to more efficient photosynthesis, ultimately leading to an increase in the number of leaves and the spread of the plant (Verma *et al.*, 2022) [39]. The beneficial microorganisms found in bio capsules may have synergistically enhanced plant nutrient uptake along with nano chitosan and Nanoparticles. Shafiei-Masouleh (2022) [30] discovered parallels in cyclamen, while Singh *et al*. (2023) [35] found similar results in strawberries.

3. Stem girth (cm)

According to the results, it was observed that the treatment T_{14} [NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles (ZnO+FeO)] recorded the maximum stem girth (cm) [33.08 cm (2021-22), 33.71 cm (2022-23) and 33.40 cm (Pooled)] significantly over all other treatments during both the years of study including pooled analysis (Table 2). Significantly lowest stem girth (cm) [26.58 cm (2021-22), 27.08 cm (2022-23) and 26.83 cm (Pooled)] cm over all other treatments was recorded in T_0 [Control (without treatment)] during both the years of study as well as pooled analysis.

The experimental results indicate that the treatment T_{14} [NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles(ZnO+FeO)] reported highest stem girth as compared to other treatments. It is possible that the observed phenomenon is due to the impact of ZnO and FeO nanoparticles, along with nano chitosan, on cellular responses at a physiological or biochemical level. This influence is probably a result of the distinct features of these nanoparticles, including their particle size, surface area, and capacity to easily pass through cellular barriers and engage with intracellular structures. As a result, these interactions might have helped improve the absorption of soil-water and nutrients, leading to a growth in stem girth (Shobha *et al*., 2014) [33]. Bio capsules may have contributed to enhancing nutrient fixation in the roots, while nano chitosan was crucial in improving nutrient availability in the soil (Bandana and Chandel, 2017) [7]. Choudhary *et al*. (2017) [8] and Kumaraswamy *et al*. (2021) [19] found similar results in Maize, while Ha *et al*. (2019) [12] reported similar findings in coffee.

Effect of Nano chitosan, Nano micronutrients and Bio capsules on flowering attributes of Kinnow Mandarin

1. Days required for flowering

According to the results, it was found that the treatment T_{14} [NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles(ZnO+FeO)] recorded the minimum days required for flowering [29.43 (2021-22), 28.80 (2022-23) and 29.11 (Pooled)] days during both the years of study including pooled analysis (Table 3). Significantly maximum days required for flowering [35.02 (2021-22), 34.28 (2022-23) and 34.65 (Pooled)] days over all other treatments was recorded in T0 [Control (without treatment)] during both the years of study as well as pooled analysis.

Based on the experimental results, treatment T_{14} showed the shortest number of days to flowering compared to the other treatments. By combining nanoparticles and nano chitosan with the appropriate amount of fertilizer, there is a possibility of decreasing the need for large amounts of traditional NPK in growing kinnow mandarin. Observations indicate that the application of chitosan can stimulate enzymatic systems by adjusting cell osmotic potential. Through this stimulation, there has been a noticeable increase in water and nutrient absorption, which has consequently boosted vegetative growth and accelerated the onset of reproductive growth (Martins *et al*., 2018; Makhlouf *et al*., 2022) [22, 21]. Bio capsules may have contributed to a positive boost in soil microbial populations, crucial for supplying essential nutrients to support plant growth and early flowering. Moreover, research has shown that supplementing with nano ZnO has higher solubility and interactivity than larger forms. These results are consistent with the studies carried out by Zagzog and Gad (2017) [41] in mango and Kumar and Dey (2011) [18] in strawberry, which also noted a reduction in the flowering period. The early flowering can be explained by the easy entry of ZnO nanoparticles due to a larger leaf surface area and the ion release through the cuticle. Shao *et al*. (2005) [31] and Singh *et al*. (2023) [35] also documented comparable findings.

2. Average number of flower per tree

During the experiment, it was found that the treatment T_{14} [NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles (ZnO+FeO)] recorded the maximum average number of flower per tree [1100.34 (2021-22), 1145.45(2022-23) and 1122.90 (Pooled)] during both the years of study as well as pooled analysis (Table 3). Minimum average number of flower per tree i.e., [911.11 (2021-22), 948.47 (2022-23) and 929.79 (Pooled)] over all other treatments was recorded in T_0 [Control (without treatment)] during both the years of study as well as pooled analysis.

Based on the experimental results, treatment T_{14} showed the highest number of flowers per tree. The increase in the number of flowers per tree may be due to the balance of nitrogen, phosphorus, and potassium (NPK) components in the medium, especially when combined with treatment T_{14}. Furthermore, the use of nano chitosan through foliar spray may have increased the availability and uptake of phosphorus, leading to enhanced bud emergence and improved blooming in Kinnow mandarin. In a study by Singh *et al*. (2018) [36], it was found that using a combination of NPK and micronutrients increased the height and number of branches of the plants, leading to a higher number of flowers per plant. Nano fertilizers have small dimensions, increased surface area, and enhanced reactivity, which may affect nutrient solubility and diffusion, ultimately influencing plant accessibility to nutrients. Researchers Górnik *et al*. (2008) [11] observed comparable results with grapes, while Abdel-Mawgoud *et al*. (2010) [2] found similar outcomes with strawberries.

Effect of Nano chitosan, Nano micronutrients and Bio capsules on physico-chemical attributes of soil

1. Available Nitrogen (N) (kg/ha)

According to the results, it was found that the treatment T_{14} [NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles (ZnO+FeO)] recorded the maximum Available Nitrogen (N) (kg/ha) i.e., [189.14(2021-22), 191.88 (2022-23) and 190.51 (Pooled)]kg/ha during both the years of study as well as pooled analysis (Table 4). Significantly lowest Available Nitrogen (N) (kg/ha) i.e., [169.44 (2021-22), 171.89 (2022-

23) and 170.67 (Pooled)] kg/ha over all other treatments was recorded in T_0 [Control (without treatment)] during both the years of study as well as pooled analysis.

Based on the experimental results, it was found that the treatment T_{14} had increased levels of available Nitrogen in the soil. Thanks to the tiny size, impressive sorption capacity, and permeable nature of chitosan nanoparticles has led to a notable increase in Nitrogen absorption and uptake by plants (Rameshaiah *et al*., 2015) [29]. Moreover, the use of bio capsules has been successful in promoting the growth of plant-enhancing rhizobacteria in the soil. The rhizosphere soil harbors a wide range of beneficial microbiomes that support plants by exhibiting various characteristics like mineral element dissolution, nitrogen fixation, siderophore production, and phytohormone synthesis (Mahmud *et al*., 2021) [20]. Moreover, it is important to highlight that microorganisms have a significant impact on balancing nutrient levels in the soil. This is accomplished through a variety of complex processes, such as nitrogen fixation, solubilization of complex inorganic compounds, and mineralization of organic materials, as emphasized by Kaviya *et al*. (2019) [15]. As a result, the soil shows a sufficient amount of Nitrogen, which helps support both microbial and plant organisms. Studies conducted by Khati *et al*. (2017) [16] in Maize and Abd-Elrahman *et al*. (2023) [3] in strawberry yielded comparable findings.

2. Available Phosphorus (P) (kg/ha)

According to the results, it was found that the treatment T_{14}[NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles (ZnO+FeO)] recorded the maximum Available Phosphorus (P) (kg/ha) i.e., [17.99(2021-22), 18.25 (2022-23) and 18.12 (Pooled)] kg/ha during both the years of study as well as pooled analysis (Table 4). Significantly lowest Available Phosphorus (P) (kg/ha) i.e., [16.11 (2021-22), 16.35 (2022-23) and 16.23 (Pooled)] kg/ha over all other treatments was recorded in T_0 [Control (without treatment)] during both the years of study as well as pooled analysis.

The experimental results indicate that the treatment T_{14} [NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles(ZnO +FeO)] reportedly had higher Available Phosphorous in Soil. The phenomenon may be due to the unique properties of nanoparticles, which show a strong attraction to different micro and macronutrients. This can be credited to their capacity to slowly release these nutrients into the soil over a prolonged period, as explained by (Mukhopadhyay *et al*. 2014) [27]. These results show that using nano chitosan, ZnO nanoparticles, and FeO nanoparticles together had a positive impact on alkaline phosphatase activity when combined with PGPR (Plant growth promoting Rhizobacteria). Because zinc is an essential component of phytase and phosphatase enzymes, which are important for phosphorous mobilization, using zinc sulphate ($ZnSO_4$) or zinc oxide (ZnO) has been found to increase the secretion of phosphorous-mobilizing enzymes. As a result, this results in an increase in the amount of phosphorous present in the soil. Upon observation, an increase in alkaline phosphatase activity suggests a greater availability of substrates in the soil treated with nano chitosan. Abd-Elrahman *et al*. (2023) [3] and Singh *et al*. (2023) [35] also observed comparable findings in strawberry.

3. Available Potassium (K) (kg/ha)

According to the results, it was found that the treatment T_{14}[NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles(ZnO +FeO)] recorded the maximum Available Phosphorus (P) (kg/ha) i.e., [254.15(2021-22), 257.83 (2022-23) and 255.99 (Pooled)] kg/ha during both the years of study as well as pooled analysis (Table 4). Significantly lowest Available Potassium (K) (kg/ha) i.e., [227.68 (2021-22), 230.97 (2022-23) and 229.32 (Pooled)] kg/ha over all other treatments was recorded in T_0 [Control (without treatment)] during both the years of study as well as pooled analysis.

Based on the experimental results, treatment T_{14} showed increased levels of Available Potassium in the soil. Nano-chitosan, a cationic biopolymer, is considered highly reactive in scientific circles. The presence of amino and hydroxyl functional groups on its backbone structure enables chemical bonding with important nutrients such as potassium (K) (Morsy *et al*. 2019) [25].

The novel nanocomposite shows effectiveness in the soil environment by acting as a slow-release fertilizer, helping to reduce the leaching of potassium (K) while also improving its bioavailability (Abdel-Aziz *et al*. 2016) [1]. Through the application of bio capsules and Nano particles ZnO and FeO, the presence of plant growth promoting rhizobacteria positively impacted soil enzymatic activities, resulting in the conversion of complex organic acids in soil to available NPK for plant nutrient uptake. In strawberry, Abd-Elrahman *et al*. (2023) [3] and Singh *et al*. (2023) [35] both reported comparable findings.

4. Organic carbon (%)

The impact of various treatments was determined to be insignificant. Based on the experimental results, it was found that the treatment T_{14} [NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles(ZnO +FeO)] had increased organic carbon in the soil (Table 5). This could be attributed to chitosan acting as a carbon source, potentially leading to an increase in organic carbon content in the soil. Through the application of bio capsules and Nano particles ZnO and FeO, plant growth promoting rhizobacteria had a positive impact on soil enzymatic activities, resulting in the solubilization of complex organic acids in soil and an increase in soil carbon content. Findings from Kondal *et al*. (2021) [17] regarding Potato and Singh *et al*. (2023) [35] in strawberry showed comparable outcomes.

5. Soil pH

The results did not show any significant findings. Based on the results obtained in an experiment, it was concluded that the treatments T12 and T14 had the lowest soil pH. Treatment T12 included N P K (Recommended dose of fertilizer) + Bio capsule 500 ppm (Soil drenching) + foliar application of nano particles (ZnO +FeO), while treatment T14 consisted of NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles (ZnO +FeO) (Table 5). This could be due to nano chitosan significantly contributing to the growth of beneficial soil microbes introduced into the soil via Bio capsules. Microbes in the soil release acidic substances that interact with different soil components to fix NPK as a result soil pH might have increased.

6. Electrical conductivity (dS/m)

According to the experimental results, treatment T14 [NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles (ZnO +FeO)] had a minimal impact on soil electrical conductivity (Table 5). It is possible that the combination of nano chitosan and bio capsules enhanced nutrient uptake in the plants, leading to a balanced nutritional status in the soil-plant system. There was a minimal decrease in electrical conductivity caused by the enhanced solubilization of oxide forms resulting from the use of ZnO nanoparticles. Consistent findings were documented by Khati *et al.* (2017) [16] in Maize, Elshamy *et al.* (2019) [10] in potato, and Singh *et al.* (2023) [35] in strawberry.

Table 2: Effect of Nano chitosan, Nano micronutrients and Bio capsules on Growth attributes of Kinnow Mandarin

Treatment Symbol	Plant Height (m)			Plant Spread (m)			Stem Girth (cm)		
	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled
T_0	3.39	3.45	3.42	2.85	2.91	2.88	26.58	27.08	26.83
T_1	3.89	3.96	3.93	3.42	3.48	3.45	30.50	31.07	30.79
T_2	3.58	3.65	3.61	3.06	3.12	3.09	28.07	28.60	28.33
T_3	3.49	3.56	3.52	2.97	3.02	2.99	27.36	27.88	27.62
T_4	3.60	3.67	3.63	3.09	3.15	3.12	28.22	28.76	28.49
T_5	3.99	4.07	4.03	3.53	3.60	3.56	31.28	31.87	31.58
T_6	3.98	4.06	4.02	3.52	3.58	3.55	31.20	31.79	31.50
T_7	4.09	4.17	4.13	3.65	3.72	3.68	32.07	32.67	32.37
T_8	4.01	4.09	4.05	3.56	3.62	3.59	31.44	32.03	31.74
T_9	3.72	3.79	3.76	3.23	3.29	3.26	29.16	29.72	29.44
T_{10}	3.69	3.76	3.72	3.19	3.25	3.22	28.93	29.48	29.20
T_{11}	3.80	3.87	3.84	3.31	3.38	3.34	29.79	30.36	30.07
T_{12}	4.12	4.20	4.16	3.68	3.75	3.72	32.30	32.91	32.61
T_{13}	4.10	4.18	4.14	3.66	3.73	3.70	32.14	32.75	32.45
T_{14}	4.22	4.30	4.26	3.80	3.87	3.83	33.08	33.71	33.40
F-test	S	S	S	S	S	S	S	S	S
S.E. (m) (±)	0.02	0.02	0.01	0.02	0.03	0.02	0.17	0.20	0.13
C.D. @ 5%	0.05	0.07	0.04	0.07	0.09	0.05	0.50	0.57	0.37

Table 3: Effect of Nano chitosan, Nano micronutrients and Bio capsules on flowering attributes of Kinnow Mandarin

Treatment Symbol	Days required for flowering			Average number of flower per tree		
	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled
T_0	35.02	34.28	34.65	911.11	948.47	929.79
T_1	31.18	30.52	30.85	1000.64	1041.67	1021.15
T_2	32.97	32.27	32.62	947.47	986.32	966.89
T_3	33.49	32.78	33.14	930.30	968.44	949.37
T_4	32.69	32.00	32.35	955.55	994.73	975.14
T_5	30.67	30.02	30.35	1020.38	1062.22	1041.30
T_6	30.76	30.11	30.44	1015.64	1057.28	1036.46
T_7	30.11	29.47	29.79	1046.13	1089.02	1067.58
T_8	30.48	29.84	30.16	1024.18	1066.17	1045.18
T_9	32.00	31.32	31.66	970.67	1010.47	990.57
T_{10}	32.27	31.59	31.93	964.65	1004.20	984.43
T_{11}	31.60	30.93	31.27	978.48	1018.60	998.54
T_{12}	29.58	28.96	29.27	1070.12	1113.99	1092.06
T_{13}	29.67	29.04	29.36	1068.13	1111.92	1090.03
T_{14}	29.42	28.80	29.11	1100.34	1145.45	1122.90
F-test	S	S	S	S	S	S
S.E. (m) (±)	0.11	0.10	0.08	7.16	8.35	5.50
C.D. @ 5%	0.33	0.29	0.22	20.73	24.18	15.57

Table 4: Effect of Nano chitosan, Nano micronutrients and Bio capsules on physico-chemical attributes of soil

Treatment Symbol	Available Nitrogen (N) (kg/ha)			Available Phosphorus (P) (kg/ha)			Available Potassium (K) (kg/ha)		
	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled
T_0	169.44	171.89	170.67	16.11	16.35	16.23	227.68	230.97	229.32
T_1	183.94	186.60	185.27	17.49	17.75	17.62	247.16	250.74	248.95
T_2	179.29	181.89	180.59	17.05	17.30	17.17	240.91	244.40	242.66
T_3	178.33	180.91	179.62	16.96	17.20	17.08	239.62	243.09	241.36
T_4	180.00	182.61	181.30	17.12	17.37	17.24	241.87	245.37	243.62
T_5	186.62	189.32	187.97	17.75	18.00	17.88	250.76	254.39	252.58
T_6	184.95	187.63	186.29	17.59	17.84	17.72	248.52	252.12	250.32
T_7	187.34	190.05	188.70	17.82	18.07	17.95	251.73	255.37	253.55
T_8	185.66	188.35	187.00	17.66	17.91	17.78	249.47	253.08	251.28
T_9	182.05	184.69	183.37	17.31	17.56	17.44	244.62	248.16	246.39
T_{10}	181.00	183.62	182.31	17.21	17.46	17.34	243.21	246.73	244.97
T_{11}	183.01	185.66	184.34	17.40	17.66	17.53	245.91	249.47	247.69
T_{12}	188.85	191.58	190.22	17.96	18.22	18.09	253.76	257.43	255.60
T_{13}	188.38	191.11	189.74	17.91	18.17	18.04	253.13	256.79	254.96
T_{14}	189.14	191.88	190.51	17.99	18.25	18.12	254.15	257.83	255.99
F-test	S	S	S	S	S	S	S	S	S
S.E. (m) (±)	0.29	0.31	0.21	0.03	0.03	0.02	0.38	0.42	0.28
C.D. @ 5%	0.83	0.91	0.60	0.08	0.09	0.06	1.11	1.22	0.81

Table 5: Effect of Nano chitosan, Nano micronutrients and Bio capsules on soil pH, Organic Carbon % and Electrical Conductivity

Treatment Symbol	Soil pH			Organic Carbon %			Electrical Conductivity (dS/m)		
	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled	2021-22	2022-23	Pooled
T_0	7.12	7.13	7.13	0.23	0.24	0.24	0.21	0.21	0.21
T_1	7.08	7.09	7.09	0.24	0.25	0.25	0.23	0.24	0.23
T_2	7.12	7.13	7.13	0.23	0.24	0.24	0.22	0.22	0.22
T_3	7.13	7.14	7.14	0.24	0.25	0.25	0.21	0.21	0.21
T_4	7.12	7.13	7.13	0.26	0.27	0.27	0.21	0.21	0.21
T_5	7.07	7.08	7.08	0.25	0.26	0.26	0.24	0.25	0.24
T_6	7.09	7.10	7.10	0.26	0.27	0.27	0.23	0.24	0.23
T_7	7.07	7.08	7.08	0.27	0.28	0.28	0.23	0.24	0.23
T_8	7.08	7.09	7.09	0.27	0.28	0.28	0.24	0.25	0.24
T_9	7.10	7.11	7.11	0.25	0.26	0.26	0.22	0.22	0.22
T_{10}	7.11	7.12	7.12	0.26	0.27	0.27	0.22	0.22	0.22
T_{11}	7.10	7.11	7.11	0.25	0.26	0.26	0.22	0.22	0.22
T_{12}	7.06	7.07	7.07	0.28	0.29	0.29	0.25	0.26	0.25
T_{13}	7.07	7.08	7.08	0.28	0.29	0.29	0.25	0.26	0.25
T_{14}	7.06	7.07	7.07	0.29	0.30	0.30	0.26	0.27	0.26
F-test	NS	NS	NS	NS	NS	NS	NS	NS	NS
S.E. (m) (±)	0.02	0.03	0.02	0.03	0.02	0.01	0.02	0.02	0.02
C.D. @ 5%	NS	NS	NS	NS	NS	NS	NS	NS	NS

7. Conclusion

Chemical fertilizers are disrupting the ecosystem and leading to negative impacts on human health. Nano fertilizers along with bio-fertilizers have been shown to enhance plant growth, improve nutritional quality, increase productivity, extend shelf life, and boost resistance to various stress factors. help to sustain nutrient levels in the soil and boost crop growth and yield through the activation of different mechanisms. A standardized amount of nano fertilizers and biofertilizers produces positive outcomes, with minimal risk of bioaccumulation in the soil due to their enhanced traverse and efficiency. They improve soil fertility and demonstrate a unique combination with beneficial interactions when compared to using just biofertilizer or nanoparticles. Overall, Treatment T_{14} [NPK (Recommended dose of fertilizer) + Soil drenching of Nano chitosan 100 ppm + Bio capsule 500 ppm + foliar application of nano particles (ZnO +FeO)] emerged as the most effective in stimulating robust growth of plant and improving soil health compared to other treatments in the experiment.

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