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# INTEGRATED NUTRIENT MANAGEMENT TECHNIQUES CAN ENHANCE GROWTH AND PHYSIO-BIOCHEMICAL RESPONSES OF *MORINGA OLEIFERA* GENOTYPES

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**Abstract**

Excessive use of synthetic fertilizers adversely affects the nutritional quality of *Moringa oleifera* L. and poses serious environmental risks. In contrast, organic amendments can sustainably enhance plant growth and nutrient accumulation by improving soil fertility and physiological efficiency. To address this, a pot experiment was conducted to evaluate the interactive effects of organic and inorganic amendments on the morpho-physiological, biochemical, and mineral responses of different *M. oleifera* genotypes. The experiment comprised two factors: (a) nutrient management strategies, including organic, inorganic, and integrated applications, and (b) genotypic variation, arranged under a completely randomized design (CRD). Results revealed that organic and inorganic amendments significantly enhanced plant height and leaf area index by 36% and 41%, respectively, compared with the control. Photosynthetic pigments and physiological parameters such as carotenoids, relative water content, photosynthetic rate, and stomatal conductance were also markedly improved, particularly under the integrated treatment (50% recommended fertilizer + *Bacillus* + compost). Antioxidant activities (SOD and CAT) and metabolite accumulation (total soluble proteins and phenolic contents) increased notably, accompanied by greater uptake of essential minerals (N, P, K, Ca, Fe, and Zn). Among the genotypes, Gulrez North Karachi (GNK) exhibited the most pronounced response across all measured attributes. Overall, the combined application of organic and inorganic amendments demonstrated superior performance over sole applications, indicating its potential as a sustainable and environmentally sound nutrient management approach to enhance *M. oleifera* growth, productivity, and nutritional value. These findings provide a scientific foundation for future studies exploring underlying molecular and signaling pathways.

**Key words:** *Moringa oleifera*; Integrated nutrient management; *Bacillus* inoculation; Antioxidant activity; Sustainable agriculture

## Introduction

Moringa (*Moringa oleifera* L.) is a tropical and subtropical bush or tree widely utilized in medicine, agriculture, food, and cosmetics due to its high nutritional content. The leaves are most often used because they contain bioactive compounds that affect osmotic adjustment and enzymatic, hormonal, and metabolic processes. It has been demonstrated to help humans with diabetes, obesity, asthma, cancer, hypertension, and microbial infections (Pasha *et al.,* 2020; Mehwish *et al.,* 2022). Moringa, a plant rich in essential nutrients like calcium, is necessary for human growth and development. Its leaves, flowers, and nutrient-dense seeds are utilized in medicine, food, cattle feed, and cosmetic products (Singh *et al.,* 2018). Moringa products have been demonstrated to improve the health of both humans and animals (Huria *et al.,* 2024). This plant contains many bioactive compounds, including carbohydrates, phenolics, isothiocyanates, flavonoids, saponins, tannins, oils, lipids, functional peptides, and proteins (Saucedo-Pompa *et al.,* 2018). Raw leaves contain a high concentration of vitamin A, which is required for eyesight, fertility, foetal growth, and cell division, making them appropriate for a variety of food product fields (Saras, 2023).

*M. oleifera* can be utilized in food security initiatives to combat malnutrition. A marginal intake can boost production, but it requires enough soil fertilization with organic fertilizers and good management (Kauser *et al.,* 2025). Due to the escalating cost of chemical fertilizers and their impact on soil health, M. oleifera may benefit from moderate doses of organic fertilizers to sustain fertilization. Sustainable agriculture attempts to preserve plant and soil health with fewer chemical inputs, and using organic resources as fertilizer has gained popularity (Christophe *et al.,* 2019; Bhardwaj *et al.,* 2019). Organic fertilizers have been proven to benefit a variety of crops (Rady *et al.,* 2016). Organic compost waste may be utilized to fertilize soil for agricultural cultivation on recovered soil. Organic manure is an efficient soil modulator that improves soil nutrient status, thereby enhancing plant growth (Ndubuaku *et al.,* 2015).

Organic fertilizers are environmentally beneficial and sustainable. However, they may not fulfill the immediate nutritional requirements of high-yielding cultivars due to delayed nutrient release (Manna *et al.,* 2021). Despite this, organic fertilizers promote soil health by improving structure, water retention, and microbial activity. However, their efficiency must be compared to that of inorganic fertilizers to maintain production. A comparison of organic and inorganic fertilizers is required to discover the best

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fertilizer for growth and yield performance (Singh *et al.,* 2020; Bhunia *et al.,* 2021). Adding organic and inorganic amendments to soil can increase soil aggregate stability, retain water, and improve crop harvest. However, the slow nutrient release from organic fertilizers raises concerns about crop yield and nutrient efficiency (Ahmad *et al*., 2024; Kauser *et al*., 2025). Evaluating the efficiency of organic and inorganic fertilizers may lead to more cost-effective, environmentally friendly agricultural methods, thereby decreasing reliance on costly chemical inputs (Abebe *et al.,* 2022). Several previous studies revealed that the application of organic and inorganic amendments improved the growth and yield of various plant species (Chen *et al.,* 2021; Wu *et al.,* 2024; Shaghaleh *et al.,* 2024; Arsalan *et al.,* 2024), while the combination of organic and inorganic fertilizers application need to be further explored, which can help to solve this issue while also maintaining the yield of moringa plants. Building on the above discussion, the present study hypothesized that combined organic and inorganic amendments may increase the nutritional value and yield of moringa plants. The current work aims to explore the effects of organic and inorganic amendments on moringa morpho-physiological attributes, nutritional value, and production, providing farmers and agricultural stakeholders with insights into sustainable cultivation methods.

## Materials and Methods

**Experimental setup, design, and treatments:** The experiment was performed in the wire house at the Department of Agronomy, University of Agriculture, Faisalabad (UAF), Pakistan. Different genotypes of *M. oleifera* seeds were procured from the Department of Agronomy, UAF. These seeds were sown in plastic pots filled with soil (8 kg per pot), of equal size and diameter. The experiment includes two factors: a) genotypes and b) organic and inorganic amendments (Table 1), and layout under a complete randomized design (CRD) with three replications of each treatment. The organic amendments (compost and *Bacillus*) were obtained from the ISES-UAF (Institute of Soil and Environmental Sciences). The standard protocol of Scott *et al*., (1991) was used for inoculating *Bacillus.* The other organic amendments and practices essential for plant health were done manually.

**Table 1. Experimental factors and treatments.**

| Factors | Treatments |
|---|---|
| Genotypes of *M. oleifera* | G1 = Gulrez North Karachi (GNK)<br>G2 = John Faisalabad (John-FSD)<br>G3 = Rahim Yar Khan (RY Khan)<br>G4 = PKM1 India |
| Organic and inorganic amendments | T0 = Control<br>T1 = *Bacillus*<br>T2 = Compost<br>T3 = 100% Recommended Fertilizer (RF)<br>T4 = 75% RF+ *Bacillus*<br>T5 = 75% RF+ Compost<br>T6 = 50% RF + *Bacillus* + Compost |

Replicate of each treatment = 3, Total no. of pots = 84

RF= Agronomic measurements and fertilizer dose @ [N=130 mg /pot, P=82 mg/pot, K=76 mg/pot]

**Growth attributes:** The fresh and dry weight of the compound leaf were recorded with an electronic weight balance (TX323L, Shimadzu, Japan), while the dry weight was obtained after drying for 3 days under shade and then placing the material in an oven (Memmert-110, Schawabach, Germany) at 70°C until constant weight. Plant height was measured with a tape measure, and the number of branches per plant and compound leaves was recorded manually. Leaf area index was measured by following the formula mentioned below:

$$\text{Leaf area} = \text{Leaf length x Leaf width x C.F}$$

Where C.F. stands for correction factor (0.75).

**Determination of photosynthetic pigment:** The methods of Aron (1949) and Davies (1976) were used to determine the concentrations of photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotene. Fresh leaf samples (1g) were taken, ground in 80% acetone, filtered, and the absorbance was measured with a spectrophotometer (UV-4000, O.R.I., Germany) at 663 nm (chlorophyll a), 645 nm (chlorophyll b), and 480 nm (carotenoids).

**Determination of physiological attributes:** The gas exchange parameters were measured according to the protocol of Ahmad *et al*., (2022), and leaf water potential was measured using an Infrared Gas Analyzer (CI-340 portable, Hoddesdon, England) and a Scholander-type pressure chamber (ARIMAD-2, ELE-International, Japan), respectively. Fresh weight (FW) of leaf samples was measured, soaked in distilled water for 24 hours to obtain turgid weight (TW), and oven-dried at 65°C for 72 hours to determine dry weight (DW). The RWC was measured by using the formula followed by Ahmad *et al*., (2021):

$$RWC = [(FW - DW)/(TW - DW)] \times 100$$

**Determination of antioxidants:** The method of Dhindsa *et al*., (1981) was used to determine superoxide dismutase (SOD) activity by grinding 100 mg of fresh leaf samples in 5 ml of extraction buffer. The mixture was centrifuged (at 10,000 rpm for 10 minutes), and the supernatant was collected and used as the enzyme source. A reaction mixture of methionine (0.2 ml), sodium carbonate (1.5 ml), EDTA (0.1 ml), NBT (0.1 ml), potassium phosphate buffer (1.5 ml), distilled water (1 ml), and enzyme extract (0.1 ml) was prepared. Two test tubes were taken without the enzyme extract as a control. The reaction was initiated by adding riboflavin and was placed under 15W fluorescent lamps (light source) for 15 minutes. After 15 minutes, the reaction was stopped by turning off the light and covering the tubes with a black cloth. The tubes with maximum color developed without the enzyme extract. A non-irradiated complete mixture was used as a blank. Absorbance was measured at 560 nm using a spectrophotometer.

Fresh leaves (0.5 g) were ground in a 50 mM phosphate buffer (5 ml) and centrifuged at 15000 rpm for 20 minutes at 4°C. The supernatants were used to determine peroxidase (POD) and catalase (CAT) activity. POD activity was determined by following the Putter (1974). A reaction mixture was prepared containing 10 mM guaiacol, 5 mM $H_2O_2$, and 50 mM phosphate buffer. The mixture was preheated to 20°C, then mixed with the reaction solution (2.8 ml) and the enzyme extract (0.2 ml). Absorbance was recorded using a spectrophotometer at 470 nm wavelength with 1-minute intervals. The CAT activity was measured using the method of Aebei (1984) with slight modifications. A reaction mixture containing 100 mM $H_2O_2$ and 50 mM phosphate buffer was preheated in a water bath at 25°C. The test tube was taken, phosphate buffer (0.2 ml), enzyme extract (0.2 ml), and $H_2O_2$ (3 ml) were added. The control tube was heated in a boiling water bath for 5 minutes to inactivate the enzyme. The absorbance at 240 nm was measured with a spectrophotometer at 1-minute intervals, and continuous determination was performed for 4 minutes.

**Determination of metabolites:** Bradford's (1976) method was used to measure total soluble proteins. Fresh leaves (0.5 g) were ground in cold phosphate buffer (8 ml) and centrifuged for 20 minutes at 4°C. The protein amount and enzyme activities were measured in the supernatants. Absorption was recorded using a spectrophotometer at 595 nm. The protocol of Singleton *et al*., (1999) was used to determine the phenolic activity. Folin-Ciocalteu method assay to measure total phenolic activity. This involves transferring electrons from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes in an alkaline solution, resulting in blue complexes that were measured at 760 nm with a spectrophotometer.

**Determination of minerals:** The different method was used to determine the minerals such as calcium (Sarkar & Chauhan, 1967), potassium (Ganesh *et al.,* 2012), nitrogen (Jackson and Burton, 1962), phosphorus (Eubank & Robert, 1949), zinc and iron (Soltanpour *et al.,* 2001), of *M. oleifera* genotypes under the impact of organic and inorganic amendments.

**Statistical analysis**

The various recorded attributes in the present work were statistically analyzed using computer-based software (Statistix, version 8.1). The means for each treatment were compared using the Tukey HSD test at the 5% significance level. The correlation and heat map were generated using Origin (version 2021) and RStudio.

**Results**

**Growth attributes:** The growth attributes of various genotypes of *M. oleifera* were significantly ($p<0.05$) affected under the influence of organic and inorganic amendments (Fig. 1). Results of the present study revealed that all the organic and inorganic amendments significantly increased the number of compound leaves per plant by 37%, number of branches per plant by 37%, fresh weight of compound leaves by 38%, dry weight of compound leaves by 34%, plant height by 36%, and leaf area index by 41% as compared to control conditions. However, among the treatments, T6 (50% RF + *Bacillus* + Compost) performed better at improving the aforementioned growth attributes. Among the genotypes, G1 (Gulrez North Karachi) showed better results, followed by G2, G4, and G3.

**Photosynthetic pigments:** Organic and inorganic amendments applications significantly ($p<0.05$) affect the photosynthetic pigments of the different *M. oleifera* genotypes. Applying all organic and inorganic amendments significantly increased chlorophyll a by 40%, chlorophyll b by 39%, and carotenoid content by 39% compared with the control. However, the treatment T6 (50% RF + *Bacillus* + Compost) showed the greatest enhancement among the treatments, while across genotypes, G1 (Gulrez North Karachi) performed best, followed by G2, G4, and G3.

**Physiological attributes:** The physiological attributes of various *M. oleifera* genotypes were significantly ($p<0.05$) affected under the influence of organic and inorganic amendments. The present work results demonstrated that all the organic and inorganic amendments improved the water potential by 16%, relative water contents by 11%, photosynthetic rate by 9%, transpiration rate by 27%, stomatal conductance by 18%, and intracellular carbon concentration by 10% as compared to control conditions (no-application). However, T6 (50% RF + *Bacillus* + Compost) outperformed the other treatments across all the above-mentioned physiological indices, whereas G1 (Gulrez North Karachi) survived best across genotypes, followed by G2, G4, and G3.

**Antioxidants:** The antioxidant activity was significantly affected by organic and inorganic amendments across different genotypes of M. oleifera ($p<0.05$). Results revealed that all organic and inorganic amendments positively improved the activity of SOD by 41%, POD by 35%, and CAT by 44% compared to the no-application (control). However, among the treatments, T6 (50% RF + *Bacillus* + Compost) improved antioxidant activity, while G1 (Gulrez North Karachi) showed better results across the other genotypes, in the following order: G2, G4, and G3.

**Metabolites:** Application of organic and inorganic amendments significantly ($p<0.05$) affected the concentration of metabolites of the various *M. oleifera* genotypes. It was found that all the organic and inorganic amendments increased the accumulation of total soluble sugar by 11% and total phenolic contents by 36% as compared to other treatments. However, among the treatments, T6 (50% RF + *Bacillus* + Compost) plays a vital role in increasing the concentration of the aforementioned metabolites. Among the various genotypes, G1 (Gulrez North Karachi) performed outclass, followed by G2, G4, and G3.

**Minerals:** Organic and inorganic amendments significantly ($p<0.05$) affected the mineral status of different genotypes of *M. oleifera*. The results of the present study demonstrated that, as compared to the control (no-application), all the organic and inorganic amendments positively increased the mineral status, such as calcium by 36%, potassium by 34%, nitrogen by 36%, phosphorus by 39%, zinc by 32%, and iron by 37%. However, the treatment T6 (50% RF + *Bacillus* + Compost) increased all the minerals mentioned above compared with the other treatments. The G1 (Gulrez North Karachi) showed better results, followed by G2, G4, and G3.

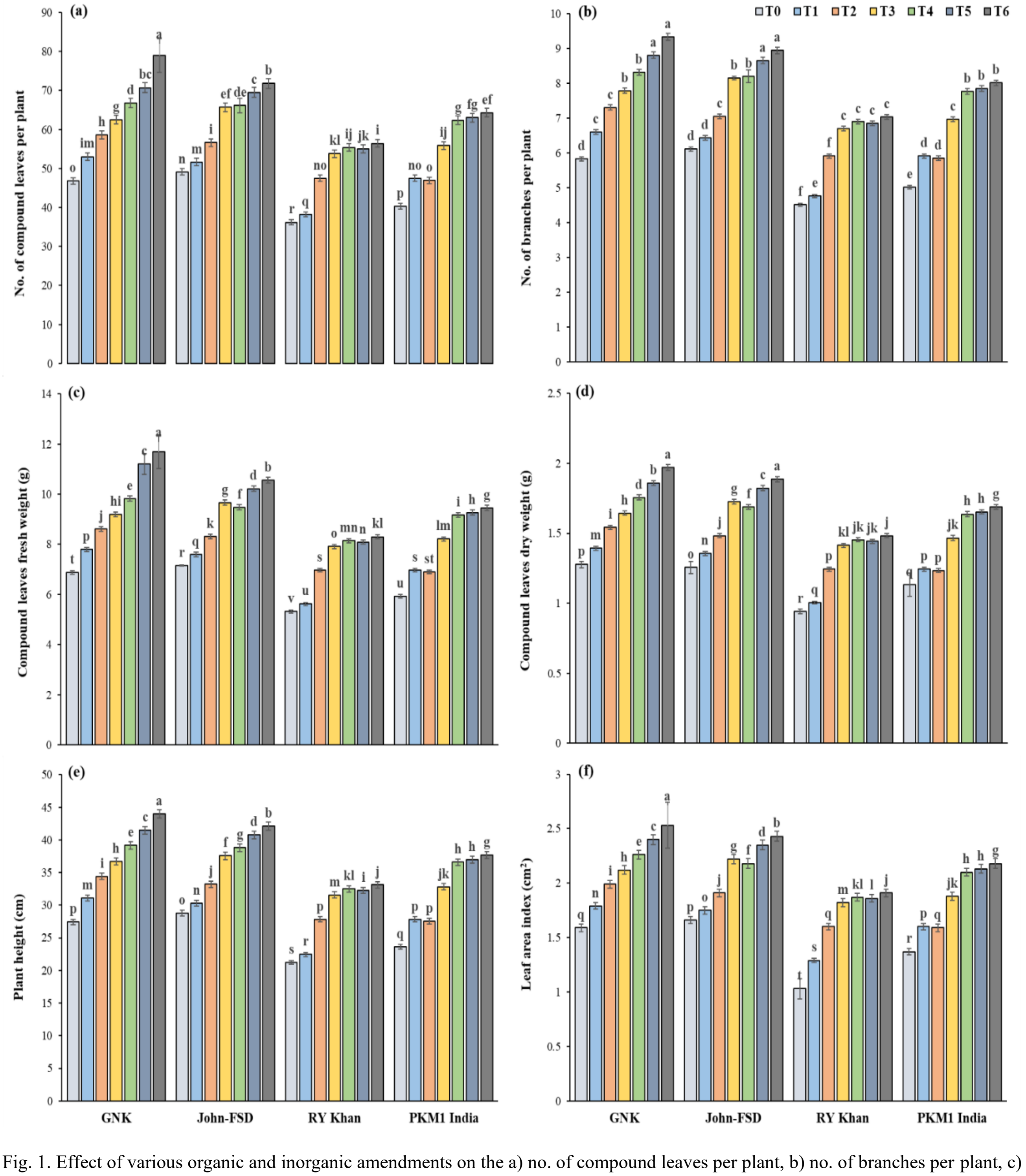


Fig. 1. Effect of various organic and inorganic amendments on the a) no. of compound leaves per plant, b) no. of branches per plant, c) compound leaves fresh weight, d) compound leaves dry weight, e) plant height, and f) leaf area index of various *M. oleifera* genotypes. Variation in the alphabets above the means (three replicates) showed a significant difference among the means according to the Tukey HSD test at a 5% probability level, and error bars represent the standard error.

**Clustered heat map and correlation analysis:** An analysis of the clustered heat map and a correlation analysis were conducted among the various recorded growth, photosynthetic pigments, physiological, biochemical attributes, and mineral contents of various M. oleifera genotypes under the influence of the solo and combined effects of organic and inorganic amendments. It was observed that a remarkable enhancement in the aforementioned indices occurred under treatment G1T6, followed by G2T6. On the other hand, less improvement was observed under the treatment G3T0, followed by G3T1 and G4T0 (Fig. 6). Correlation analysis was performed among the various recorded attributes of *M. oleifera* genotypes under the influence of organic and inorganic amendments. The growth attributes showed strong positive correlations with photosynthetic pigments and physiological attributes. Moreover, antioxidants, metabolites, and mineral contents also showed strong positive correlation with the above-mentioned indices (Fig. 7).

**Table 2. Impact of organic and inorganic amendments on the mineral status of various *M. oleifera* genotypes.**

| Genotypes | Treatments | Calcium (mg kg$^{-1}$) | Potassium (mg kg$^{-1}$) | Nitrogen (mg kg$^{-1}$) | Phosphorus (mg kg$^{-1}$) | Zinc (mg kg$^{-1}$) | Iron (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|
| GNK | T0 | 1.10 ± 0.03n | 11.42 ± 0.06n | 53.56 ± 2.27l | 0.53 ± 0.02m | 18.70 ± 0.77l | 17.90 ± 0.26n |
| | T1 | 1.25 ± 0.03kl | 12.94 ± 0.06k | 60.68 ± 2.36jk | 0.60 ± 0.02jk | 21.18 ± 0.88ij | 20.27 ± 0.28k |
| | T2 | 1.39 ± 0.03g | 14.39 ± 0.01g | 67.42 ± 2.59g | 0.66 ± 0.02g | 23.60 ± 0.87f | 22.59 ± 0.26g |
| | T3 | 1.48 ± 0.03f | 15.25 ± 0.03f | 71.65 ± 2.92f | 0.70 ± 0.02f | 25.00 ± 0.93e | 23.94 ± 0.22f |
| | T4 | 1.58 ± 0.03d | 16.34 ± 0.06d | 76.58 ± 3.05d | 0.75 ± 0.02d | 26.81 ± 1.02c | 25.66 ± 0.23d |
| | T5 | 1.67 ± 0.04bc | 17.28 ± 0.06b | 81.11 ± 3.29bc | 0.80 ± 0.02bc | 28.33 ± 1.13b | 27.12 ± 0.30b |
| | T6 | 1.77 ± 0.03a | 18.29 ± 0.01a | 85.81 ± 3.39a | 0.84 ± 0.03a | 29.96 ± 1.14a | 28.68 ± 0.33a |
| Jhon-FSD | T0 | 1.16 ± 0.02m | 12.00 ± 0.06m | 56.10 ± 2.29l | 0.55 ± 0.01l | 19.66 ± 0.77k | 18.81 ± 0.12m |
| | T1 | 1.22 ± 0.02l | 12.58 ± 0.02l | 59.11 ± 2.41k | 0.58 ± 0.02k | 20.61 ± 0.76j | 19.73 ± 0.24l |
| | T2 | 1.33 ± 0.03h | 13.81 ± 0.07h | 64.63 ± 2.61g | 0.64 ± 0.02h | 22.59 ± 0.93g | 21.62 ± 0.25h |
| | T3 | 1.49 ± 0.06e | 15.72 ± 0.03e | 75.15 ± 2.96ef | 0.71 ± 0.05ef | 25.61 ± 0.85de | 24.95 ± 0.47e |
| | T4 | 1.55 ± 0.05d | 16.02 ± 0.01d | 73.60 ± 3.01de | 0.76 ± 0.04de | 26.00 ± 1.12cd | 25.54 ± 0.53d |
| | T5 | 1.64 ± 0.03c | 16.98 ± 0.04c | 79.60 ± 3.25c | 0.78 ± 0.03c | 27.82 ± 1.05b | 26.63 ± 0.41c |
| | T6 | 1.69 ± 0.04b | 17.50 ± 0.06b | 82.23 ± 3.27b | 0.81 ± 0.02b | 28.67 ± 1.14b | 27.44 ±0.30b |
| RY Khan | T0 | 0.86 ± 0.02q | 8.82 ± 0.06q | 41.45 ± 1.69n | 0.41 ± 0.01p | 14.54 ± 0.63n | 13.91 ± 0.18q |
| | T1 | 0.90 ± 0.02p | 9.33 ± 0.04p | 43.67 ± 1.69mn | 0.43 ± 0.01o | 15.26 ± 0.59mn | 14.61 ± 0.15p |
| | T2 | 1.12 ± 0.02n | 11.56 ± 0.05n | 54.04 ± 2.21l | 0.53 ± 0.01lm | 18.92 ± 0.72kl | 18.11 ± 0.16n |
| | T3 | 1.27 ± 0.03jk | 13.16 ± 0.06jk | 61.68 ± 2.49j-k | 0.61 ± 0.02ij | 21.57 ± 0.91hi | 20.64 ± 0.25jk |
| | T4 | 1.31 ± 0.03h-j | 13.51 ± 0.03i | 63.41 ± 2.58hi | 0.62 ± 0.02hi | 22.13 ± 0.87gh | 21.19 ± 0.30hi |
| | T5 | 1.29 ± 0.03ij | 13.37 ± 0.06ij | 62.90 ± 2.52h-j | 0.62 ± 0.02h-j | 21.91 ± 0.92g-i | 20.97 ± 0.25ij |
| | T6 | 1.33 ± 0.03h | 13.81 ± 0.07h | 64.63 ± 2.61h | 0.64 ± 0.02h | 22.59 ± 0.93g | 21.62 ± 0.25h |
| PKM1 India | T0 | 0.95 ± 0.02o | 9.83 ± 0.02o | 46.20 ± 1.91m | 0.45 ± 0.01n | 16.10 ± 0.61m | 15.42 ± 0.23o |
| | T1 | 1.12 ± 0.02n | 11.56 ± 0.05n | 54.04 ± 2.21l | 0.53 ± 0.01lm | 18.92 ± 0.72kl | 18.11 ± 0.16n |
| | T2 | 1.11 ± 0.02n | 11.49 ± 0.03n | 53.72 ± 2.28l | 0.53 ± 0.02m | 18.81 ± 0.66kl | 18.01 ± 0.24n |
| | T3 | 1.31 ± 0.02hi | 13.59 ± 0.06hi | 63.80 ± 2.59hi | 0.63 ± 0.02h | 22.24 ± 0.77gh | 21.30 ± 0.29hi |
| | T4 | 1.48 ± 0.03f | 15.25 ± 0.03f | 71.36 ± 2.79f | 0.70 ± 0.02f | 25.00 ± 0.93e | 23.94 ± 0.22f |
| | T5 | 1.48 ± 0.03f | 15.32 ± 0.06f | 71.90 ± 2.89f | 0.71 ± 0.02f | 25.12 ± 0.99e | 24.04 ± 0.32f |
| | T6 | 1.52 ± 0.03e | 15.69 ± 0.05e | 73.60 ± 3.01de | 0.72 ± 0.02ef | 25.73 ± 0.97de | 24.64 ± 0.27e |

Variation in the alphabets after the means (three replicates) ± standard error showed significant differences among the means according to the Tukey HSD test ($p<0.05$). For the treatment group, please see Table 1

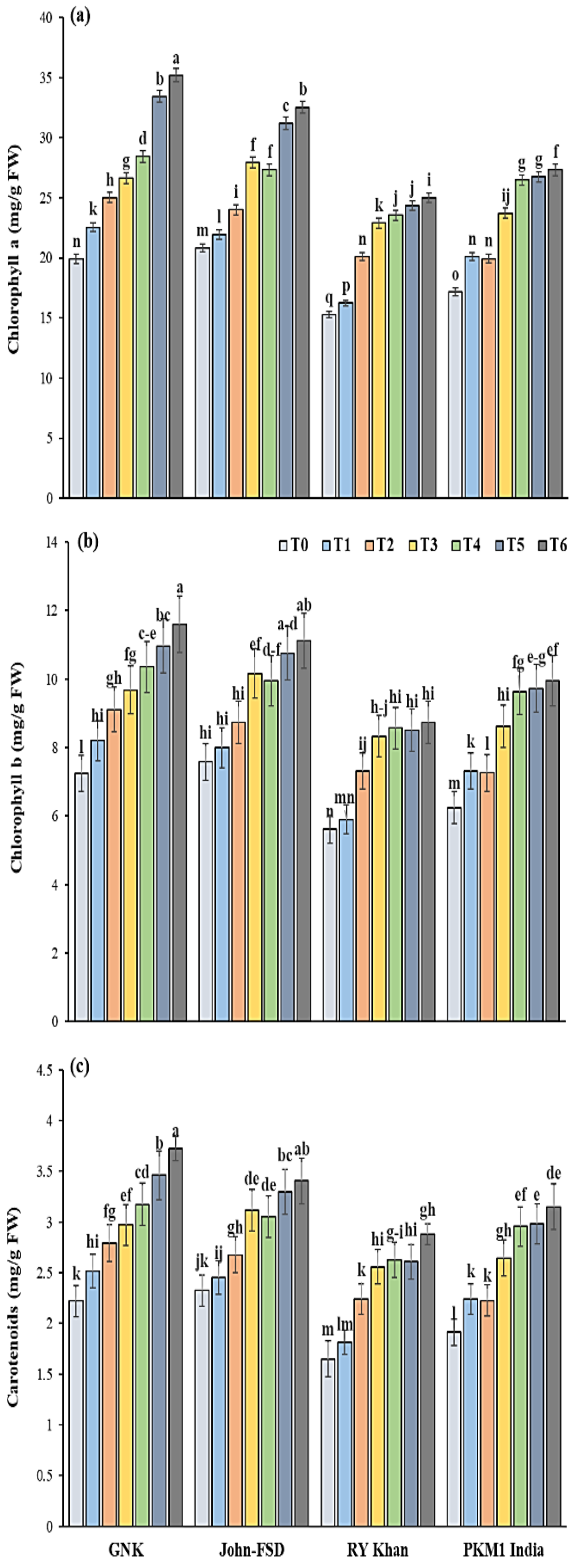


Fig. 2. Effect of various organic and inorganic amendments on the a) chlorophyll a, b) chlorophyll b, and c) carotenoids of various *M. oleifera* genotypes. Variation in the alphabets above the means (three replicates) showed a significant difference among the means according to the Tukey HSD test at a 5% probability level, and error bars represent the standard error.

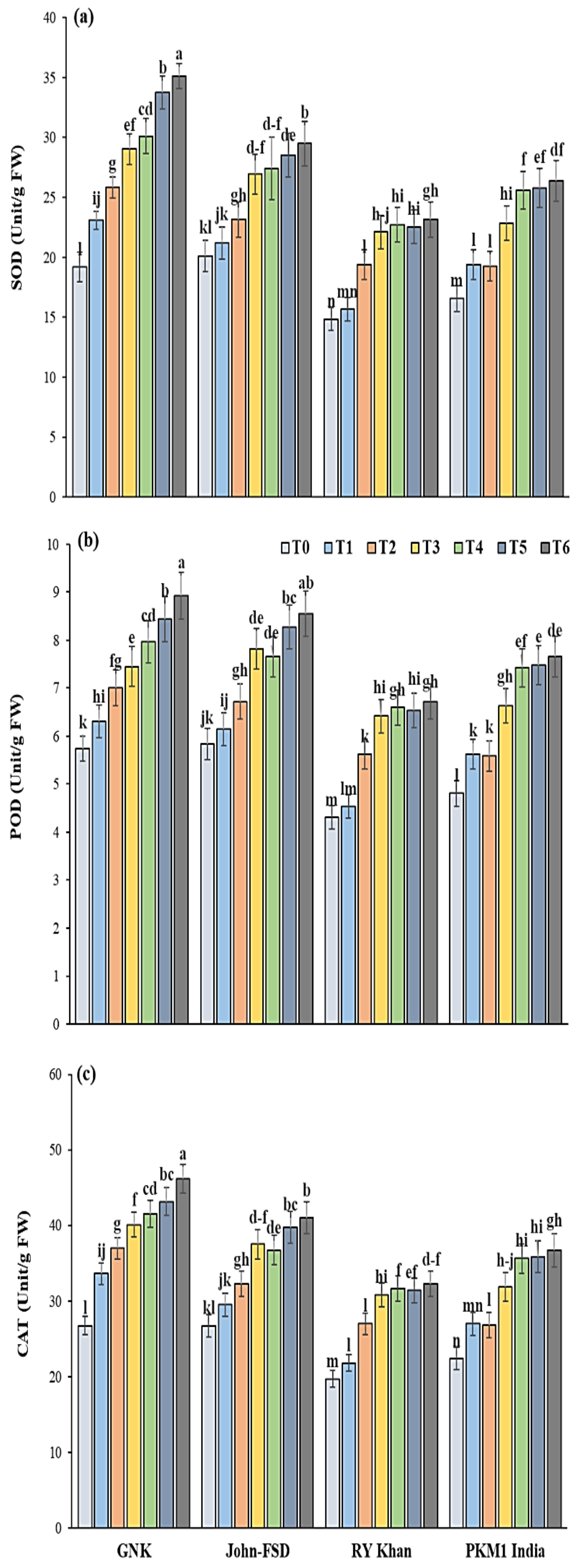


Fig. 4. Effect of various organic and inorganic amendments on the a) SOD, b) POD, and c) CAT of various *M. oleifera* genotypes. Variation in the alphabets above the means (three replicates) showed a significant difference among the means according to the Tukey HSD test at a 5% probability level, and error bars represent the standard error.

## Discussion

The growth attributes of various M. oleifera genotypes were positively affected by the application of organic and inorganic amendments (Fig. 1), as reported in previous investigations (Gad *et al.,* 2019; Kauser *et al.,* 2025). This is due to organic additions improving soil structure by enhancing permeability, water retention, and aggregate stability. These physical enhancements improve the environment for root growth and microbial colonization, leading to greater plant development. Improved soil structure reduces compaction and enhances oxygenation, thereby supporting greater plant growth (Pantelides *et al.,* 2023). Furthermore, the application of organic amendments, such as compost, increases soil enzyme activity, leading to more effective nutrient mineralization and greater availability. This is associated with higher microbial biomass and diversity, which transform organic matter into nutrients that plants can use, thereby enhancing growth-contributing indices (Khan *et al.,* 2022). The combination of organic and inorganic amendments can boost plant growth attributes as observed in the present study (Fig. 1) by restoring bacterial community diversity, increasing soil biochemical properties, and stimulating microbial activity. The combined effect results from the rapid nutrient delivery from inorganic fertilizers and the long-term release from organic matter inputs (Wei *et al.,* 2016).

Results of the present work demonstrated improvements in the photosynthetic pigments of M. oleifera genotypes with the application of organic and inorganic amendments (Fig. 2). Compost is well known for its capacity to enhance soil structure, increase organic matter, and provide important macro- and micronutrients that play a key role in chlorophyll production. Compost treatment, as demonstrated in the present study, greatly increases plant height and leaf area (Fig. 1), which are associated with enhanced photosynthetic capacity and pigment formation. Organic matter from compost not only provides minerals such as magnesium and iron (Table 2), which are essential for chlorophyll production, but also increases soil water-holding capacity, indirectly aiding pigment stability (Alori *et al.,* 2023). The inoculation of *Bacillus* spp., a type of plant growth-promoting rhizobacteria (PGR), improves plant development by creating phytohormones (auxins) that help in nutrient solubility, which is required for the production and maintenance of photosynthetic pigments. They also increase antioxidant enzyme activity, as observed in the current study (Fig. 4), thereby protecting chlorophyll molecules and increasing phosphorus uptake (Table 2), which is required for photosynthesis and pigment maintenance (Ahmad *et al.,* 2018).

The physiological attributes of M. oleifera genotypes were improved by organic and inorganic amendments (Fig. 3), as reported in previous investigations. Compost increases soil organic matter, porosity, and water-holding capacity, resulting in higher relative water content (RWC) and better water potential (WP) in plant tissues. When combined with Bacillus spp., it promotes root growth and nutrient uptake, improving the plant's capacity to take up and retain water. The same combination was seen in different plant species (Omara *et al.,* 2022; Moraes *et al.,* 2025). Moreover, the combination of amendments improves stomatal behavior by regulating plant water status via phytohormone signaling. This leads to higher stomatal conductance, improved photosynthesis, and increased photosynthetic and transpiration rates (Naveed *et al.,* 2021).

Organic and inorganic amendments significantly increased antioxidant activity and metabolite accumulation, whereas combined application showed greater enhancement, as observed in the present work (Figs. 4 and 5). Compost increases soil organic matter and micronutrients, such as iron and zinc, as observed in the current work (Table 1), which function as cofactors for antioxidant enzymes such as SOD, POD, and CAT. *Bacillus* spp. activate the plant's antioxidant mechanisms by increasing systemic resistance and generating signaling chemicals. This activates the signaling pathway, leading to the expression of antioxidant enzymes (Masters-Clark *et al.,* 2020; Zhang *et al.,* 2023). Compost offers precursors and humic compounds that induce phenolic metabolism. *Bacillus* spp. amplify this effect by influencing the phenylpropanoid pathway, which generates secondary metabolites and stimulants. Compost also provides vital elements, such as nitrogen (Table 2), which is required for protein synthesis. *Bacillus* spp. improve nitrogen mineralization and absorption by dissolving phosphorus and fixing atmospheric nitrogen contributes to the accumulation of total soluble proteins (Li *et al.,* 2023; Lerma-Moliz *et al.,* 2024).

The mineral status of the various *M. oleifera* genotypes was significantly enhanced by the application of all organic and inorganic amendments, with the combined application showing greater mineral uptake (Table 2), in line with previous studies (Kauser *et al.,* 2025). This is because compost is a rich source of both micro- and macro-nutrients, directly adding to the soil's nutrient pool. Inoculating with *Bacillus* spp. accelerates the breakdown and mineralization of organic matter, resulting in faster transformation of complex organic substances into plant-available mineral forms (Simol *et al.,* 2023). *Bacillus* spp. are well-known for fixing atmospheric nitrogen, utilizing soluble inorganic phosphate, and producing siderophores that bind and release micronutrients such as zinc and iron. They also produce organic acids and enzymes, which aid in the solubilization of insoluble mineral components in soil. The combination of compost and *Bacillus* improves nitrogen cycling and mineral absorption (Patani *et al.,* 2024).

The results of the present study revealed that the sole application of organic and inorganic amendments plays a vital role in improving the morpho-physiological, biochemical mechanisms, and mineral status of the different genotypes of M. oleifera, with genotype G1 performing better across these indices. The combined application of organic and inorganic amendments showed better results than a single application. The present study suggests that combining organic and inorganic fertilizers may not only prevent ecological damage but also enhance soil quality, making it a viable growing mode with excellent productivity and profitability.

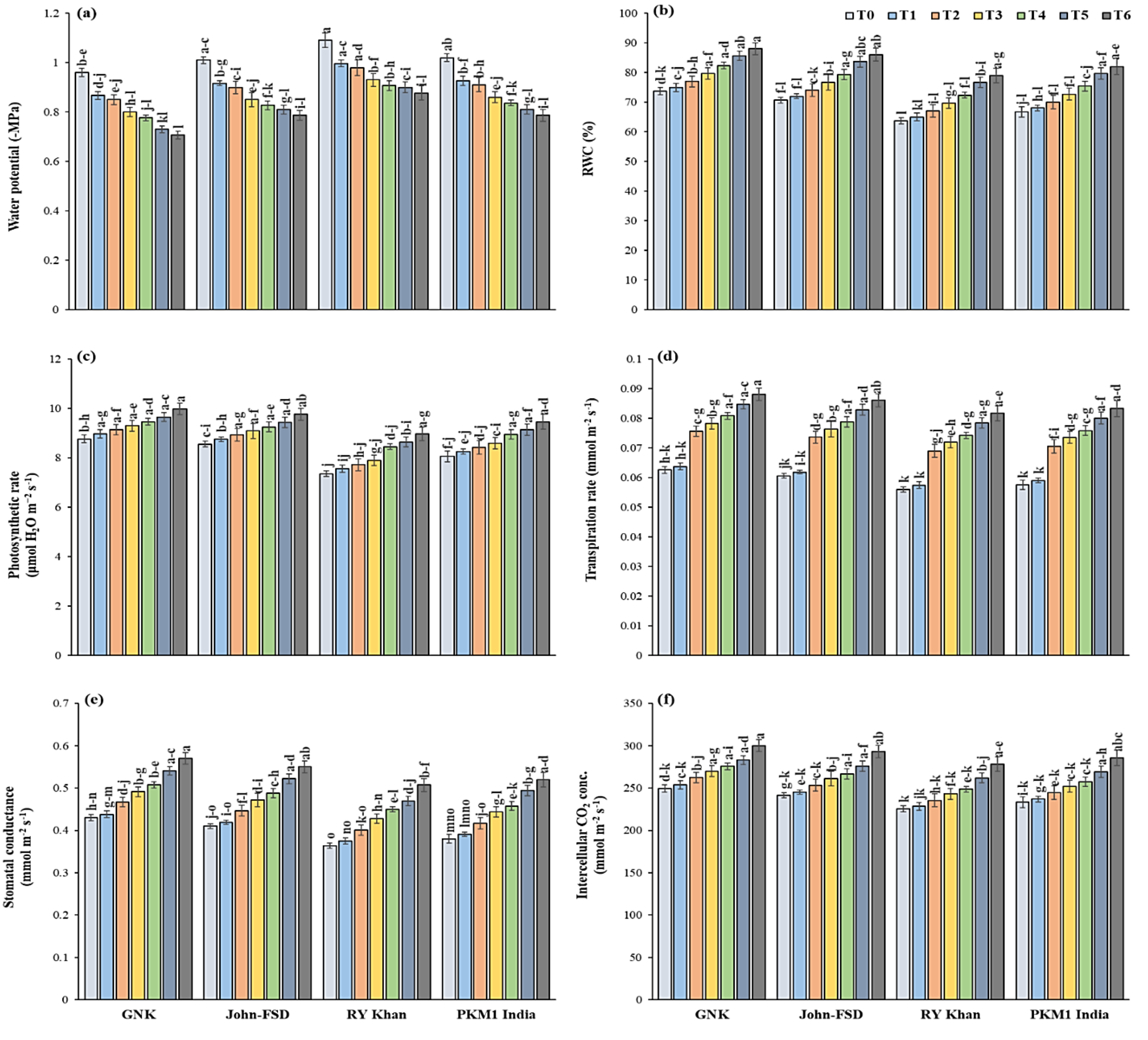


Fig. 3. Effect of various organic and inorganic amendments on the a) water potential, b) relative water contents, c) photosynthetic rate, d) transpiration rate, e) stomatal conductance, and f) intercellular carbon concentration of various *M. oleifera* genotypes. Variation in the alphabets above the means (three replicates) showed a significant difference among the means according to the Tukey HSD test at a 5% probability level, and error bars represent the standard error.

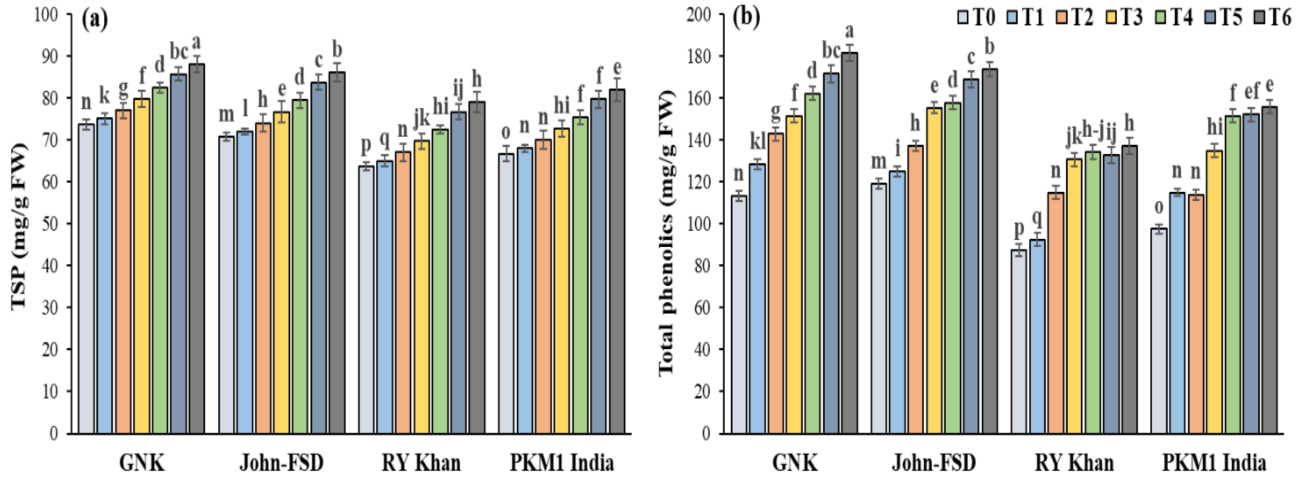


Fig. 5. Effect of various organic and inorganic amendments on the a) total soluble protein, and b) total phenolic contents of various *M. oleifera* genotypes. Variation in the alphabets above the means (three replicates) showed a significant difference among the means according to the Tukey HSD test at a 5% probability level, and error bars represent the standard error.

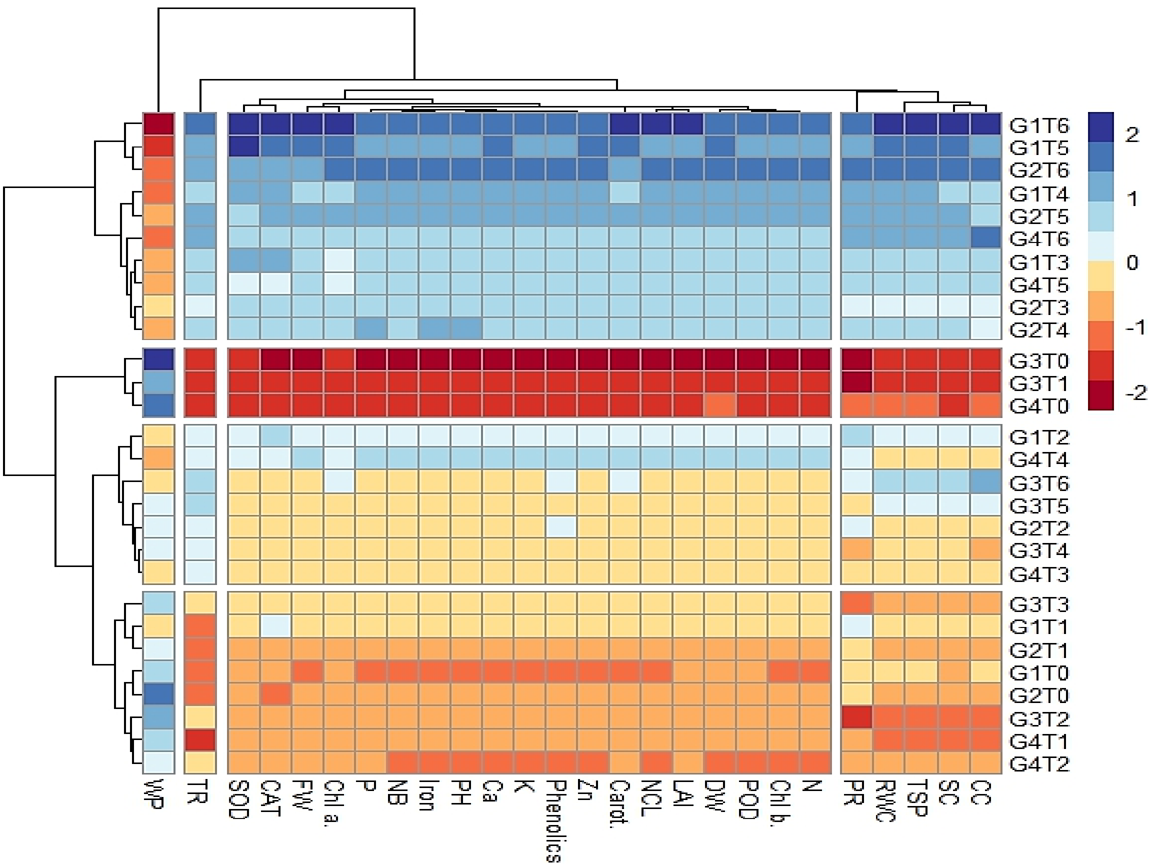


Fig. 6. Heat map analysis was done among the various recorded attributes of different *M. oleifer*a genotypes under the influence of organic and inorganic amendments. The abbreviations of the indices are NCL (Number of compound leaves per plant), NB (Number of branches per plant), FW (Fresh weight of compound leaves), DW (Dry weight of compound leaves), PH (Plant height), LAI (Leaf area index), Chl a. (Chlorophyll a), Chl b. (Chlorophyll b), Carot. (Carotenoids), WP (Water potential), RWC (Relative water contents), PR (Photosynthetic rate), TR (Transpiration rate), SC (Stomatal conductance), CC (Intracellular carbon concentration), SOD (Superoxide dismutase), POD (Peroxidase), CAT (Catalase), TSP (Total soluble proteins), Phenolics, Ca (Calcium contents), K (Potassium contents), N (Nitrogen contents), P (Phosphorus contents), Zn (Zinc contents), Iron contents. Please see Table 1 for the treatment group.

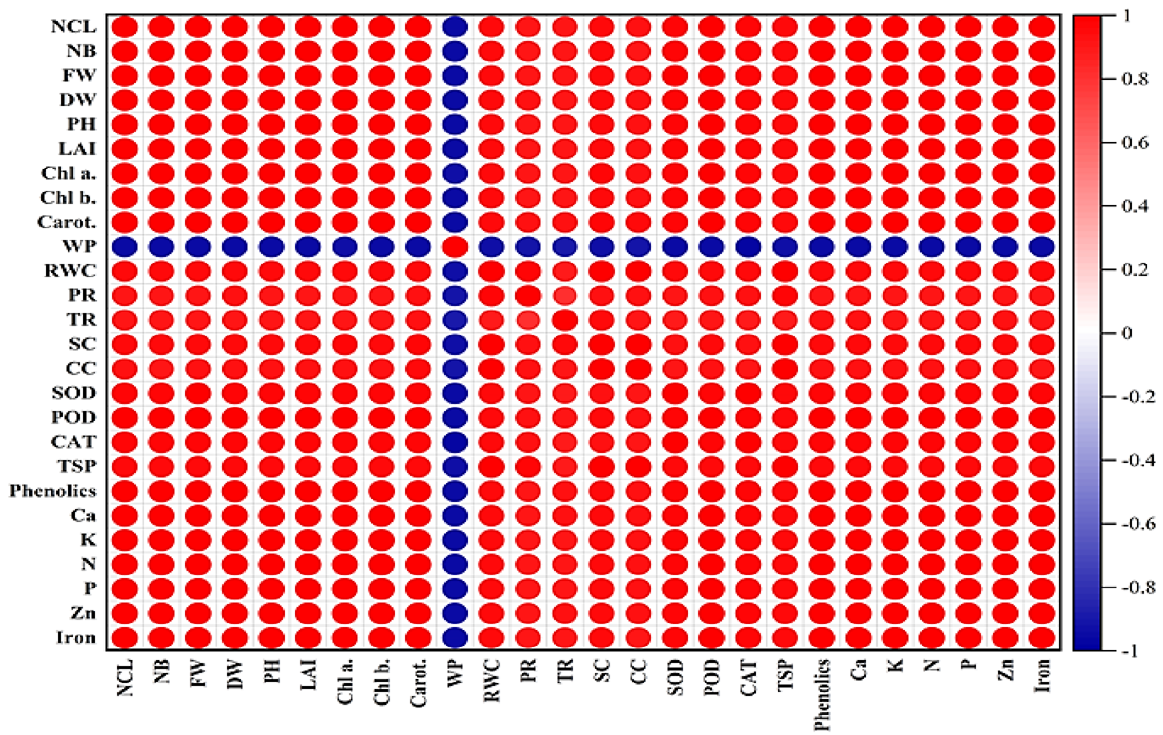


Fig. 7. Correlation analysis was done among the various recorded attributes of different *M. oleifera* genotypes under the influence of organic and inorganic amendments. Please see the caption of Fig. 6 for the abbreviations of the recorded attributes

## Conclusion

The present study demonstrated that the application of organic and inorganic amendments significantly increased the growth and mineral status of M. oleifera genotypes. The combined effect of organic and inorganic amendments was more efficient than the single application, while the genotype (Gulrez North Karachi) showed better results among the other *M. oleifera* genotypes in the following order: G1>G2>G4>G3. Organic and inorganic amendments improved the photosynthetic efficiency by increasing the photosynthetic pigments and physiological attributes that contribute to enhanced growth. Moreover, the combined (50% RF + *Bacillus* + Compost) application increased the uptake of essential minerals that act as cofactors for antioxidants and during the biosynthesis of photosynthetic pigments and metabolites, ultimately increasing the growth and development of *M. oleifera* genotypes. Future research should focus on optimizing organic-inorganic amendment combinations for M. oleifera production and adaptability in diverse agro-climatic conditions and soil types. Furthermore, exploring the long-term soil conditions, bacterial diversity, and carbon sequestration capacity may be crucial for sustainable agriculture, with genetic variation aiding in cultivar development.

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