Research Article

Synergistic Effects of Organic Amendments and Plant Extracts on Growth, Minerals Content, and Phytohormones of Pomegranate cv. Wonderful

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Abstract |Pomegranate is an important fruit crop because of its higher nutritional and phytochemical properties. Different management practices can be employed to improve the growth and yield of pomegranate cultivated in arid and semi-arid regions globally. The aim of the present research was to improve the growth, minerals status, and phytohormones of pomegranate seedlings with the synergistic impact of organic fertilizers (vermicompost and nano organic fertilizer), and plant extracts (moringa and roselle extracts) repetition removed to improve conciseness . Different treatments such as vermicompost (250 g L^{-1}), nano organic fertilizer (5 mL L^{-1}), moringa leaf extract (20 mL L^{-1}), and roselle leaf extract (20 mL L^{-1}) along with control were arranged under a complete randomized design (CRD). Regarding the pomegranate growth traits, seedlings' height, number of leaves, and chlorophyll content were greater in moringa leaf extract, while seedling height, number of leaves, and chlorophyll content were lower in control pomegranate seedlings as compared to other applied organic treatments. Concerning minerals status, pomegranate leaves gained higher nitrogen, phosphorus, and potassium contents measured in moringa leaf extract-treated seedlings, while lower nitrogen, phosphorus, and potassium contents were determined from in control of seedlings than other applied compared to other treatments. Regarding phytohormones, auxin, gibberellins, and cytokinins were higher in seedlings treated with moringa leaf extract than control and other organic amendments. Hence, all the applied treatments (organic amendments and plant extracts) improved pomegranate seedlings' growth, mineral status, and phytohormones. These organic amendments and plant extracts had significant contributions in improving the growth, yield, and quality of pomegranate traits in the future.

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Introduction

Pomegranate (*Punica granatum L.*) is fruit crop native to arid and semi-arid regions. Although the tree is often thought to have originated in Persia, it was also first identified in China and Iraq (Stover and Mercure, 2007). It is widely distributed in temperate regions. The tree thrives in cold climates and often appears green or semi deciduous in tropical and subtropical regions (Mir *et al*., 2012). Its fruit has a long shelf life in the market which allows it to be transported over long distances and kept for a long period. Pomegranate fruits are rich in minerals and vitamins necessary for a healthy life. Its peel is also nutritionally rich, especially in vitamin C, pigments, lipids, carbohydrates, organic acids, proteins, and minerals. However, nutritional value may vary depending on cultivar, agricultural practices, and fertilizer balance (Al-Muslhi *et al*., 2024; Hamid, 2025a). Pomegranate productivity is still low due to poor management practices (Hamid, 2025b). Hence, organic amendments are an effective way to improve fruit crop productivity. Organic amendments are environmentally friendly, easy to use, and cost-effective (Malik *et al*., 2025).

Nano-bio fertilizers have the potential to be a major technological advancement because they activate soil microorganisms in the soil and efficiently supply nutrients, especially vital elements like potassium, phosphorus, and nitrogen, which are essential for plant growth and development processes (Hassan *et al*., 2021; Hundi *et al*., 2025). The biological process of making compost and fertilizer requires the biological dissolution of organic materials in the presence of oxygen. This is carried out in a controlled environment that encourages the growth of aerobic microorganisms, and the transformation produces a fully decomposed, humus-like material that can be used without having an adverse effect on the environment (Lin, 2008). By improving the physical characteristics of the soil, adding more organic matter promotes the growth of clusters and makes them more stable (Hamid *et al*., 2025).

Fruit crop production, yield, and quality can be improved with the application of nanotechnology. It involves materials with nanoscale dimensions (Oudah and Saleh, 2024; Wang *et al*., 2025). One of the most recent technological developments that might lead to a new scientific revolution is nanotechnology, which makes it possible to create nanoparticles from diverse materials (Muhammad *et al*., 2022; Al-Janabi, 2024). The use of nanotechnology brings a greater revolution for plant researchers to improve soil and plant health focusing on sustainable farming (Mushtaq *et al*., 2010). Numerous industries, including agriculture, have reported using them in the production of pesticides and fertilizers that are sprayed on plants or applied to the soil to improve its fertility or other properties. Nanoparticles are more advantageous than conventional pellets or supplements (Kaira *et al*., 2012). Chemical fertilization had adverse effects on soil and plant health. Soil compaction and environmental pollution are major threats to fruit crops which majorly occur from bombardment of chemical fertilization. Advancement in fruit production is necessary for higher growth, yield, and quality (Drebee *et al*., 2021).

It is important to develop realistic, efficient and cost effective techniques to improve soil fertility and yield for sustainable agriculture. One effective approach to address this issue is application of organic fertilizers and plant based extracts, which positively influence One effective approach and agricultural productivity. Vermicomposting, in particular, is an organic technique that enhances soil structural integrity and nutrient availability (Drebee *et al*., 2022).

The rising concern throughout the globe about the environment and the desire to preserve it has led to a decline in the use of chemical fertilizers, this, particularly when applied in large amounts, has resulted in air and soil pollution. This has caused some individuals to investigate safe alternatives to the conventional way of fertilization, as well as reverting to the organic approach and eliminating chemicals completely. This is because the European Union has chosen to outlaw the usage of artificial growth boosters, , Instead, they promote the use of plant-based alternatives (El-Motty *et al*., 2010; Grashorn, 2010). These extracts obtained from plants are utilized by adding them to the soil or by spraying them onto other plants following the process of drying or extraction since they contain nutrients and different chemicals that can combat pests and diseases. However, it is also regarded as an option for biostimulants that enhance growth (Bulgari *et al*., 2019).

Moringa plant extract is effective in enhancing the growth of plants, while roselle extract is important to protect plants from diseases because roselle extract has excellent antimicrobial properties. Taking into consideration the relevance of these aspects, the present research aimed to evaluate the effects of mixing vermicompost and organic fertilizers as well as the foliar application of organic plant extracts on the development of pomegranate plants by improvement of minerals content and phytohormones.

Materials and Methods

Study area

The experiment was conducted from December 2022 to June 2023 near a private daycare in the Qasim neighborhood of Babylon to evaluate the effects of vermicompost, organic fertilizers, and foliar applications of plant extracts on the growth of pomegranate seedlings.

Research design and treatment arrangements

The current research was organized under a complete randomized design (CRD) with three biological replicates. Different treatments such as no spray (control), vermicompost (250 g L^{-1}), nano organic fertilizer (5 mL L^{-1}), moringa leaf extract (20 mL L^{-1}), and roselle leaf extract (20 mL L^{-1}) were used on pomegranate seedlings in the current work. The organic treatments were applied to seedlings in the early morning hours. Moreover, control seedlings were treated with distilled water. Leaf minerals and hormonal analyses were performed at the Department of Plant Production Technology, Musaiyab University of Technology, Iraq.

Growth traits

Seedling height, number of leaves, and chlorophyll content were measured to assess the growth of pomegranate under different organic treatments. Seedling height was measured using a measuring scale (Ghaffar *et al*., 2024). The number of leaves was counted from every seedling (Batool *et al*., 2023). Chlorophyll content was measured with SPAD-502 (Minolta, Japan) meter from young mature leaves (Li *et al*., 2023).

Estimation of minerals

Different minerals (nitrogen, phosphorus, and potassium) were measured in pomegranate leaves. Mature leaves were collected from each experimental unit in duplicates, rinsed with distilled water to remove surface contaminants, and placed in perforated paper bags. Leaf samples were dried, and ground using an electric mill, and 0.5 g of each sample was digested with sulfuric and perchloric acids to produce a colorless extract for mineral analysis. Mineral content (nitrogen, phosphorus, and potassium) was determined following standard methods (Nafees *et al*., 2020; Batool *et al*., 2023).

Phytohormones quantification

Approximately 1 g of fresh leaf tissue was homogenized and extracted with a solvent mixture containing methanol (12 mL), chloroform (5 mL), and ammonium hydroxide (3 mL). The extract volume was adjusted to between 25 and 50 mL with distilled water, and the solution pH was carefully adjusted using hydrochloric acid. Auxin and gibberellin levels were determined by measuring absorbance at 254 and 222 nm, respectively, using a spectrophotometer calibrated with standard curves. For cytokinin determination, the extract was partitioned with ethyl acetate, and its pH was adjusted to 7.0 Cytokinin content was measured at 269 nm. This protocol allows precise quantification of key growth hormones in leaf samples (Nuray *et al*., 2002; Permatasari *et al*., 2025).

Statistical analysis

The collected data were evaluated using analysis of variance (ANOVA) to assess growth traits, mineral status, and phytohormones in pomegranate seedlings with the computational software Statistix 8.1. Means were separated using the Least Significant Difference (LSD) test at probability 5%. Pearson's correlation matrix was also calculated using Statistix 8.1.

Results and Discussion

Vermicompost, nano organic fertilizer, and plant extracts influenced pomegranate growth performance

Pomegranate seedling's growth such as seedlings height, number of leaves per seedling, and chlorophyll content were markedly different among the applied treatments. Different treatments showed a significant impact on the growth of pomegranate seedlings. Pomegranate seedlings' height was longer in moringa leaf extract (143 cm), while the shorter seedlings' height was measured in control (98 cm) pomegranate seedlings as compared to other applied organic treatments (Table 1). Different treatments such as vermicompost, nano organic fertilizer, and plant extracts significantly improved the number of leaves of each seedling in the present study. The maximum number of leaves in pomegranate seedlings was counted in moringa leaf extract (328), while the minimum number of leaves in pomegranate seedlings number (230) was recorded in control seedlings, compared to other applied organic treatments (Table 1). Different treatments such as vermicompost, nano organic fertilizer, and plant extracts substantially enhanced chlorophyll content in the leaves of

Table 1: *Effect of vermicompost, nano organic fertilizer, and plant extracts on growth attributes of pomegranate seedlings*

Treatments application	Seedlings height (cm)	Number of leaves per seedling	Chlorophyll content (SPAD)
Control (T0)	98 d	230 e	32 d
Vermicompost (T1)	120 c	251 d	42 c
Nano organic fertilizer (T2)	124 c	286 c	49 b
Roselle extract (T3)	129 b	301 b	56 ab
Moringa leaf extract (T4)	143 a	328 a	58 a
LSD value at 5% probability	12.22	14.96	21.16

Table 2: *Effect of vermicompost, nano organic fertilizer, and plant extracts on minerals contents in pomegranate seedlings*

Treatments application	Nitrogen level in leaves (%)	Phosphorus level in leaves (%)	Potassium level in leaves (%)
Control (T0)	0.95 e	0.11 c	1.14 e
Vermicompost (T1)	1.01 d	0.13 b	1.25 d
Nano organic fertilizer (T2)	1.35 c	0.21 ab	1.48 c
Roselle extract (T3)	1.54 b	0.26 ab	1.72 b
Moringa leaf extract (T4)	1.72 a	0.29 a	1.95 a
LSD value at 5% probability	11.62	16.43	20.13

pomegranate seedlings. The greater chlorophyll content in pomegranate leaves was measured in moringa leaf extract (58 SPAD), followed by roselle extract (56 SPAD), while lower chlorophyll content in pomegranate leaves was measured in control seedlings (32 SPAD) (Table 1).

Vermicompost, nano organic fertilizer, and plant extracts influenced mineral contents in pomegranate leaves

Minerals content such as nitrogen, phosphorus, and potassium present in leaves of pomegranate seedlings were significantly different among the applied organic treatments. Different organic treatments had a significant effect on mineral levels in of pomegranate seedlings. Pomegranate seedling leaves had the highest nitrogen content with moringa leaf extract (1.72%), while the lowest nitrogen content (0.95%) was recorded in the control compared to other than other applied organic treatments (Table 2).

All the studied organic treatments such as vermicompost, nano organic fertilizer, and plant extracts significantly improved the phosphorus content in pomegranate seedlings. The maximum phosphorus content in pomegranate leaves was determined in moringa leaf extract (0.29%), followed by roselle extract (0.26%) and nano organic fertilizer (0.21%), while the minimum phosphorus content in pomegranate leaves was recorded in control seedlings (0.11%) of pomegranate seedlings as compared to other applied organic treatments (Table 2). Potassium content was substantially improved in the leaves of pomegranate seedlings. The greater potassium content in pomegranate leaves was measured in moringa leaf extract (1.95%), while lower potassium content in the lowest content (1.14%) was recorded in control seedlings (Table 2).

Vermicompost, nano organic fertilizer, and plant extracts influenced phytohormones in pomegranate leaves

Phytohormones such as auxin, gibberellins, and cytokinin were improved in pomegranate leaves with the supplementation of vermicompost, nano-organic fertilizer, and plant extracts. Different organic treatments showed a significant influence on the phytohormones of pomegranate seedlings. Pomegranate leaves of seedlings recorded the highest auxin levels with moringa leaf extract (17.25 mg kg^{-1}) and roselle extract (16.12 mg kg^{-1}), followed by nano organic fertilizer (14.51 mg kg^{-1}), while lower auxin was measured from the control of seedlings (10.28 mg kg^{-1}) than other applied organic treatments (Table 3). All the organic treatments such as vermicompost, nano organic fertilizer, and plant extracts significantly improved the gibberellins level in pomegranate leaves. The maximum gibberellins level in pomegranate leaves was determined in moringa leaf extract (19.46 mg kg^{-1}), followed by roselle extract (17.57 mg kg^{-1}), while

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Table 3: *Effect of vermicompost, nano organic fertilizer and plant extracts on phytohormones in pomegranate seedlings*

Treatments application	Auxin in leaves (mg kg^{-1})	Gibberellins in leaves (mg kg^{-1})	Cytokinin in leaves (mg kg^{-1})
Control (T0)	10.28 c	12.16 c	9.56 c
Vermicompost (T1)	11.05 b	13.24 b	10.22 b
Nano organic fertilizer (T2)	14.51 ab	14.84 b	13.36 ab
Roselle extract (T3)	16.12 a	17.57 ab	15.12 a
Moringa leaf extract (T4)	17.25 a	19.46 a	16.92 a
LSD value at 5% probability	4.38	5.05	6.19

the lowest level (12.16 mg kg^{-1}) was recorded in the control (Table 3). Cytokinin level was substantially improved in the leaves of pomegranate seedlings in the present study. The greater cytokinin level in pomegranate leaves was measured in moringa leaf extract (16.92 mg kg^{-1}), roselle extract (15.12 mg kg^{-1}), followed by nano organic fertilizer (13.36 mg kg^{-1}), while the lowest value (9.56 mg kg^{-1}) was recorded in control seedlings (9.56 mg kg^{-1}) (Table 3).

Trait relationships developed from different organic treatments

Pearson's correlation matrix was developed from the collected data of different organic treatments. Seedlings height illustrated significant association with number of leaves, chlorophyll content, auxin, gibberellins, and cytokinins, while non-significant association with nitrogen, phosphorus, and potassium. The number of leaves proved a significant association with chlorophyll content, auxin, gibberellins, and cytokinins, while non-significant association with nitrogen, phosphorus, and potassium. Chlorophyll content exhibited significant association with auxin, gibberellins, and cytokinins, while non-significant association with nitrogen, phosphorus, and potassium. Nitrogen showed a significant correlation with phosphorus, and potassium, while non-significant association with auxin, gibberellins, and cytokinins (Table 4).

A re-examination of prior studies indicated that the plant growth traits, chlorophyll content, nutrient up take, and growth regulators (auxins, cytokinins, and gibberellins were boosted by the addition of vermicompost (Mubdir *et al*., 2024). The cause of the enhanced studied traits is likely the significant role of vermicomposting, as vermicompost is an organic fertilizer that is produced by earthworms that have a long-term commitment to the study, this chemical is composed of many of the micronutrients in the form of chelates, which are important to the plants because of their long-term commitment to the study. These amendments also improves availability of essential nutrients such as nitrates, phosphorus, potassium, calcium and magnesium that are designed for plant consumption. Additionally, these substances contain plant growth regulators, helpful for enhancing plant growth and development (Durak *et al*., 2017; Torres-Rodriguez, 2024).

Table 4: *Pearson's correlation matrix among the growth, minerals, and phytohormones of pomegranate seedlings*

Traits	SH	NL	CC	N	P	K	AX	GB	CK
SH	1.00								
NL	0.95**	1.00							
CC	0.96**	0.98**	1.00						
N	0.73ns	0.74ns	0.63ns	1.00					
P	0.70ns	0.70ns	0.59ns	0.99**	1.00				
K	0.71ns	0.72ns	0.61ns	0.99**	0.99**	1.00			
AX	0.89*	0.99**	0.97**	0.66ns	0.62ns	0.64ns	1.00		
GB	0.91*	0.97**	0.95**	0.77ns	0.74ns	0.76ns	0.97**	1.00	
CK	0.90**	0.99**	0.96**	0.72ns	0.70ns	0.71ns	0.99**	0.99**	1.00

*** = significant at P < 0.01; ** = significant at P < 0.05, ns = non-significant, seedlings height (SH), number of leaves (NL), chlorophyll contents (CC), nitrogen (N), phosphorus (P), potassium (K), auxin (AX), gibberellin (GB), and cytokinin (CK). Values in bold illustrated significant correlation among the studied traits of pomegranate.*

Earthworms are considered as important source of fertilizers essential for plant growth and development (Jabbar and Marhoon, 2022). Various hormones were found in vermicompost that promote growth by regulating cell division and physiological activity in plants in plants. These studies demonstrated that organic amendments including vermicompost are helpful in improving growth and development, Results showed improvements in leaf area, plant height, root length, biomass, and yield due to organic amendments. Vermicompost enhance plant development, for example through increasing dry weight and number of leaves. Moreover, vermicompost is rich in nutrients that are released throughout degradation process, This contributes to increased photosynthesis, higher carbohydrate production, and accumulation of biomass and nutrient groups (Joshi *et al*., 2015; Chuong, 2024).

Development of aforementioned qualities by adding organic fertilizers may be linked to substantial function it plays in enhancing cell proliferation and cell expansion, this directly influences many processes of life. This may be related to fact that these fertilizers aid development of soil and boost availability of nutrients, as well as prevent them from being carried away from the root region owing to their capability to connect to ions on their surface. They also operate as a buffer for fluctuations in the pH of the soil, which permits the best possible consumption of nutrients and guarantees the appropriate nutritional composition of plants. plants were able to grow vigorously and create a significant number of carbon metabolites, which in turn helps accumulation of carbohydrates, proteins and enzymes (Ye *et al*., 2020). Similar findings were confirmed in case of potato (Tiwari *et al*., 2025), Furthermore, different rootstocks and genotypes were also tested the results were also aligned with current study (Omari and Beniken, 2025).

Previous studies have reported significant improvements in vegetative parameters, as well as increased chlorophyll content and leaf nutrient concentrations with foliar application of plant extracts. These effects were primarily attributed to bioactive compounds present in *Moringa oleifera* leaf extract. This effect may be attributed to the presence of essential nutrients in extract, such as iron and magnesium, which play important roles in photosynthesis, respiration and other key metabolic processes in plants.

Furthermore, moringa leaf extract contains growth promoting compounds, including vitamins B1, B2 and B3, which are essential for carbohydrate metabolism and amino acid synthesis, fundamental building blocks of proteins thereby contributing to improved plant growth (Opara *et al*., 2009; Al-Muhe and Abdul-Wahid, 2024). Extract also contains significant amounts of proteins and carbohydrates, which serve as critical energy sources for biosynthesis and development, thereby enhancing the plant growth characteristics. Additionally, moringa extract applies for multiple physiological effects that improve plant vigor, seedling development, increased structural strength and improved growth parameters (Hegazi *et al*., 2015).

Conclusions and Recommendations

The current study concluded that the combination of adding vermicompost to soil (average 250 gm per seedling) and foliar spraying of moringa leaf extract (20 ml/L) and nano-biofertilizer (5 ml/L) significantly improved plant growth characteristics and chlorophyll content, nutrients in leaves, and growth regulators. Therefore, use of organic amendments is recommended, these are environmentl friendly and natural as compared to chemical compounds. These natural substances improves the nutritional status of Plants and indirectly enhance the growth and nutrient uptake by pomegranate seedlings.

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Novelty Statement

This study introduces a unique approach by combining vermicompost, nano-organic fertilizers, and plant-based extracts (moringa and roselle) to significantly improve mineral uptake and phytohormonal levels in pomegranate seedlings.

Author's Contribution

Akram A.A. Hadi: Designed the experiment, conducted data analysis, and wrote the manuscript.
Mawaheb M. Hussein: Was responsible for field-

work, sample collec-tion, and literature review. Both authors read and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement
The authors have declared there is no generative AI and AI-assisted technology used in this manuscript.

Conflict of interest
The authors have no conflict of interest.

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