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Enhancing Fruit Yield and Citrus Quality through Integrated Application of Organic Fertilizers and Zinc

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Abstract

Maximization of citrus fruit yield and enhancing their quality are ambitious objectives for citrus growers and scientist across the globe. This study explores the synergistic potential of organic manures and zinc to achieve these objectives. This integrated approach represents a sustainable and environmentally friendly strategy for citrus cultivation. For this, a study was conducted with different treatments of zinc and farm yard manure. The trial was conducted using randomized complete block design with five repeats. Results showed that the highest fruit yield (58.23 kg plant^{-1}), fruit weight (169.40 g fruit^{-1}), fruit diameter (72.80 mm), juice content (46.64%), TSS (10.87 ˚Brix), citric acid (645.22 mg 100 mL^{-1}), ascorbic acid (593.45 mg 100 mL^{-1}), total sugar content (7.05%), Zn content in leaf (19.46 mg kg^{-1}), photosynthetic rate (14.68 µmol m^{-2} s^{-1}), transpiration rate (3.95 mmol m^{-2} s^{-1}), WUE (3.72 µmol CO_2 mmol^{-1} H_2O), stomata conductance (0.465 mmol m^{-2} s^{-1}) and total chlorophyll (4.41 mg g^{-1}) were obtained by application of T8: (Zn @ 50 g plant^{-1} + FYM @ 25 kg plant^{-1} + PM @ 10 kg plant^{-1}). On the basis of our results, it is concluded that integrated use of Zn coupled with organic manures was the most effective combination of fertilization for improving physiological functioning and ionic metabolism, yield contributing attributes and fruit quality of citrus.

Keywords: Farm manures, micronutrient, synergistic effect, ionic concentration, fruit crop
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Introduction

Citrus is amongst the world's foremost fruit crops with worldwide availability and popularity donating to human nutrition. Citrus fruits are important and rapid source of nutrients, minerals, fiber, vitamins and various other essential compounds compulsory for human body (Liu et al., 2022; Richa et al., 2023). Citrus fruit comprises a healthy amount of vitamins like A, B, C, foliate and minerals nutrients like Fe, P and Ca (Khan et al., 2015). In our food, citrus helps against diseases like influenza, diabetics and malaria because of the existence of various phenolic compounds and vitamins in them (Gonzalez-Molina et al., 2010; Abobatta, 2019). It is grown on an area of around 0.2 million hectares with a production of 2.33 million tons and a productivity of 9.2 tons per hectare in Pakistan (FAO, 2021). Feutrell and Kinnow are two major varieties of mandarins which are mostly grow in 80% growing area of Punjab (Altaf, 2006). The types of soils in Pakistan and climate have gave a distinctive taste and flavor to Kinnow. Hence, Kinnow has developed a trademark of Pakistan.

Presently, Pakistan produced around 95% of the total word's production of Kinnow (ACIAR, 2008). Pakistan stands at sixth place amongst the citrus producing countries of the world but the citrus production in general is almost stagnant, or even declining in some parts of the country (Ilyas et al., 2015; Liu et al., 2022). The reasons for this decline may be numerous but traditional nutrient management approach based mostly on the application of macronutrients in citrus orchards, which is not very effective in improving the citrus yield as well as quality (Ilyas et al., 2015; Bastakoti et al., 2022). According to a survey based on leaf analysis, described that in the citrus orchards of Malakand Division, Zn is deficient in 100%, manganese in 96%, boron in 24% and copper in 16% of citrus orchards (Shah et al., 2012).

Citrus is a comparatively high nutrient required crop and extremely responsive to added nutrients in the form of fertilizers (Ashraf et al., 2010; Srivastava, 2012). Higher growth with enhanced fruit quality and yield can be gained by adequate and balanced fertilization as any nutrient either excess or deficient can lead to a decrease in crop yield joined with lesser fruit quality. Yaseen and Ahmad (2010) suggested that nutrient management of citrus might have significant effect on vegetative growth as well as flowering, fruit quality, fruit development and fruit set characteristics. Many studies have demonstrated that appropriate application of micronutrients might help to increase the fruit quality and yield (Noor et al., 2013; Ghayekhloo and Sedaghathoor, 2015; Sabahat et al., 2021). However, bioavailability of micronutrients is very limited in our soils because of less organic matter in soil, calcareous soil nature and alkaline pH of soil (Ashraf et al., 2012; Bastakoti et al., 2022). The soils in Pakistan are commonly lacking organic matter because of arid climatic conditions resulting in a quick decomposition of organic matter, and since very low amount of organic matter is applied to the soil. This situation can be overcome by the application of micronutrients with organic manures into the soil which increases soil organic matter contents while decreases soil pH, and thus leading to enhanced micronutrients use efficiency (Rutkowska et al., 2014). Organic manures are not only the direct source of micronutrients but also increase the bioavailability of applied micronutrients by lowering soil pH, improving C status of soil and binding the fixing agents (Saquee et al., 2023). In order to obtain desired quality and productivity of fruit, judicious management of micronutrients is required.

Zinc is a vital nutrient for all living organisms, with latent roles in 1000s of proteins in humans and plants (Broadley et al., 2007). Zinc is a very significant nutrient for the development and growth of plant. Plants take zinc in divalent form as Zn^{2+} at normal pH and take in monovalent form as $ZnOH^{+}$ under high pH. Zinc deficiency is very severe in Pakistani soil because of the presence of large quantities of calcium in soils. There are numerous reasons of this reduction like high pH, less organic matter, high amount of calcium, salinity and sodicity (Singh, 2005). It is predicted that around one third of cultivated soils worldwide have low available Zn content which results in lessened crop production as well as low nutritional quality of the cultivated crops (Cakmak, 2008; Hacisalihoglu, 2020).

The present research was planned to determine the current status of micronutrients, especially zinc, in soil and citrus plants. It also aimed to evaluate the efficacy of soil application of zinc, both with and without various organic manures, in order to enhance the quality, growth and fruit yield of citrus.

Materials and Methods

Experimental site

A field experiment was conducted in the Research area, College of Agriculture, University of Sargodha, Sargodha, Punjab, Pakistan (Latitude 32.083ºN, Longitude 72.0669ºE and altitude 190 m) to compare the nutrient use efficiency of zinc applied through soil application as well as in combination with farm yard manure (FYM) and poultry manure (PM) for improving fruit yield and quality of citrus. For this study, about 8-10 years old plants from citrus orchard were selected from the research area of College of Agriculture. Prior to experimentation, soil was analyzed for physicochemical properties using standard techniques of analysis. For this, soil samples were collected from the selected fields at the depth of 0-30 cm (Table 1).

Table 1. Physicochemical properties of soil to be used in study

Soil properties	**Value**
Physical properties	
Sand (g kg^{-1})	580
Silt (g kg^{-1})	182
Clay (g kg^{-1})	238
Textural class	Sandy clay loam
Saturation percentage (%)	33.50
Chemical properties	
pH	7.82 ± 0.06
Electrical conductivity (µS cm^{-1})	1239 ± 39.39
Organic matter (g kg^{-1})	9.98 ± 1.18
TOC (g kg^{-1})	5.81 ± 0.26
DOC (mg kg^{-1})	46.43 ± 4.64
Total N (mg kg^{-1})	265.04 ± 10.13
Available P (mg kg^{-1})	8.94 ± 0.63
Available K (mg kg^{-1})	194.74 ± 9.32

TOC: Total organic-C, DOC: Dissolved organic-C

Experiment description

A field experiment was performed to evaluate the efficacy of integrated application of Zn and organic manures for improving fruit yield and quality of citrus during 2020. The study was comprised of two level of Zn (i.e. 0 and 50 g plant^{-1}), two levels of FYM (0.0 and 50 kg plant^{-1}, two levels of PM (i.e. 0 and 20 kg plant^{-1}) and their integrated application with all possible combinations of Zn (Zn was applied as $ZnSO_4.7H_2O$) and manures using Randomized Complete Block Design (RCBD) with five repeats per treatment.

The textural class of the experimental field was sandy clay loam soil that belong to the Lyallpur soil series (Aridisols fine silty, hyperthermic haplic-vermosols according to FAO Soil Classification System). Ten different soil samples from 0-30 cm depth were taken and hand-sorted to remove any visible stones in the field, complete/or intact dead and/ or live vegetation before they were carefully mixed to develop a composite soil sample. That composite sample of soil was air-dried, and then sieved through 2mm mesh-screen and analyzed for physicochemical properties of soil to be used in study (Table 2).

Table 2. Physicochemical properties of farm yard manure (FYM) used in the study

Properties of manure	Farm manures (Mean values)		References
	FYM	**PM**	
Physical properties			
Moisture (%)	33.29 ± 1.96	29.13 ± 1.19	Willits (1951)
Color	Light to dark brown		
Chemical Properties			
pH	7.23 ± 0.02	7.69 ± 0.03	Schofield and Taylor (1995)
EC (µS cm^{-1})	1234 ± 56.42	1169 ± 60.37	US Salinity Laboratory Staff (1954)
Organic matter (%)	50.94 ± 2.39	48.26 ± 1.69	Walkley and Black (1934)
Total organic-C (%)	29.62 ± 1.32	28.06 ± 1.54	
DOC (mg g^{-1})	24.19 ± 1.16	25.04 ± 1.63	
Total N (%)	0.72 ± 0.06	0.79 ± 0.05	Bremner and Tabatabai (1972)
Total P (%)	0.44 ± 0.04	0.53 ± 0.06	Allen et al. (19860
Total K (%)	0.57 ± 0.05	0.42 ± 0.03	
Zn (mg kg^{-1})	65.36 ± 3.23	32.15 ± 2.56	Lindsay and Norvell (1978)
Fe (mg kg^{-1})	158.45 ± 10.29	89.24 ± 8.26	
Mn (mg kg^{-1})	45.76 ± 3.04	19.32 ± 1.59	
Cu (mg kg^{-1})	12.46 ± 1.86	9.36 ± 0.69	
B (mg kg^{-1})	32.54 ± 2.17	27.63 ± 1.74	Gaines and Mitchell (1979)

FYM: Farm yard manure, PM: Poultry manure

While, the chemical fertilizers N: P: K @ 1000: 500: 500 g plant^{-1} were applied as urea, di-ammonium phosphate (DAP) and sulfate of potash (SOP). But one third (1/3th) of N and full dose of P and K fertilizers were applied as a basal dose before initiation of flowering on plants and remaining quantity of N was top-dressed in to two equal splits at different growth stages i.e. after fruit setting (April) and at the time of fruit color changing (1^{st} week of September) of citrus fruit. Good quality irrigation water was applied throughout the research that having EC 0.075 dS m^{-1}, SAR 0.34 (mmol $L^{-1})^{1/2}$ and zero RSC. In addition, all agronomic practices were used to overcome the plant diseases and weeds during study.

Different physiological parameters [i.e. chlorophyll contents (*a*, *b* and total), net photosynthetic rate, water use efficiency (WUE), transpiration rate, and stomata conductance], were taken after 60 days of treatments application. Yield contributing parameters (i.e. fruit yield, fruit weight, fruit diameter, number of seed $fruit^{-1}$, rag weight $fruit^{-1}$, peel thickness, peel weight $fruit^{-1}$), quality attributes (i.e. Juice content, total soluble solids , pH), biochemical attributes (i.e. ascorbic acid, citric acid, total sugar content, reducing sugar content, non-reducing sugar content, zinc content in fruit and leaf were determined using standard procedures.

Soil analysis

Soil samples were taken from research area at the depth of 0-30 cm during the month of September before and after the research experiments and analyzed in order to determine physicochemical and biochemical characteristics of soil. The soil samples were collected from 5-8 spots randomly to represent the soil of whole orchard.

For determination of soil texture hydrometer method was used (Moodie et al., 1959). Finally, soil textural class was determined by using international textural triangle (Brady, 1999). Soil pH was measured by using pH meter (JENWAY-3510). For this, suspension ratio of soil and water (1: 5) was prepared to analyze pH of soil. Soil EC was measured by using the conductivity bridge method described by Jackson (1967). According to this method soil sample was prepared for determination of soluble salts in soil using EC meter (HANNA EC-215). While, saturation percentage was determined by using prescribed formula. A fraction of soil saturated paste was oven dried 105ºC to a constant weight. Then saturation percentage was then determined by using following formula.

$$Water\ content\ (\%) = \frac{\text{loss in weight on drying}}{\text{Oven dry weight of soil}} \times 100$$

Total organic – C content in soil was measured by using the method defined by Walkley and Black (1934). For estimation of N from soil, two steps are involved (digestion and distillation) in estimation of total N from soil using Kjeldhal's distillation apparatus (Bremner and Tabatabai, 1972). While, Olsen's method was applied to soil sample for determination of available soil phosphorus (Jackson, 1967) using Spectrophotometer (Model Hitachi-120). Extractable - K from soil sample was determined using 1.0 N ammonium acetate (NH_4OAc) solution by performing repeated extractions in soil sample (Richard, 1954).

Plant analysis

During period of plant growth on field experiments, we observed yield contributing parameters (i.e. fruit yield, fruit weight, fruit diameter, number of seed $fruit^{-1}$, rag weight $fruit^{-1}$, peel thickness, peel weight $fruit^{-1}$), different physiological parameters [i.e. chlorophyll contents (*a*, *b* and total), net photosynthetic rate, water use efficiency, transpiration rate, and stomata conductance], were taken after 60 days of treatments application. quality attributes [i.e. juice content, total soluble solids, pH], biochemical attributes (i.e. citric acid, ascorbic acid, reducing sugar content, non-reducing sugar content, total sugar content, zinc content in fruit and leaf were determined using standard procedures. For analysis five number of fruits of average size were collected from every tree. Data regarding these parameters were estimated by using following standard procedures.

Yield and contributing attributes of plant

Fruit weight was calculated from randomly selected fruits from the sample picked and weighed on top pan electronic balance. Diameter of fruits in every sample was noted with digital Vernier Callipers and mean value was taken in millimeters (mm). The peel thickness was measured with the help of digital Vernier Callipers at the equator of fruit and expressed in mm. Number of healthy and aborted seeds of each fruit of sample was counted separately and their average is taken into consideration.

Plant physiological parameters

Transpiration rate, stomatal conductance and photosynthetic rate, (at leaf stage) were measured by adopting Infra- red Gas Analyzer (IRGA, LCA-4, ADC, Hoddesdon UK) and measured readings were noted by using procedure recommended by Long and Bernacchi, (2003). Water use efficiency (WUE) was calculated by dividing net photosynthetic rate by transpiration rate (μmol CO_2 mmol H_2O m^{-2} s^{-1}). While, chlorophyll pigments were extracted from leaves of citrus plant with aqueous acetone, and estimated by Spectrophotometer (Model Hitachi-120, Japan) is according to Vernon's models which is modified of Arnon's models.

Determination of fruit quality attributes

Total soluble solids content was measured at 20°C with a Schmidt and Haensch HR32B handheld Refractometer (Schmid and Haensch GmbH and Co., 13403 Berlin, Germany) having a sensitivity of 0.2 °Brix. Juice content of fruit was extracted with help of juicer machine and pH of fruit juice was measured by using pH meter (JENWAY-3510). But fruits were peeled off and their juice was extracted with the help of simple juice extractor. Then weighed the juice with electronic balance and percentage of juice was calculated on the basis of following formulae:

$$Juice\ content\ (\%) = \frac{\text{Juice weight}}{\text{Fruit weight}} \times 100$$

After extraction of juice, the left over residue was considered as rag content. The per cent rag content was calculated by using the formula:

$$Rag\ weight\ (g) = [Fruit\ weight - (Peel\ weight + Juice\ weight)$$

Randomly selected fruits were peeled manually. Peel was weighed with electronic balance and the per cent peel content was calculated by using the formula:

$$Peel\ weight\ (g) = [Fruit\ weight - (Rag\ weight + Juice\ weight)$$

Determination of biochemical attributes of fruit

The micronutrient (Zn), citric acid, ascorbic acid, reducing sugar content, non-reducing sugar content, total sugar content in fruit samples were determined using standard procedures. Zinc was analyzed after digestion of dry and milled plant material with 2.0 N HCl solution. Zn concentration was determined by atomic absorption spectrophotometer (Benton Jones, 2001). The total sugar content was determined by Anthrone method (Hodge and Hofreiter, 1962), reducing sugar content by Spectrophotometric method

(Somogyi, 1952) while, ascorbic acid and citric acid contents were estimated with titration method described by Ranganna (1986).

Statistical analysis

All the data were analyzed by using Statistix 10 Statistical Package and to compare the difference among the treatment means we use least significant difference (LSD) test at 0.05 P (Steel et al., 1997).

Results

A study was designed that having different eight treatments which was conducted in the Research Farm, College of Agriculture to compare the Zn applied through soil application and in combination with farm yard manure and poultry manure for improving fruit yield and quality of citrus. The results of this study are given below.

Integrated effect of Zn and farm manures on growth and fruit of citrus

Results showed that yield components of citrus in terms of fruit weight, fruit diameter, fruit yield as well as peel weight fruit^{-1} and peel thickness fruit^{-1} and rag weight fruit^{-1} were significantly ($p \leq 0.05$) improved with increasing exogenous zinc in soil amended FYM except number of seed fruit^{-1} showing non-significant results (Table 3). Maximum yield (58.23 kg plant^{-1}) was recorded by (T8) integrated use of FYM @ 50 kg plant^{-1} + PM @ 10 kg plant^{-1} and application of Zn @ 50 g plant^{-1} followed by T7 (53.80 kg plant^{-1}) and T6 (50.75 kg ha^{-1}) while, minimum value (34.44 kg plant^{-1}) of fruit yield had shown by T1 treatment (untreated). Results of all treatments were at par significantly relative to untreated control. While, maximum fruit weight was observed in T8 treatment (Zn @ 50 g plant^{-1} + FYM @ 25 kg plant^{-1} + PM @ 10 kg plant^{-1}) with value of 169.40 g fruit^{-1} while, minimum value (116.60 g fruit^{-1}) of fruit weight was obtained from control. All the treatments had improved fruit weight in citrus relative to untreated control. Data in Table 3 had demonstrated that all the treatments were different from each other significantly and had improved fruit diameter in citrus. Maximum citrus fruit diameter (72.80 mm) was indicated by T8 treatment while, minimum fruit diameter (59.88 mm) was obtained by respective control treatment.

Number of seeds fruit^{-1} in citrus fruit had improved with integrated application of FYM and zinc through soil application. However highest value (9.64) seeds fruit^{-1} had recorded in T8 treatment while, minimum (8.75) of number of seeds fruit^{-1} had shown by untreated control. All the treatments had shown non-significant difference among each other relative to control. It had confirmed from the data of rag weight of citrus fruit (Table 3) that all the single and combined treatments had increased rag weight of citrus fruit relative to control. However maximum rag weight (42.39 g fruit^{-1}) was observed in T8 treatment where integrated supply of FYM @ 50 kg plant^{-1} along with PM @ 10 kg plant^{-1} and Zn @ 50 g plant^{-1} was applied and minimum rag weight (32.56 g fruit^{-1}) was obtained by respective control. But result of peel thickness had shown that minimum peel thickness (3.62 mm) was of citrus fruit was observed in untreated control while, highest value (4.48 mm) of peel thickness was recorded by T8 treatment where FYM and Zn were applied combinedly. All the treatments had increased peel thickness significantly over control treatment except T2 treatment where only Zn was applied @ 50 g plant^{-1}. While, peel weight of citrus fruit had revealed that highest weight (39.67 g fruit^{-1}) of peel had recognized in T8 treatment where Zn was fertilized along with FYM and PM through soil application followed by T7 (38.17 g fruit^{-1}) and T6 (37.82 g fruit^{-1}) while, minimum concentration of peel weight had determined in respective control (35.78 g fruit^{-1}).

Table 3. Integrated effect of Zn and different manures on fruit yield (FY kg plant^{-1}), Fruit weight (FW g), fruit diameter (FD mm), number of seed fruit (NSF^{-1}), rag weight (g fruit^{-1}), peel thickness (PT mm) and peel weight (PW g fruit^{-1})

Treatments	FY	FW	FD	NSF^{-1}	RW	PT	PW
T1	34.44g	116.60f	59.88e	8.75	32.56e	3.62e	35.78d
T2	38.39f	134.87e	63.94de	9.02	35.06d	3.65e	36.47cd
T3	41.79ef	138.57e	66.45c	9.87	35.84d	3.68de	36.90bc
T4	45.78d	140.90de	67.29bc	9.05	36.54cd	3.77cd	37.46b
T5	48.23cd	145.72d	66.70c	9.78	37.65c	3.85c	37.79b
T6	50.75bc	149.68cd	68.74b	9.53	38.09bc	4.07bc	37.82b
T7	53.80b	158.23b	69.39ab	9.40	39.72ab	4.27ab	38.17ab
T8	58.23a	169.40a	72.80a	9.64NS	42.39a	4.48a	39.67a

Values are means of four replicates. For every parameter, under each column, values sharing dissimilar letters differ significantly from each other at $p<0.05$. NS: Non-significant, T1: Control (untreated), T2: Zn @ 50 g plant^{-1}, T3: FYM @ 50 kg plant^{-1}, T4: PM @ 20 kg plant^{-1}, T5: Zn @ 50 g plant^{-1} + FYM @ 50 kg plant^{-1} T6: Zn @ 50 g plant^{-1} + PM @ 20 kg plant^{-1}, T7: FYM @ 50 kg plant^{-1} + PM @ 20 kg plant^{-1}, T8: Zn @ 50 g plant^{-1} + FYM @ 25 kg plant^{-1} + PM @ 10 kg plant^{-1}

Integrated effect of zinc and organic manure on fruit quality attributes of citrus

Results regarding Juice contents in citrus fruit in Table 4 had confirmed that in all treatments integrated concentration of Zn, FYM and PM had improved percentage of juice contents in citrus fruit over respective control treatment. However highest percentage (46.64%) of juice contents had determined by T8 treatment followed by T7 (43.54%) and T6 (42.36%) treatment while, minimum percentage value (34.95%) of juice contents had found in T1 treatment. As like to fruit juice, the results regarding TSS had revealed that maximum TSS were observed in treatment (T8) where combined fertilization technique was performed followed by T7 and T6 treatments while, lowest TSS had found in untreated control treatment. Overall, all the treatments had improved TSS concentration significantly over control treatment in citrus fruits. Results regarding to pH of juice had suggested that in all treatments improved concentration of single and combined fertilizers and FYM had decreased pH values relative to untreated control. Although, maximum pH value was observed in control treatment and minimum value was observed in T8 and T7, respectively where combine use of FYM and micronutrients were performed through soil and foliar application.

This attribute (citric acid) in citrus plant (Table 4) had confirmed that maximum citric acid concentration had found where FYM and micronutrients were applied in combined form in T8 (645.22 mg 100 mL^{-1}) and T7 (622.54 mg 100 mL^{-1}) treatments, respectively while, lowest citric acid (493.57 mg 100 mL^{-1}) had found in control treatment. Generally, all the treatments had found significantly different form each other relative to control and had improved citric acid gradually. As like to citric acid, the value of ascorbic acid found minimum (455.90 mg 100 mL^{-1}) in control treatment while, maximum value (593.45 mg 100 mL^{-1}) of ascorbic acid had found in T8 treatment where Zn, FYM and PM had applied in combined form followed by T7 (576.77 mg 100 mL^{-1}) and T6 (568.33 mg 100 mL^{-1}) treatments. All the treatments had proved best to improve ascorbic acid content with respect to control.

Table 4. Integrated effect of Zn and different manures on fruit quality Juice content (JC%), TSS (Brix), pH, citric acid (CA), ascorbic acid (AA mg 100 mg ML^{-1}), reducing sugar (RS %), non-reducing sugar (NR %), total sugar content (TC %) of citrus plants

Treatments	JC	TSS	pH	CA	AA	RS	NR	TC
T1	34.95e	8.54e	7.82a	493.57d	455.90e	2.87f	2.94e	5.81f
T2	37.60d	8.70d	7.75a	562.42c	523.67d	3.04de	3.26d	6.30e
T3	37.96d	8.79cd	7.69ab	570.73c	540.12cd	3.09d	3.29cd	6.38e
T4	39.56cd	8.85c	7.58b	580.44bc	552.80bc	3.20cd	3.31c	6.51d
T5	40.32c	8.89c	7.52b	587.36b	561.51b	3.29bc	3.35c	6.64cd
T6	42.36b	9.44bc	7.37c	599.54b	568.33b	3.35ab	3.43bc	6.78bc
T7	43.54b	9.96b	7.24d	622.54ab	576.77ab	3.41a	3.47b	6.88ab
T8	46.64a	10.87a	6.98e	645.22a	593.45a	3.46a	3.61a	7.05a

Values are means of four replicates. For every parameter, under each column, values sharing dissimilar letters differ significantly from each other at $p<0.05$. NS: Non-significant, T1: Control (untreated), T2: Zn @ 50 g plant^{-1}, T3: FYM @ 50 kg plant^{-1}, T4: PM @ 20 kg plant^{-1}, T5: Zn @ 50 g plant^{-1} + FYM @ 50 kg plant^{-1} T6: Zn @ 50 g plant^{-1} + PM @ 20 kg plant^{-1}, T7: FYM @ 50 kg plant^{-1} + PM @ 20 kg plant^{-1}, T8: Zn @ 50 g plant^{-1} + FYM @ 25 kg plant^{-1} + PM @ 10 kg plant^{-1}

Data of reducing sugar (Table 4) had demonstrated that maximum reducing sugar in citrus fruit had found in T8 (Zn + FYM + PM) were applied in combined form showing the value of 3.46% followed by T7 and T6, respectively while, lowest sugar percentage had appeared in control treatment (2.87%). However, all the treatments had increased percentage of reducing sugar significantly over control. While, results of non-reducing sugar presented in Table 4 had revealed that all the treatments had improved non reducing sugar in citrus fruit with improved concentration of OM and Zn. Minimum percentage (2.94%) of non-reducing sugar had calculated in untreated control while, highest percentage (3.61%) of non-reducing sugar had found in treatment T8 compared to control and subsequent readings were noted in T7 (3.47%) and T6 (3.43%), respectively. Similarly, results of total sugar in citrus fruit interpreted in Table 4 that maximum percentage of total sugar had recorded in combined application of FYM and micronutrients in high concentration in T8 treatment with values of 7.05% followed by T7 (6.88%) and T6 (6.78%) while, minimum readings had found in untreated control.

Integrated effect of zinc and organic manure on ionic concentration of citrus plants

Data regarding to Zn content in citrus plant leaves had shown that that maximum value of Zn concentration (19.46 mg kg^{-1}) in citrus leaf had found in combined application of OM and Zn (T8) while minimum concentration (6.43 mg kg^{-1}) had observed in control treatment (Fig. 1a). All the treatments had proved to best in increasing micronutrient (Zn) concentration in citrus leaf over control, as like to Zn concentration in plants.

Integrated effect of zinc and organic manure on physiological attributes of citrus plants

Results regarding chlorophyll *a* content mentioned in Fig. 1b 5 had revealed that all the treatments significantly improved the chlorophyll *a* content in citrus plant with integrated use of organic manures and Zn over control. Minimum percentage (1.77 mg g^{-1}) of chlorophyll *a* content had calculated in untreated control while, highest value (2.64 mg g^{-1}) of chlorophyll *a* content had found in T8 where OM and Zn had applied in combined form over control treatment and subsequent readings were noted in T7 (2.56 mg g^{-1}) and T6 (2.50 mg g^{-1}), respectively.

According to the results about chlorophyll *b* content in Fig. 1c had depicted that control treatment (T1) had shown lowest chlorophyll *b* content (1.22 mg g^{-1}) in citrus leaves while, highest value (1.77 mg g^{-1}) of chlorophyll *b* content had presented in T8 (50 g Zn + 25 kg FYM + 10 kg PM $plant^{-1}$) treatment. All the treatments had increased chlorophyll *b* content with increased concentration of OM and Zn over control treatment.

Total chlorophyll content in citrus plant leaves had improved significantly with gradually increase in single and combined fertilizers to citrus plant over control. Maximum value (4.41 mg g^{-1}) of total chlorophyll content was observed in T8 treatment due to integrated use of FYM+PM and Zn while, lowest value (2.99 mg g^{-1}) of total chlorophyll content was recorded in untreated control.

Results regarding net photosynthetic rate mentioned in Table 5 had revealed that all the treatments had improved Net photosynthetic rate in citrus plant with combined concentration of OM and Zn over control treatment. Minimum value (9.64 µmol m^{-2} s^{-1}) of net photosynthetic rate had calculated in untreated control (T1) while, highest value (14.68 µmol m^{-2} s^{-1}) of net photosynthetic rate had found in T8 where OM and Zn had applied in combined form over respective control and subsequent readings were recorded in T7 (13.74 µmol m^{-2} s^{-1}) and T6 (13.08 µmol m^{-2} s^{-1}), respectively.

Table 5. Integrated effect of Zn and different manures on physiological attributes of citrus plants

Treatments	Net photosynthetic rate (µmol m^{-2} s^{-1})	Transpiration rate (mmol m^{-2} s^{-1})	WUE (µmol CO_2 $mmol^{-1}$ H_2O)	Stomata conductance (mmol m^{-2} s^{-1})
T1	9.64d	3.12d	3.09d	0.328f
T2	10.70d	3.37c	3.18cd	0.370e
T3	10.96cd	3.43c	3.20c	0.386de
T4	11.21c	3.48bc	3.22c	0.393d
T5	11.60c	3.59b	3.23c	0.401c
T6	13.08b	3.73ab	3.51b	0.424bc
T7	13.74ab	3.88a	3.54b	0.443b
T8	14.68a	3.95a	3.72a	0.465a

Values are means of four replicates. For every parameter, under each column, values sharing dissimilar letters differ significantly from each other at $p<0.05$. NS: Non-significant, T1: Control (untreated), T2: Zn @ 50 g $plant^{-1}$, T3: FYM @ 50 kg $plant^{-1}$, T4: PM @ 20 kg $plant^{-1}$, T5: Zn @ 50 g $plant^{-1}$ + FYM @ 50 kg $plant^{-1}$ T6: Zn @ 50 g $plant^{-1}$ + PM @ 20 kg $plant^{-1}$, T7: FYM @ 50 kg $plant^{-1}$ + PM @ 20 kg $plant^{-1}$, T8: Zn @ 50 g $plant^{-1}$ + FYM @ 25 kg $plant^{-1}$ + PM @ 10 kg $plant^{-1}$

Data of transpiration rate (Table 5) had demonstrated that maximum transpiration rate in citrus plant had found in T8 where OM and Zn were applied in combined form showing the value of 3.95 mmol m^{-2} s^{-1} followed by T7 and T6, respectively while lowest transpiration rate had appeared in control (T1) treatment where no single and combined fertilizer had applied. However, all the treatments had increased value of transpiration rate significantly over control.

Results regarding WUE of citrus plant in Table 5 had confirmed that in all treatments the combined concentration of OM and Zn had improved WUE in citrus plant over untreated control. However highest WUE (3.72 µmol CO_2 mmol[1] H_2O) was recorded in T8 followed by T7 (3.54 µmol CO_2 $mmol^{-1}$ H_2O) and T6 (3.51 µmol CO_2 $mmol^{-1}$ H_2O) treatments while, minimum WUE (3.09 µmol CO_2 $mmol^{-1}$ H_2O) was found in untreated control. Similarly, stomatal conductance had increased in all treatments treated with increasing concentration of single and combined use of OM and Zn. Highest stomata conductance (0.465 mmol m^{-2} s^{-1}) recorded in T8 where amount of OM and Zn had fertilized in combined form whereas control treatment had shown lowest concentration (0.328 mmol m^{-2} s^{-1}) of stomata conductance.

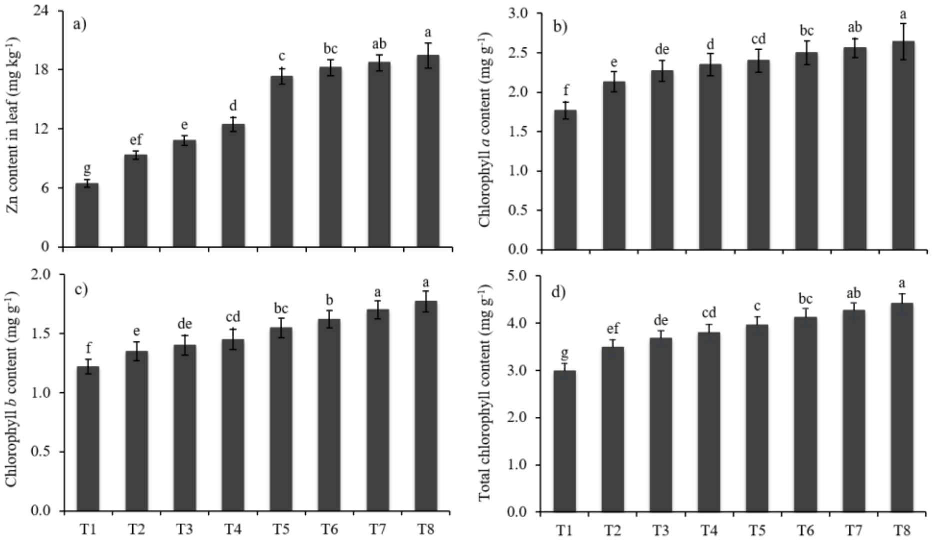

Figure 1. Integrated effect of Zn and different manures on zinc and chlorophyll contents of citrus plants. Values are means of four replicates. For every parameter, under each column, values sharing dissimilar letters differ significantly from each other at $p<0.05$. NS: Non-significant, T1: Control (untreated), T2: Zn @ 50 g $plant^{-1}$, T3: FYM @ 50 kg $plant^{-1}$, T4: PM @ 20 kg $plant^{-1}$, T5: Zn @ 50 g $plant^{-1}$ + FYM @ 50 kg $plant^{-1}$ T6: Zn @ 50 g $plant^{-1}$ + PM @ 20 kg $plant^{-1}$, T7: FYM @ 50 kg $plant^{-1}$ + PM @ 20 kg $plant^{-1}$, T8: Zn @ 50 g $plant^{-1}$ + FYM @ 25 kg $plant^{-1}$ + PM @ 10 kg $plant^{-1}$

Discussion

Overall, it is observed that the integrated use of Zn, FYM and PM had shown a very effective results regarding yield and contributing attributes (fruit weight, fruit diameter, fruit yield as well as peel weight fruit[1] and peel thickness $fruit^{-1}$ and rag weight $fruit^{-1}$) and our finding are matched with Saquee et al. (2023) who described that combined use of micronutrients (Fe, Zn, Mn, Cu and B) significantly increased the fruit weight, fruit yield, peel thickness $fruit^{-1}$ and rag weight $fruit^{-1}$ compared to respective control. Many researches have verified that appropriate application of micronutrients might help to increase the fruit yield and quality (Noor et al., 2013; Ghayekhloo and Sedaghathoor, 2015). However, bioavailability of micronutrients is very limited in our soils because of calcareous soil nature, low organic matter in soil and alkaline soil pH (Ashraf et al., 2012). However, adequate regulation of micronutrients improved fruit quality, growth and yield of citrus (Ashraf et al., 2013; Ilyas et al., 2015). Omar et al. (2014) informed that addition of boric acid alone (1500 ppm) or combined with Zn (300 ppm) improved the yield and quality of date palm. Ghayekhloo and Sedaghathoor (2015) reported that application of Zn + Fe + Mn significantly improved dry and fresh fruit weight, Mn content, Fe content, vitamin-C and sugar of *Citrus reticulata* fruits. Comparing the efficiency of foliar and soil application of micronutrients, it has been found that addition of some nutrients via foliar can be 10-20 times more effective than its addition via soil application (Alva et al., 2006). Camberato (2004) found that foliar spray of Zn, B, Mg, Cu and Mn was more effective compared to soil application for quick remedy of their deficiencies, decreasing the toxicity in soil and avoiding their fixation. Similarly, Srivastava and Singh (2008) also documented that integrated application of micronutrients along with manure depicted highly significant improvement in fruit weight, fruit yield, TSS, and organic acids (citric acid and ascorbic acid) and total sugar contents as compared to untreated control treatment. However, numerous other researchers (Monga and Josan, 2000; Aular et al., 2017; Liu et al., 2022) they also stated that combined application of micronutrients in soil amended with manures showed highly significant results fruit weight, fruit yield, peel weight, rag weight, TSS, juice content, and organic acids (citric acid and ascorbic acid), total sugar contents, as well as physiological attributes citrus plants as compared to control treatments.

The results of our study are in line with Bastakoti et al. (2022) who detected that integrated addition of micronutrients (Zn, Cu, B, Fe, and Mn) along with manure depicted highly significant improvement in fruit weight, fruit yield, TSS, and organic acids (citric acid and ascorbic acid) and total sugar contents as compared to untreated control. Saquee et al. (2023) reported that combined or alone application of Cu, Mn and Fe in the concentrations 0.5 to 1% as foliar spray, improved quality of orange juice. Zinc deficiency has traditionally been the most widespread in citrus orchard soils, worldwide. However, Zn application rates from 4-12 kg ha^{-1} may have positive effects on fruit quality characteristics, best results in terms of juice contents and flavor of juice, total sugars, and vitamin-C were attained at 4 kg ha^{-1} (Mdwaradze, 1981). Batru et al. (1984) found that application of Zn at 0.6% markedly improved fruit diameter, weight, juice percentage, ascorbic acid content and TSS and in Kagzi lime. However, numerous other researchers (Monga and Josan, 2000; Aular et al., 2017; Bastakoti et al., 2022) they also described that mutual application of micronutrients in soil amended with manures showed highly significant results TSS, juice content, and organic acids (citric acid and ascorbic acid), reducing sugar content, non-reducing sugar content and total sugar contents, as well as physiological attributes citrus plants as compared to untreated control treatments.

Similar to every fruit tree, citrus tree also needs essential nutrients for normal production, growth, and quality regardless of the source (Zekri, 1995). Renewed and strengthened hard work have been in progress during the last 10-15 years to cultivate citrus organically ever then exhausting soil fertility produced serious anxiety with the repetition of high density orcharding joined with heavy use of inorganic fertilizers that were instantly available to plants for the uptake of nutrient (Saquee et al., 2023) carrying extraordinary decrease in organic matter in soil (Intrigliolo and Stagno, 2001). Organic manuring is frequently considered as a maintainable agricultural practice, and, if used properly, potentials to offer ridiculous outputs on lasting basis (Akanmu et al., 2023).

Larger accessibility of Zn, B, Fe, and Cu is general probability in soils where addition of FYM or any other organic sources have been applied for many years. A substantial increase in DTPA-extractable Mn, Zn, Fe, and Cu in the upper layer loamy sand soil was described by Shivay et al. (2010) who observed that quantity of Zn, B and Mn in fruit and leaf was greater in organically produced (with farmyard manure) than in normally grown (with inorganic fertilizers) crops. A number of researches have confirmed that appropriate addition of micronutrients might help to increase the fruit yield and quality (Noor et al., 2013; Ghayekhloo and Sedaghathoor, 2015; Liu et al., 2022). However, bioavailability of micronutrients is very limited in our soils because of calcareous soil nature, low organic matter in soil, and alkaline soil pH (Ashraf et al., 2012). However, Dhaliwal et al. (2013) described the weighty gain of Zn in soil throughout 1st, 2nd and 3rd sampling results when organic manures were applied as compared to untreated control treatments. Past studies had highlighted the insufficiency of mineral nutrients, mainly, micronutrients (Zn, Cu, Fe, Mn and B) in the citrus orchards of this part of the world (Ilyas et al., 2015).

The results of our study are in line with Monga and Josan (2000) who reported that the combined use of manures and micronutrients improved significantly fruit yield, quality as well as physiological attributes (total chlorophyll contents, net photosynthetic rate, transpiration rate and stomata conductance) of fruit plants as compared to respective control treatments. Bastakoti et al., (2022) who observed that integrated use of micronutrients (Cu, Zn, Fe, Mn and B) along with manure depicted highly significant improvement in fruit quality and physiological attributes of fruit crop plants as compared to untreated control treatment. However, many other researchers (Aular et al., 2017; Liu et al., 2022) they also reported that combined application of micronutrients in soil amended with manures showed highly significant results TSS, juice content, and organic acids (citric acid and ascorbic acid), reducing sugar content, non-reducing sugar content and total sugar contents, as well as physiological attributes citrus plants compared to untreated control. Organic manuring is frequently measured as a sustainable agricultural practice and, if used properly, potentials to give rich outputs on lasting basis (Saquee et al., 2023; Akanmu et al., 2023).

Conclusion

In conclusion, the integrated use of organic fertilizers and Zn is a promising approach to boost fruit yield and improve citrus quality. By harnessing the nutrient-rich benefits of organic fertilizers and the essential micronutrient zinc, growers can achieve healthier and more abundant citrus crops. This approach not only increases productivity but also enhances fruit quality, making it a sustainable and economically viable solution for citrus farming. Additionally, this approach promotes environmental sustainability by reducing the reliance on chemical fertilizers. Continued research and adoption of this integrated approach hold great potential for optimizing citrus cultivation and meeting growing market demands.

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