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Optimizing bio-chemical fertilizer treatments for quantitative and qualitative traits of *Artemisia annua* L. using graphical analysis

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ABSTRACT

Purpose: *Artemisia annua* L., a medicinal herb of significant importance due to its high artemisinin content, a potent antimalarial compound. This study aimed to investigate the influence of various bio-chemical fertilizer combinations on the growth characteristics and artemisinin content of *Artemisia annua* L. Graphical analysis techniques were employed to visualize and optimize fertilizer treatments for achieving the desired balance between quantitative (biomass) and qualitative (artemisinin content) traits. **Research Method:** This experiment was conducted as a factorial experiment with a basic randomized complete block design (RCBD) with four replications in a research greenhouse. The main factors in this experiment included the application of bio-fertilizers (control, nitroxin, bio-phosphorus, and vermicompost), and the sub-factor consisted of four levels of chemical phosphorus (P) and nitrogen (N) fertilizers (0, N40 P40, N80 P40, and N80 P80). **Findings:** The results of the combined analysis for all traits revealed significant effects of bio-fertilizer and chemical fertilizer at the 5% and 1% probability levels, respectively. The interaction of treatments also exhibited significant differences for most traits. Mean comparison using LSD showed that vermicompost and $N_{80}P_{80}$ treatments were superior to other treatments. Correlation analysis revealed a positive and significant correlation between the traits, as evidenced by the correlation coefficients and correlation charts. Graphical analysis identified treatments Vermicompost + $N_{80}P_{80}$ and Nitroxin + $N_{80}P_{80}$ as optimal based on trait desirability. The results of the focused scatter plot analysis further confirmed Vermicompost + $N_{80}P_{80}$ as the most favorable treatment. The findings revealed a strong correlation among the evaluated traits. **Research limitations:** There was no limitation. **Originality/Value:** Treatments Vermicompost + $N_{80}P_{80}$ and Nitroxin + $N_{80}P_{80}$ emerged as the most favorable options based on the assessed traits, demonstrating remarkable efficacy in augmenting artemisinin levels.

INTRODUCTION

Malaria, a devastating disease that continues to claim lives, resulted in nearly 429,000 fatalities worldwide in 2015. Predominantly prevalent in African countries (accounting for approximately 90% of global cases), malaria poses a significant barrier to social and economic progress in these regions.

One of the most lethal malaria parasites has developed resistance to commonly used antimalarial drugs such as quinine, chloroquine, mefloquine, and sulfadoxine-pyrimethamine in Asia and Africa. This resistance poses a major challenge to malaria control and treatment (Brisibe et al., 2012; Kumar & Rathinam, 2013).

Numerous studies conducted by the World Health Organization (WHO) since 2001 have demonstrated the efficacy of artemisinin-based combination therapy (ACT) in combating malaria (World Health Organization, 2016). Artemisinin, a molecule extracted from the Chinese annual herb *Artemisia annua* L., holds immense promise in the battle against this devastating disease (Aftab et al., 2014).

Artemisia annua L., an ancient herb belonging to the Asteraceae family, has been utilized in traditional Chinese medicine for centuries to treat fevers. The discovery of the antimalarial properties of its extracts in the 1970s marked a turning point in the fight against malaria. The plant's active compound, artemisinin, emerged as a new generation of antimalarial drugs, and in combination with other medications, significantly improved the treatment of uncomplicated malaria cases (Shahrajabian et al., 2020; Siddiqui et al., 2018).

Artemisinin has not only proven its effectiveness but also exhibits minimal side effects. Unlike conventional chemical drugs, this plant-derived compound exhibits low toxicity and has demonstrated superior efficacy in malaria treatment (Aftab et al., 2014).

Despite these advantages, the WHO does not recommend direct consumption of *Artemisia annua* L. for malaria treatment (World Health Organization, 2016). This stems from the insufficient production of artemisinin to meet the growing global demand and its high price in China, rendering this treatment inaccessible to low-income populations. To address these limitations, production chains for this compound were established in the early 2000s, initially in Vietnam and later in East Africa (Konaré et al., 2023).

In the world, the use of chemical fertilizers has been well-documented as a key factor in the remarkable increase in crop yields. This holds true for a wide range of crops cultivated under diverse climatic and soil conditions. Evidence from numerous field trials suggests that in many soils, nutrient deficiencies, particularly nitrogen, pose a major constraint to plant growth, and chemical fertilization can significantly alleviate this limitation (Nyoni et al., 2020).

Nitrogen, an essential element for plant growth, plays a crucial role in the synthesis of proteins and nucleic acids. Studies by Singh (2000) have demonstrated that the application of varying levels of nitrogen fertilizer positively impacts artemisinin content and essential oil in *Artemisia annua* L. plants. This is due to the direct role of nitrogen in the structure of the chlorophyll molecule; a strong positive correlation exists between leaf nitrogen content and plant chlorophyll levels (Jia et al., 2021).

In today's agricultural landscape, there is a growing demand for sustainable and environmentally friendly solutions to enhance crop production. In this context, biological fertilizers have emerged as a new generation of fertilizers that utilize beneficial microbes instead of chemicals to promote plant growth. These fertilizers employ various mechanisms, including nitrogen fixation from the air, enhancing nutrient solubility and uptake from the soil, producing plant hormones, and diversifying soil microbial communities, which stimulate plant growth directly and indirectly (Kumar, 2004; Raimi et al., 2021).

Unlike chemical fertilizers, which are not only expensive but also pose environmental risks, biological fertilizers are cost-effective, eco-friendly, and sustainable, contributing to long-term soil health (Raimi et al., 2021).

Artemisia annua L., a remarkable medicinal herb, has gained significant attention for its potent antimalarial compound, artemisinin. While chemical fertilizers have played a crucial role in boosting crop yields, their environmental impact and potential drawbacks have prompted a shift towards sustainable alternatives. In this context, biological fertilizers, enriched with beneficial microbes, have emerged as a promising approach for enhancing *Artemisia annua* L. cultivation and artemisinin production (Bijeh Keshavarzi & Omidi, 2025).

A diverse array of microorganisms, particularly fungi and certain bacteria, have demonstrated the potential to augment the production of bioactive compounds in *Artemisia annua* L. Among the common bacteria employed in *Artemisia annua* L. biofertilizers are *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, Streptomyces, and Rhizobium (Aidah et al., 2023).

The utilization of biological fertilizers in *Artemisia annua* L. cultivation offers not only a means to increase the yield of this valuable medicinal herb but also presents a sustainable and environmentally friendly approach to promote soil health and foster sustainable agricultural practices. Yazdani et al. (2009) demonstrated that the application of phosphate-solubilizing microorganisms and plant growth-promoting rhizobacteria (PGPR) in conjunction with chemical fertilizers (nitrogen, phosphorus, and potassium) in maize led to significant improvements in cob weight, number of rows per cob, and number of grains per row. Galindo et al. (2022) investigated the effects of nitrogen fertilizer, *Azospirillum* inoculation, and their combined application on cowpea growth. Their findings demonstrated that the combined application of nitrogen fertilizer and *Azospirillum* inoculation significantly increased both cowpea nitrogen and grain yield compared to the control and other treatments. When *Azospirillum* co-inoculation is employed in cowpea cultivation, nitrogen supplementation via mineral fertilizers becomes unnecessary.

The low artemisinin content in *Artemisia annua* L. and the economic challenges associated with large-scale production prompted the researchers to undertake this study. The objective was to investigate the influence of biofertilizers, chemical fertilizers, and their combinations on artemisinin content.

MATERIALS AND METHODS

This experiment was conducted as a factorial experiment with a basic randomized complete block design (RCBD) with four replications in a research greenhouse located in Tehran province during the summer of 2011. The main factors in this experiment included the application of bio-fertilizers, and the sub-factor consisted of four levels of chemical phosphorus and nitrogen fertilizers. Nitrogen fertilizer was applied in the form of urea, and phosphorus fertilizer was applied as triple superphosphate. All related laboratory analyses were performed at the Biotechnology Laboratory of Hamdard University, India.

Experimental factors

Experimental factors included bio-fertilizer at 4 levels: control (without fertilizer), Nitroxin (Containing Azotobacter and Azospirillum Bacteria), Biophosphorus (Containing Bacillus and Pseudomonas Bacteria), Vermicompost (10 tons per hectare) and N and P chemical fertilizer at 4 levels: Control (No Fertilizer), Nitrogen 40 and Phosphorus 40 kg/ha ($N_{40}P_{40}$), Nitrogen 80 and Phosphorus 40 kg/ha ($N_{80}P_{40}$), Nitrogen 80 and Phosphorus 80 kg/ha ($N_{80}P_{80}$).

Table 1. Treatment characteristics, names, and measured traits.

Treatment code	Treatment characteristics	Traits code	Traits
tr_1	Control	C_1	Artemisinin – Pre flowering
tr_2	$N_{40}P_{40}$	C_2	Artemisinin – Post flowering
tr_3	$N_{80}P_{40}$	C_3	Leaf Water Content – Pre flowering
tr_4	$N_{80}P_{80}$	C_4	Leaf Water Content – Post flowering
tr_5	Nitroxin	C_5	Leaf Number – Pre flowering
tr_6	Nitroxin + $N_{40}P_{40}$	C_6	Leaf Number – Post flowering
tr_7	Nitroxin + $N_{80}P_{40}$	C_7	Fresh Leaf Weight – Pre flowering
tr_8	Nitroxin + $N_{80}P_{80}$	C_8	Fresh Leaf Weight – Post flowering
tr_9	Biophosphorus	C_9	Dry Leaf Weight – Pre flowering
tr_{10}	Biophosphorus + $N_{40}P_{40}$	C_{10}	Dry Leaf Weight – Post flowering
tr_{11}	Biophosphorus + $N_{80}P_{40}$	C_{11}	Protein
tr_{12}	Biophosphorus + $N_{80}P_{80}$	C_{12}	Chlorophyll a
tr_{13}	Vermicompost	C_{13}	Chlorophyll b
tr_{14}	Vermicompost + $N_{40}P_{40}$	C_{14}	Total Chlorophyll
tr_{15}	Vermicompost + $N_{80}P_{40}$	C_{15}	Stem Height – Pre flowering
tr_{16}	Vermicompost + $N_{80}P_{80}$	C_{16}	Stem Height – Post flowering
		C_{17}	Number of Lateral Stems - Pre flowering
		C_{18}	Number of Lateral Stems - Post flowering
		C_{19}	Stem Water Content – Pre flowering
		C_{20}	Stem Water Content – Post flowering
		C_{21}	Fresh Stem Weight – Pre flowering
		C_{22}	Fresh Stem Weight – Post flowering
		C_{23}	Dry Stem Weight – Pre flowering
		C_{24}	Dry Stem Weight – Pre flowering

Planting

For seed mixing and inoculation, the desired *Artemisia annua* L. seeds were first spread on a clean, wide plastic sheet. The inoculant was then sprinkled on the seeds and the seeds were mixed to ensure even inoculation. The inoculated seeds were then placed in the shade for 1 hour to allow the inoculant to dry, and were then ready for planting (Shakouri & Keshavarzi, 2020).

Nitrogen fertilizer was applied from urea (with 48% nitrogen) and phosphorus fertilizer from triple superphosphate (with 46% phosphorus). All phosphorus fertilizer was applied before planting, and nitrogen fertilizer was applied in 3 split applications based on soil testing. Table 1 shows the characteristics and names of the treatments and the traits measured in the experiment.

Planting was conducted on the first of September 2011. Prior to planting, a plastic bag (with drainage holes) was placed in each of the pre-prepared plastic pots (20 cm diameter and 25 cm height) to prevent fertilizers from leaching out of the bottom of the pots. The desired soil was then filled into the pots. After the potential risk of pests was eliminated, thinning was performed by retaining three healthy plants and removing the remaining plants from the pots. The soil used had a sandy loam texture with a pH of 7. The plant's light requirement was met artificially for 16 hours per day. The set temperature was 18°C for the day and 22°C for the night. The relative air humidity was also set at 65% (Zhang et al., 2023).

Trait measurement

To measure stem height, the plants were cut at ground level and the exact height of each plant was individually measured using a ruler. This was done both before and after flowering.

For measuring the fresh and dry weight of the entire aboveground biomass, the plants were cut at ground level. Then, using a digital scale, the fresh weight of each plant organ (leaf, stem) was individually measured and recorded. The separated organs were then placed in separate bags and dried in an oven at 70°C for 24 hours. The dry weight of each organ was then individually measured and recorded using a digital scale.

To measure the water content of each plant organ (leaf, stem), the dry weight was subtracted from the fresh weight to determine the water content (in milligrams). Chlorophyll content was measured using the method of Hiscox and Israelstam (1979), and protein content was measured using the Bradford method (1976).

Chlorophyll content

100 mg of fresh Artemisia leaves were placed in a test tube, and 10 mL of Dimethyl Sulfoxide (DMSO) was added. The mixture was then incubated at 65°C for 1 hour to ensure complete extraction of chlorophylls and to achieve complete bleaching of the leaves. Subsequently, aliquots of the samples were transferred to spectrophotometer cuvettes, and absorbance was measured separately at wavelengths of 663 nm for chlorophyll a and 645 nm for chlorophyll b. The recorded absorbance values were then used in the following formulas (1, 2 & 3) to calculate the concentrations of chlorophyll a, chlorophyll b, and total chlorophyll.

$$\text{Chlorophyll a (mg/g)} = 12.7\,(OD663) - 2.69(OD645) \times (V/(1000 \times wt)) \quad (1)$$

$$\text{Chlorophyll b (mg/g)} = 22.9\,(OD645) - 4.68(OD663) \times (V/(1000 \times wt)) \quad (2)$$

$$\text{Total chlorophyll (mg/g)} = 20.2\,(OD645) + 8.02(OD663) \times (V/(1000 \times wt)) \quad (3)$$

Where V represents the volume of the filtered solution, wt represents the fresh weight of the sample used, and OD represents the optical density (absorbance) at wavelengths of 663 nm and 645 nm (Hiscox & Israelstam, 1979).

Protein content

0.5 g of fresh plant sample was added to 5 mL of Buffer Solution No. 1 and thoroughly ground until the sample was completely dissolved in the buffer. The resulting solution was centrifuged at 12,000 rpm for 20 minutes. 1 mL of the supernatant was carefully collected using a micropipette and transferred to a separate Eppendorf tube. Then, 1 mL of 20% Trichloroacetic Acid was added, and the mixture was incubated at 20°C for 2 hours. Subsequently, the mixture was centrifuged again at 10,000 rpm for 10 minutes. After centrifugation, 1 mL of 0.1 M NaOH was added to the pellet and dissolved. 100 µL of the resulting solution was then added to 5 mL of Buffer Solution No. 2 (Bradford reagent). By adding Milli-Q water (double-distilled water), the final volume was adjusted to 6 mL. Finally, the sample was placed in a spectrophotometer at a wavelength of 595 nm, and the obtained absorbance was recorded.

Artemisinin content was determined in the samples using the Gupta et al. (1996) method and HPLC. 1 g of dried material was finely ground and 20 ml of petroleum ether was added. The samples were shaken at 37°C for 12 hours, and the resulting solution was collected. This process was repeated three times. The collected material was placed on a heater to evaporate the solvent and dry the sample. Four ml of ethanol was added to the sample and after filtration; the residue was washed with 2 ml of ethanol. The entire filtered solution was made up to 10 ml with ethanol. One ml of the final solution was diluted with 4 ml of 2% NaOH and heated in a water bath at 50°C for 30 minutes. After cooling, 1 ml of ethanol was added and the volume was made up to 10 ml with 2 N acetic acid.

Statistical analysis

To gain deeper insights from the composite analysis, we employed various techniques, which were included comparing treatment means using the LSD method, evaluating the interaction effects of treatments on averages, performing correlation analysis, and conducting graphical analysis. The graphical analysis utilized multivariate diagrams and correlation plots to identify

the optimal treatment based on an ideal standard. Additionally, concentrated scatter plots visualized the relationships between desired treatments and traits. Software like Excel, SAS, V9, and Genstat V. 12.1 were used for the data analysis.

RESULTS AND DISCUSSION

The results of the analysis of variance revealed significant effects of replication on all traits except artemisinin (pre-flowering), artemisinin (post-flowering), chlorophyll a, chlorophyll b and total chlorophyll at the 0.05 and 0.01 probability levels. Bio-fertilizer and chemical fertilizer effects were also significantly different for all traits. The interaction effect of bio-fertilizer and chemical fertilizer was also significantly different for all traits except Leaf Water Content (post-flowering), leaf number (pre-flowering), leaf number (post-flowering), fresh leaf weight (pre-flowering), dry leaf weight (pre-flowering), chlorophyll b, and number of lateral stems (pre-flowering). The highest coefficient of variation percentage was observed for trait number of lateral stems (pre-flowering) (17.20%), while the lowest was for trait fresh stem weight (pre-flowering) (8.30%). Additionally, the highest R-squared value was for trait artemisinin (post-flowering) (99.0%) and the lowest was for trait c13 (71.0%) (Table 2).

Mean comparison using the LSD method was employed to select the most suitable treatment based on trait means. The results indicated that the vermicompost and N80P80 treatments exhibited greater desirability compared to the other treatments under investigation for all traits. Furthermore, the nitroxin treatment demonstrated superior performance for traits such as leaf water content (before flowering), leaf fresh weight (after flowering), leaf dry weight (before flowering), protein content, number of lateral branches (before flowering), stem water content (before flowering), stem water content (after flowering), and stem dry weight (after flowering) (Table 3). In a study aimed at selecting iron and zinc nanofertilizers for morphological and biological traits of rice plants under drought stress conditions, LSD mean comparison was utilized to evaluate the treatments, and the most desirable treatments for various traits were selected based on this analysis (Jafarsalehi et al., 2024).

Table 2. Analysis of variance of treatments for evaluated traits in the experiment.

S.O.V	df	Artemisinin – pre flowering	Artemisinin – post flowering	Leaf water content - before flowering	Leaf Water Content – Post flowering	Leaf Number – Pre flowering	Leaf Number – Post flowering
Repetition (R)	3	0.0001^{ns}	0.0001^{ns}	1.12^{**}	1.69^{**}	348^{**}	504.08^{**}
Bio-Fertilizer (A)	3	0.02^{**}	0.026^{**}	4.66^{**}	11.1^{**}	1286.8^{**}	1762.04^{**}
Error1	9	0.0002	0.0009	1.18	0.67	19.6	26.4
Chemical Fertilizer (B)	3	0.04^{**}	0.1^{**}	8.43^{**}	12.1^{**}	1246.37^{**}	1698^{**}
Bio-Fertilizer × Chemical Fertilizer (A×B)	9	0.0008^{**}	0.0019^{**}	0.12^{**}	0.38^{ns}	36.2^{ns}	53.82^{ns}
Error 2	36	0.0001	0.00005	0.22	0.21	25.01	34.5
CV%	-	5.58	3.69	18	14.2	8.28	8.3
R-Square	-	0.96	0.99	0.86	0.91	0.91	0.91

Table 2. *Continued.*

S.O.V	df	Fresh Leaf Weight – Pre flowering	Fresh Leaf Weight – Post flowering	Dry Leaf Weight – Pre flowering	Dry Leaf Weight – Post flowering	Protein	Chlorophyll a	Chlorophyll b
Repetition (R)	3	0.06^{**}	0.31^{**}	0.75^{*}	1.91^{**}	2.34^{**}	0.002^{ns}	0.014^{ns}
Bio-Fertilizer (A)	3	0.047^{**}	1.5^{**}	8.06^{**}	20.78^{**}	22.3^{**}	0.48^{**}	0.36^{**}
Error1	9	0.07	0.13	1.44	0.48	1.6	0.005	0.009^{*}
Chemical Fertilizer (B)	3	0.57^{**}	1.06^{**}	13.4^{**}	20.3^{**}	19.29^{**}	0.7^{**}	0.21^{**}
Bio-Fertilizer × Chemical Fertilizer (A×B)	9	0.018^{ns}	0.09^{**}	1.47^{ns}	0.67^{*}	1.02^{**}	0.02^{**}	0.02^{ns}
Error 2	36	0.011	0.017	0.22	0.23	0.1	0.005	0.023
CV%	-	12.39	12.58	13.37	11.33	5.4	3.43	17.05
R-Square	-	0.91	0.94	0.91	0.94	0.97	0.95	0.71

Table 2. *Continued.*

S.O.V	df	Total Chlorophyll	Stem Height – Pre flowering	Stem Height – Post flowering	Number of Lateral Stems - Pre flowering	Number of Lateral Stems - Post flowering
Repetition (R)	3	0.01^{ns}	12.22^{**}	236.1^{**}	5.93^{*}	68.59^{*}
Bio-Fertilizer (A)	3	1.66^{**}	156.9^{**}	266.16^{**}	67.55^{**}	631.8^{**}
Error1	9	0.01	1.65	2.9	4.14	3.36
Chemical Fertilizer (B)	3	2.11^{**}	255.9^{**}	458.2^{**}	76.76^{**}	571.18^{**}
Bio-Fertilizer × Chemical Fertilizer (A×B)	9	0.08^{**}	6.3^{**}	5.6^{**}	2.19^{ns}	30.11^{*}
Error 2	36	0.01	0.9	1.8	1.72	15.78
CV%	-	3.7	3.03	3.42	20.17	13.64
R-Square	-	0.96	0.97	0.97	0.89	0.87

Table 2. *Continued.*

S.O.V	df	Stem Water Content – Pre flowering	Stem Water Content – Post flowering	Fresh Stem Weight – Pre flowering	Fresh Stem Weight – Post flowering	Dry Stem Weight – Pre flowering	Dry Stem Weight – Post flowering
Repetition (R)	3	0.012^{**}	0.014^{**}	0.024^{**}	0.02^{**}	0.001^{**}	0.001^{**}
Bio-Fertilizer (A)	3	0.12^{**}	0.16^{**}	0.32^{**}	0.24^{**}	0.026^{**}	0.017^{**}
Error1	9	0.007	0.009	0.01	0.009	0.0004	0.0002
Chemical Fertilizer (B)	3	0.32^{**}	0.38^{**}	0.66^{**}	0.58^{**}	0.04^{**}	0.035^{**}
Bio-Fertilizer × Chemical Fertilizer (A×B)	9	0.006^{**}	0.009^{**}	0.012^{**}	0.008^{**}	0.0007^{**}	0.0004^{*}
Error 2	36	0.001	0.003	0.003	0.002	0.0001	0.0001
CV%	-	12.67	14.5	0.83	10.69	8.11	10.6
R-Square	-	0.96	0.94	0.96	0.97	0.97	0.96

Table 3. Mean comparison of treatments for evaluated traits in the experiment.

Bio-Fertilizer (A)	Artemisinin – pre flowering	Artemisinin – post flowering	Leaf water content - before flowering	Leaf Water Content – Post flowering	Leaf Number – Pre flowering	Leaf Number – Post flowering
Control (A_1)	0.2^{d}	0.14^{d}	2.08^{b}	2.4^{c}	50.12^{d}	58.75^{d}
Nitroxin (A_2)	0.26^{b}	0.16^{b}	2.7^{ab}	3.47^{b}	65.2^{b}	76.25^{b}
Bio-Phosphorus (A_3)	0.22^{b}	0.18^{c}	2.43^{b}	2.82^{bc}	56.06^{c}	65.43^{c}
Vermicompost (V_4)	0.28^{a}	0.24^{a}	3.36^{a}	4.31^{a}	70.06^{a}	82.06^{a}
LSD	0.01	0.007	0.86	0.65	3.54	4.1
Chemical Fertilizer (B)	Artemisinin – pre flowering	Artemisinin – post flowering	Leaf water content - before flowering	Leaf Water Content – Post flowering	Leaf Number – Pre flowering	Leaf Number – Post flowering
Control (B_1)	0.18^{d}	0.086^{d}	1.8^{d}	2.34^{c}	49.75^{c}	58.12^{c}
$N_{40}P_{40}$ (B_2)	0.22^{c}	0.18^{c}	2.38^{c}	2.8^{c}	57.93^{b}	67.87^{b}
$N_{80}P_{40}$ (B_3)	0.27^{b}	0.22^{b}	2.92^{b}	3.55^{b}	63.06^{b}	73.87^{b}
$N_{80}P_{80}$ (B_4)	0.29^{a}	0.27^{a}	3.48^{a}	4.31^{a}	70.75^{a}	82.66^{a}
LSD	0.01	0.003	0.43	0.49	5.65	6.46

Table 3. *Continued.*

Bio-Fertilizer (A)	Fresh Leaf Weight – Pre flowering	Fresh Leaf Weight – Post flowering	Dry Leaf Weight – Pre flowering	Dry Leaf Weight – Post flowering	Protein	Chlorophyll a
Control (A_1)	0.65^{c}	0.73^{c}	2.74^{b}	3.14^{d}	4.71^{c}	1.89^{d}
Nitroxin (A_2)	0.92^{b}	1.15^{ab}	3.63^{ab}	4.62^{b}	6.49^{ab}	2.2^{b}
Bio-Phosphorus (A_3)	0.79^{bc}	0.91^{bc}	3.22^{b}	3.73^{c}	5.5^{bc}	2.02^{c}
Vermicompost (V_4)	1.05^{a}	1.44^{a}	4.42^{a}	5.75^{a}	7.43^{a}	2.27^{a}
LSD	0.65	0.73	2.74	3.14	1.01	0.05
Chemical Fertilizer (B)	Fresh Leaf Weight – Pre flowering	Fresh Leaf Weight – Post flowering	Dry Leaf Weight – Pre flowering	Dry Leaf Weight – Post flowering	Protein	Chlorophyll a
Control (B_1)	0.63^{d}	0.77^{d}	2.43^{d}	3.11^{d}	4.8^{d}	1.86^{d}
$N_{40}P_{40}$ (B_2)	0.78^{c}	0.93^{c}	3.16^{c}	3.72^{c}	5.52^{c}	1.99^{c}
$N_{80}P_{40}$ (B_3)	0.94^{b}	1.19^{b}	3.87^{b}	4.75^{b}	6.5^{b}	2.2^{b}
$N_{80}P_{80}$ (B_4)	1.06^{a}	1.34^{a}	4.55^{a}	5.66^{a}	7.31^{a}	2.33^{a}
LSD	0.1	0.12	0.36	0.46	0.23	0.04

Table 3. *Continued.*

Bio-Fertilizer (A)	Chlorophyll b	Total Chlorophyll	Stem Height – Pre flowering	Stem Height – Post flowering	Number of Lateral Stems - Pre flowering	Number of Lateral Stems - Post flowering
Control (A_1)	0.744^{d}	2.64^{d}	28.29^{c}	35.05^{d}	3.93^{c}	22.43^{d}
Nitroxin (A_2)	0.919^{b}	3.13^{b}	32.55^{b}	4.97^{b}	7.5^{ab}	30^{b}
Bio-Phosphorus (A_3)	0.838^{c}	2.86^{c}	29^{c}	36.96^{c}	5.93^{b}	26.68^{c}
Vermicompost (V_4)	1.1^{a}	3.38^{a}	34.98^{a}	44.15^{a}	8.68^{a}	37.31^{a}
LSD	0.07	0.08	1.02	1.36	1.62	1.46
Chemical Fertilizer (B)	Chlorophyll b	Total Chlorophyll	Stem Height – Pre flowering	Stem Height – Post flowering	Number of Lateral Stems - Pre flowering	Number of Lateral Stems - Post flowering
Control (B_1)	0.74^{b}	2.58^{d}	26.59^{d}	32.95^{d}	3.87^{a}	22.5^{d}
$N_{40}P_{40}$ (B_2)	0.89^{b}	2.84^{c}	29.45^{c}	37.26^{c}	5.75^{c}	26.06^{c}
$N_{80}P_{40}$ (B_3)	1.003^{a}	3.17^{b}	33.1^{b}	41.6^{b}	7.5^{b}	32^{b}
$N_{80}P_{80}$ (B_4)	0.96^{a}	3.41^{a}	35.68^{a}	45.31^{a}	8.93^{a}	35.87^{a}
LSD	0.17	0.1	0.68	1.46	0.92	1.25

Table 3. *Continued.*

Bio-Fertilizer (A)	Stem Water Content – Pre flowering	Stem Water Content – Post flowering	Fresh Stem Weight – Pre flowering	Fresh Stem Weight – Post flowering	Dry Stem Weight – Pre flowering	Dry Stem Weight – Post flowering
Control (A_1)	0.22^{b}	0.25^{c}	0.36^{d}	0.3^{b}	0.1^{d}	0.077^{d}
Nitroxin (A_2)	0.36^{a}	0.42^{a}	0.58^{b}	0.49^{a}	0.15^{b}	0.128^{b}
Bio-Phosphorus (A_3)	0.25^{b}	0.33^{b}	0.47^{c}	0.35^{b}	0.13^{c}	0.1^{c}
Vermicompost (V_4)	0.41^{aa}	0.49^{aa}	0.69^{a}	0.57^{aa}	0.19^{a}	0.154^{a}
LSD	0.068	0.078	0.08	0.079	0.016	0.013
Chemical Fertilizer (B)	Stem Water Content – Pre flowering	Stem Water Content – Post flowering	Fresh Stem Weight – Pre flowering	Fresh Stem Weight – Post flowering	Dry Stem Weight – Pre flowering	Dry Stem Weight – Post flowering
Control (B_1)	0.15^{d}	0.199^{d}	0.28^{d}	0.21^{d}	0.08^{d}	0.06^{d}
$N_{40}P_{40}$ (B_2)	0.24^{c}	0.31^{c}	0.44^{c}	0.34^{c}	0.136^{c}	0.09^{c}
$N_{80}P_{40}$ (B_3)	0.38^{b}	0.46^{b}	0.62^{b}	0.52^{b}	0.167^{b}	0.14^{b}
$N_{80}P_{80}$ (B_4)	0.47^{a}	0.54^{a}	0.74^{a}	0.64^{a}	0.202^{a}	0.16^{a}
LSD	0.038	0.051	0.053	0.045	0.009	0.009

Considering the significance and widespread application of traits artemisinin (pre-flowering) and artemisinin (post-flowering) in this experiment, and since identifying suitable treatments based on these traits is a primary objective of this research, mean comparison plots were employed to visualize the interaction effect of bio-fertilizer and chemical fertilizer on these two traits. According to the plotted graph for trait artemisinin (pre-flowering), treatments tr16, tr8, and tr15 were identified as desirable treatments. Conversely, treatments tr1, tr9, and tr2 were categorized as undesirable treatments (Fig. 1a). Similarly, based on the graph for trait artemisinin (post-flowering), treatments tr16 and tr8 were identified as desirable treatments, while treatments tr1, tr9, and tr5 were classified as undesirable treatments for this trait (Fig. 1b). Several researchers have utilized this type of graph to evaluate their treatments (Khatibi et al., 2023; Omrani et al., 2022; Shojaei et al., 2022).

The results obtained from the mean comparison chart of the interaction between bio-fertilizer and chemical fertilizer showed similar results compared to those obtained from the mean comparison analysis using the LSD method (Table 4). These results indicated that the application of vermicompost + N80P80 treatment can have a positive effect on increasing the performance of traits in growth and enhancing the content of the active ingredient in this plant.

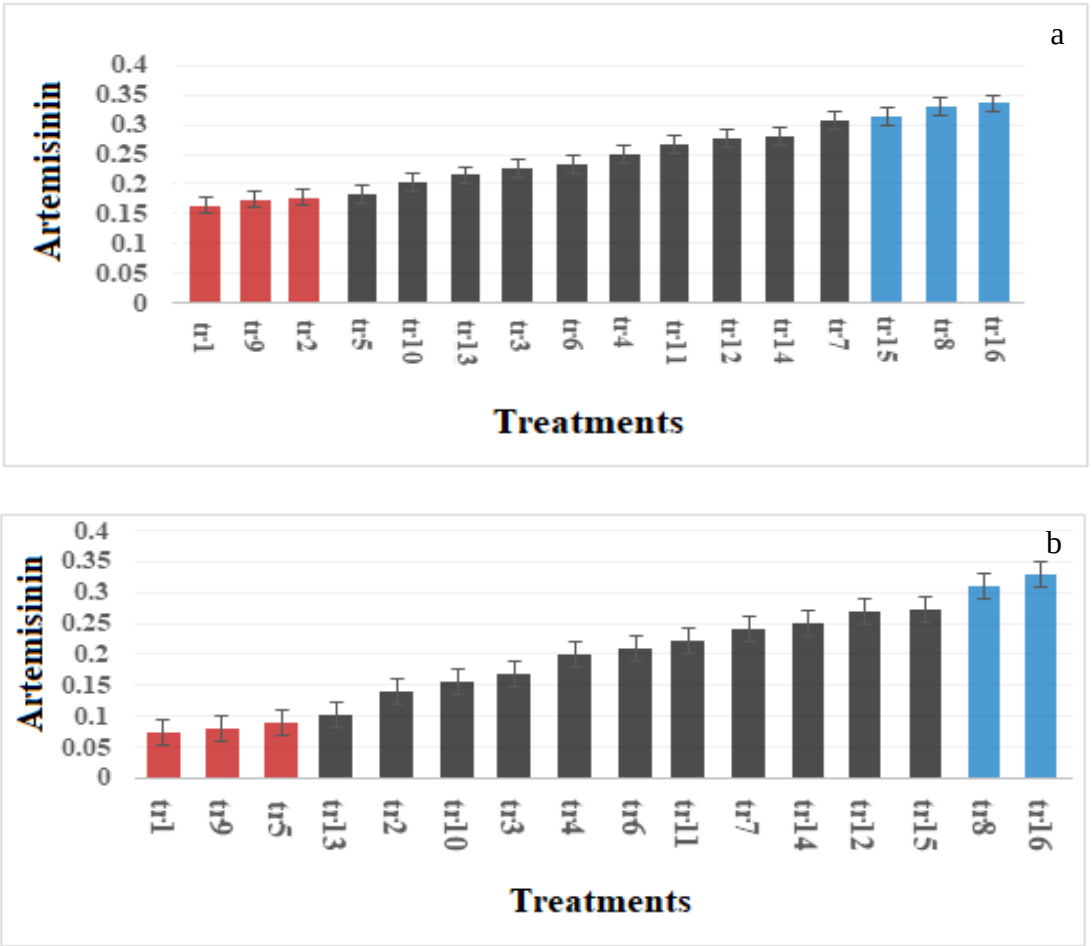


Fig. 1. Mean comparison plots of the interaction effect of bio-fertilizer and chemical fertilizer, a: Based on Trait c1 (Artemisinin (pre-flowering)), b: Based on Trait c2 (Artemisinin (post-flowering)).
(tr_1: Control; tr_2: $N_{40}P_{40}$; tr_3: $N_{80}P_{40}$; tr_4: $N_{80}P_{80}$; tr_5: Nitroxin; tr_6: Nitroxin + $N_{40}P_{40}$; tr_7: Nitroxin + $N_{80}P_{40}$; tr_8: Nitroxin + $N_{80}P_{80}$; tr_9: Biophosphorus; tr_{10}: Biophosphorus + $N_{40}P_{40}$; tr_{11}: Biophosphorus + $N_{80}P_{40}$; tr_{12}: Biophosphorus + $N_{80}P_{80}$; tr_{13}: Vermicompost; tr_{14}: Vermicompost + $N_{40}P_{40}$; tr_{15}: Vermicompost + $N_{80}P_{40}$; tr_{16}: Vermicompost + $N_{80}P_{80}$)

Table 4. Means comparison of interaction effects of treatments for evaluated traits in the experiment.

	Artemisinin – pre flowering	Artemisinin – post flowering	Leaf water content - before flowering	Leaf water content – Post flowering	Leaf number – Pre flowering	Leaf number – Post flowering	Fresh leaf weight – Pre flowering	Fresh leaf weight – Post flowering
Control	0.16^{j}	0.074^{l}	1.28^{g}	1.78^{h}	42.5^{i}	49.5^{i}	0.5^{h}	0.55^{i}
$N_{40}P_{40}$	0.17^{j}	0.13^{i}	1.75^{gf}	1.79^{h}	44.75^{hi}	53^{hi}	0.59^{gh}	0.68^{hi}
$N_{80}P_{40}$	0.22^{g}	0.16^{g}	2.53^{def}	2.37^{ghf}	5.075^{gh}	59.5^{gh}	0.58^{fgh}	0.79^{fghi}
$N_{80}P_{80}$	0.25^{ef}	0.19^{f}	2.78^{cde}	3.65^{cd}	62.25^{def}	73^{def}	0.83^{def}	0.9^{efgh}
Nitroxin	0.18^{ij}	0.088^{k}	1.7^{gf}	2.37^{fgh}	5.075^{gh}	58.5^{gh}	0.72^{gf}	0.83^{fghi}
Nitroxin + $N_{40}P_{40}$	0.23^{gh}	0.2^{f}	2.29^{ef}	2.84^{efg}	65.25^{cd}	76.5^{cd}	0.81^{ef}	0.99^{efg}
Nitroxin + $N_{80}P_{40}$	0.3^{c}	0.24^{d}	2.99^{bcde}	4.04^{bc}	69.75^{bc}	84.75^{bc}	1.03^{bcd}	1.31^{cd}
Nitroxin + $N_{80}P_{80}$	0.331^{ab}	0.308^{b}	3.81^{ab}	4.6^{b}	75.25^{ab}	88.25^{ab}	1.14^{abc}	1.47^{bc}
Biophosphorus	0.17^{j}	0.081^{kl}	1.75^{gh}	2.2^{gh}	47.5^{hi}	55.75^{hi}	0.59^{gh}	0.73^{ghi}
Biophosphorus + $N_{40}P_{40}$	0.2^{hi}	0.15^{h}	2.25^{ef}	2.47^{fgh}	56^{fg}	65.5^{fg}	0.76^{efg}	0.87^{fgh}
Biophosphorus + $N_{80}P_{40}$	0.26^{de}	0.22^{e}	2.61^{def}	3.07^{def}	56.5^{fg}	66.25^{efg}	0.85^{def}	0.96^{efgh}
Biophosphorus + $N_{80}P_{80}$	0.27^{d}	0.26^{c}	3.1^{bcde}	3.52^{cde}	64.25^{cde}	74.25^{cde}	0.95^{cde}	1.07^{de}
Vermicompost	0.21^{gh}	0.1^{j}	2.43^{def}	2.99^{defg}	58.25^{ef}	68.75^{def}	0.71^{ghf}	0.95^{efgh}
Vermicompost + $N_{40}P_{40}$	0.27^{d}	0.25^{d}	3.22^{bcd}	4.05^{bc}	65.75^{cd}	76.5^{cd}	0.96^{cde}	1.17^{ef}
Vermicompost + $N_{80}P_{40}$	0.31^{bc}	0.27^{c}	3.54^{abc}	4.71^{ab}	75.25^{ab}	88^{ab}	1.21^{ab}	1.72^{ab}
Vermicompost + $N_{80}P_{80}$	0.335^{a}	0.328^{a}	4.25^{a}	5.49^{a}	81^{a}	95^{a}	1.34^{a}	1.92^{a}

Table 4. *Continued.*

	Dry leaf weight – Pre flowering	Dry leaf weight – Post flowering	Protein	Chlorophyll a	Chlorophyll b	Total chlorophyll	Stem height – Pre flowering	Stem height – Post flowering
Control	1.79^{g}	2.34^{l}	4.08^{l}	1.74^{b}	0.67^{f}	2.4^{j}	26.62^{jk}	29.27^{j}
$N_{40}P_{40}$	2.34^{fg}	2.47^{kl}	4.42^{kl}	1.84^{gf}	0.73^{ef}	2.56^{ij}	26.42^{ij}	33.35^{hi}
$N_{80}P_{40}$	3.22^{cdef}	3.17^{ijk}	4.93^{ijk}	1.94^{ef}	0.81^{def}	2.73^{gh}	28.97^{h}	36.5^{fg}
$N_{80}P_{80}$	3.62^{cde}	4.55^{ef}	5.43^{ghij}	2.02^{de}	0.75^{ef}	285^{fg}	32.15^{f}	41.07^{e}
Nitroxin	2.43^{fg}	3.21^{hijk}	5.02^{hij}	1.88^{fg}	0.74^{ef}	2.63^{hi}	26.72^{ij}	33.67^{hi}
Nitroxin + $N_{40}P_{40}$	3.1^{def}	3.84^{fghi}	5.99^{efg}	2.15^{c}	0.87^{cdef}	2.96^{ef}	30.55^{g}	38.32^{f}
Nitroxin + $N_{80}P_{40}$	4.13^{bcd}	5.36^{cd}	7.04^{cd}	2.31^{b}	1.01^{bcd}	3.25^{cd}	35.95^{cd}	45.31^{bc}
Nitroxin + $N_{80}P_{80}$	4.95^{ab}	6.08^{bc}	7.92^{bc}	2.45^{a}	1.05^{bc}	3.66^{b}	37^{bc}	46.57^{b}
Biophosphorus	2.34^{fg}	3.17^{ijk}	4.56^{ijkl}	1.78^{gh}	0.74^{ef}	2.48^{ij}	24.65^{k}	31.75^{i}
Biophosphorus + $N_{40}P_{40}$	3.01^{ef}	3.35^{ghij}	5.14^{ghijk}	1.87^{fg}	0.85^{cdef}	2.72^{gh}	27.17^{i}	34.95^{gh}
Biophosphorus + $N_{80}P_{40}$	3.46^{cde}	4.04^{fg}	5.86^{efgh}	2.09^{cd}	0.86^{cdef}	2.96^{ef}	29.67^{gh}	37.5^{f}
Biophosphorus + $N_{80}P_{80}$	4.06^{bcd}	4.59^{def}	6.43^{def}	2.34^{b}	0.89^{cde}	3.28^{c}	34.5^{de}	43.65^{cd}
Vermicompost	3.14^{def}	3.94^{fgh}	5.56^{fghi}	2.04^{de}	0.81^{def}	2.82^{fg}	29.37^{gh}	37.12^{f}
Vermicompost + $N_{40}P_{40}$	4.18^{bc}	5.22^{de}	6.53^{de}	2.09^{cd}	1.12^{ab}	3.11^{de}	33.67^{e}	42.42^{de}
Vermicompost + $N_{80}P_{40}$	4.75^{ab}	6.43^{b}	8.16^{b}	2.45^{a}	1.32^{a}	3.75^{ab}	37.77^{ab}	47.1^{b}
Vermicompost + $N_{80}P_{80}$	5.59^{a}	7.41^{a}	9.45^{a}	2.5^{a}	1.16^{ab}	3.84^{a}	39.1^{a}	49.97^{a}

Table 4. *Continued.*

	Number of Lateral Stems - Pre flowering	Number of Lateral Stems - Post flowering	Stem Water Content – Pre flowering	Stem Water Content – Post flowering	Fresh Stem Weight – Pre flowering	Fresh Stem Weight – Post flowering	Dry Stem Weight – Pre flowering	Dry Stem Weight – Post flowering
Control	1.75^{i}	19^{i}	0.11^{i}	0.13^{k}	0.18^{j}	0.14^{j}	0.05^{j}	0.034^{k}
$N_{40}P_{40}$	3.25^{hi}	18.25^{i}	0.15^{hi}	0.18^{ijk}	0.28^{ij}	0.22^{hij}	0.092^{h}	0.06^{j}
$N_{80}P_{40}$	5^{gh}	25.5^{gh}	0.26^{gh}	0.34^{fgh}	0.42^{fg}	0.36^{fg}	0.12^{fg}	0.09^{gh}
$N_{80}P_{80}$	5.75^{efg}	27^{efgh}	0.37^{de}	0.41^{def}	0.55^{de}	0.49^{d}	0.14^{e}	0.11^{ef}
Nitroxin	5^{gh}	25.75^{gh}	0.17^{hi}	0.21^{ijk}	0.3^{hi}	0.24^{hi}	0.095^{h}	0.066^{ij}
Nitroxin + $N_{40}P_{40}$	6.25^{defg}	25.75^{gh}	0.27^{f}	0.33^{fgh}	0.47^{ef}	0.38^{ef}	0.13^{ef}	0.1^{fg}
Nitroxin + $N_{80}P_{40}$	8^{cd}	31^{def}	0.44^{cd}	0.54^{bc}	0.72^{c}	0.6^{c}	0.18^{d}	0.16^{c}
Nitroxin + $N_{80}P_{80}$	10.75^{ab}	37.5^{bc}	0.56^{ab}	0.62^{ab}	0.82^{b}	0.75^{ab}	0.2^{bc}	0.18^{b}
Biophosphorus	3.5^{hi}	19.25^{i}	0.13^{hi}	0.21^{ijk}	0.28^{ij}	0.18^{ij}	0.07^{i}	0.05^{j}
Biophosphorus + $N_{40}P_{40}$	5.75^{efg}	25.25^{h}	0.17^{hi}	0.27^{hij}	0.39^{fgh}	0.25^{hi}	0.12^{fg}	0.08^{hi}
Biophosphorus + $N_{80}P_{40}$	7.25^{def}	30.5^{defg}	0.33^{de}	0.38^{efg}	0.53^{e}	0.45^{de}	0.15^{e}	0.12^{de}
Biophosphorus + $N_{80}P_{80}$	7.25^{def}	31.75^{de}	0.37^{de}	0.48^{cd}	0.66^{c}	0.52^{cd}	0.18^{d}	0.14^{cd}
Vermicompost	5.25^{fgh}	27^{efgh}	0.19^{gh}	0.24^{hij}	0.35^{ghi}	0.28^{gh}	0.11^{gh}	0.08^{hi}
Vermicompost + $N_{40}P_{40}$	7.75^{cde}	35^{cd}	0.37^{de}	0.44^{de}	0.64^{cd}	0.51^{d}	0.19^{cd}	0.13^{de}
Vermicompost + $N_{80}P_{40}$	9.75^{bc}	41^{b}	0.49^{bc}	0.61^{ab}	0.83^{b}	0.68^{b}	0.21^{b}	0.19^{b}
Vermicompost + $N_{80}P_{80}$	12^{a}	47.25^{a}	0.59^{a}	0.66^{a}	0.94^{a}	0.8^{a}	0.27^{a}	0.21^{a}

Trait Correlation

As all traits exhibited significant positive correlations with each other, the magnitude of the correlation coefficient between two traits reflected the strength of the association between them (Table 5).

Artemisinin (pre-flowering) demonstrated the strongest correlations with artemisinin (post-flowering), stem height (pre-flowering), and stem dry weight (post-flowering). Artemisinin (post-flowering) exhibited the highest correlations with chlorophyll b, stem fresh weight (pre-flowering), stem fresh weight (post-flowering), stem dry weight (pre-flowering), and stem dry weight (post-flowering). Leaf water content (pre-flowering) displayed the strongest positive correlation with leaf dry weight (pre-flowering), while leaf water content (post-flowering) exhibited the highest correlation with leaf dry weight (post-flowering) compared to other traits (Table 5).

Number of leaves (pre-flowering) showed the strongest correlation with number of leaves (post-flowering); number of leaves (post-flowering) exhibited the highest correlation with stem height (post-flowering); leaf fresh weight (pre-flowering) demonstrated the strongest correlation with leaf dry weight (post-flowering); leaf fresh weight (post-flowering) exhibited the highest correlations with protein, stem fresh weight (post-flowering), stem dry weight (pre-flowering), stem dry weight (post-flowering), leaf dry weight (pre-flowering) with leaf dry weight (post-flowering), protein, number of lateral branches (pre-flowering), stem dry weight (pre-flowering), and stem dry weight (post-flowering); and leaf dry weight (post-flowering) exhibited the strongest correlation with stem dry weight (pre-flowering) (Table 5).

In the analysis of protein correlations, protein displayed the strongest correlation with chlorophyll a. Chlorophyll a and chlorophyll b exhibited the strongest correlations with chlorophyll a; chlorophyll a with stem height (pre-flowering), stem dry weight (pre-flowering), and stem dry weight (post-flowering); stem height (pre-flowering) with stem fresh weight (pre-flowering), stem fresh weight (post-flowering), stem dry weight (pre-flowering), and stem dry weight (post-flowering); stem height (post-flowering) with stem dry weight (post-flowering); number of lateral branches (pre-flowering) with number of lateral branches (post-flowering); number of lateral branches (post-flowering) with stem dry weight (post-flowering); stem water content (pre-flowering) with stem fresh weight (post-flowering); stem water content (post-flowering) with stem fresh weight (pre-flowering); leaf fresh weight (pre-flowering) with leaf fresh weight (post-flowering) and stem dry weight (post-flowering); and leaf fresh weight (post-flowering) with stem dry weight (post-flowering) (Table 5).

Table 5. Correlation analysis of evaluated traits in the experiment.

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
C1	1											
C2	0.931**	1										
C3	0.712**	0.737**	1									
C4	0.839**	0.792**	0.724**	1								
C5	0.801**	0.791**	0.741**	0.814**	1							
C6	0.799**	0.788**	0.731**	0.809**	0.997**	1						
C7	0.819**	0.793**	0.647**	0.844**	0.701**	0.692**	1					
C8	0.75**	0.731**	0.734**	0.691**	0.774**	0.774**	0.712**	1				
C9	0.782**	0.798**	0.984**	0.798**	0.78**	0.77**	0.769**	0.777**	1			
C10	0.869**	0.827**	0.774**	0.979**	0.856**	0.852**	0.863**	0.821**	0.848**	1		
C11	0.835**	0.806**	0.803**	0.834**	0.825**	0.821**	0.795**	0.848**	0.853**	0.892**	1	
C12	0.906**	0.888**	0.708**	0.786**	0.782**	0.778**	0.796**	0.797**	0.774**	084**	0.856**	1

Table 5. *Continued.*

	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24
C13	1											
C14	0.741**	1										
C15	0.688**	0.924**	1									
C16	0.659**	0.831**	0.841**	1								
C17	0.566**	0.819**	0.827**	0.767**	1							
C18	0.657**	0.868**	0.838**	0.798**	0.853**	1						
C19	0.623**	0.878**	0.898**	0.844**	0.784**	0.81**	1					
C20	0.622**	0.876**	0.898**	0.832**	0.809**	0.819**	0.96**	1				
C21	0.654**	0.903**	0.92**	0.858**	0.834**	0.851**	0.965**	0.993	1			
C22	0.64**	0.897**	0.914**	0.856**	0.805**	0.83**	0.997**	0.968**	0.976**	1		
C23	0.697**	0.914**	0.913**	0.871**	0.843**	0.881**	0.904**	0.897**	0.941**	0.924**	1	
C24	0.669**	0.928**	0.93**	0.864**	0.839**	0.863**	0.954**	0.959**	0.977**	0.974**	0.953**	1

(C1:, C2:, C3:, C4: Leaf Water Content – Post flowering, C5: Leaf Number – Pre flowering, C6: Leaf Number – Post flowering, C7: Fresh Leaf Weight – Pre flowering, C8: Fresh Leaf Weight – Post flowering, C9: Dry Leaf Weight – Pre flowering, C10: Dry Leaf Weight – Post flowering, C11: Protein, C12: Chlorophyll a, C13: Chlorophyll b, C14: Total Chlorophyll, C15: Stem Height – Pre flowering, C16: Stem Height – Post flowering, C17: Number of Lateral Stems - Pre flowering, C18: Number of Lateral Stems - Post flowering, C19: Stem Water Content – Pre flowering, C20: Stem Water Content – Post flowering, C21: Fresh Stem Weight – Pre flowering, C22: Fresh Stem Weight – Post flowering, C23: Dry Stem Weight – Pre flowering, C24: Dry Stem Weight – Post flowering)

Correlation plots were employed to further investigate the relationships between traits. In these plots, the smaller the angle between traits vectors, the stronger the correlation between the corresponding traits. The cosine of the angle between vectors represents the correlation coefficient. A positive correlation is indicated by an angle between vectors smaller than 90 degrees. A 90-degree angle between vectors implies no correlation between genotypes, signifying their independence. Conversely, an angle larger than 90 degrees between vectors indicates a negative correlation between the corresponding genotypes (Ghasemi et al., 2021; Khatamain et al., 2011).

The correlation plot derived from the experimental data also revealed positive correlations among all traits. The results obtained from the correlation plot were consistent with those presented in the correlation coefficient table. According to the correlation plot, the correlation between the traits "number of leaves (post-flowering)" and "number of leaves (pre-flowering)" with the trait "number of lateral branches (post-flowering)" was relatively weaker compared to other trait pairs (Fig. 2).

Graphical Analysis

Biplot polygons are a crucial feature of the biplot method for identifying the best treatment for evaluated traits. The polygon observed in this plot is formed by connecting the treatments that are farthest from the origin, such that all other treatments lie within this polygon. Perpendicular vectors are then drawn from the origin to the edges of this polygon, representing the interactions between treatments and traits (Yan et al., 2000).

The first principal component accounted for over 97% of the data variance, according to the constructed biplot polygon, while the second principal component explained nearly 2%. Based on this plot, treatments tr16, tr8, tr7, tr6, tr9, tr2, tr5, and tr11 exhibited superior performance compared to the other treatments.

Within each section, treatment tr16 demonstrated the highest desirability for the trait "number of lateral branches (post-flowering)," while treatment tr8 exhibited the highest desirability for the traits "stem height (post-flowering)," "number of leaf (pre-flowering)," and "number of leaves (post-flowering)" compared to the other treatments. Treatments that fell on the origin of the plot, such as treatment tr12, displayed no significant response to changes in performance (Fig. 3a).

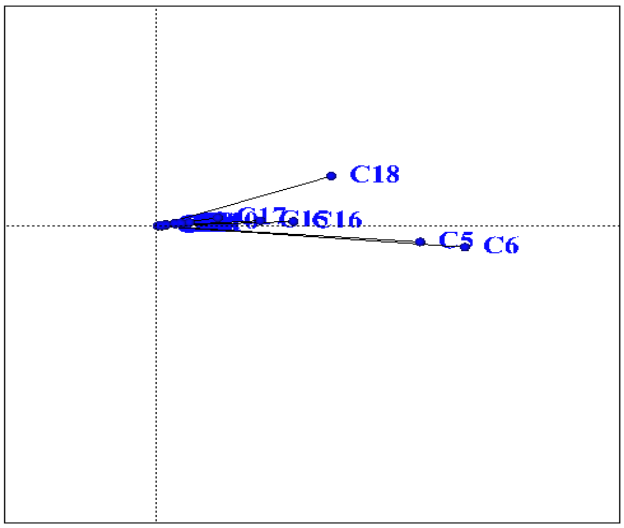


Fig. 2. Correlation plot of evaluated traits in the experiment.

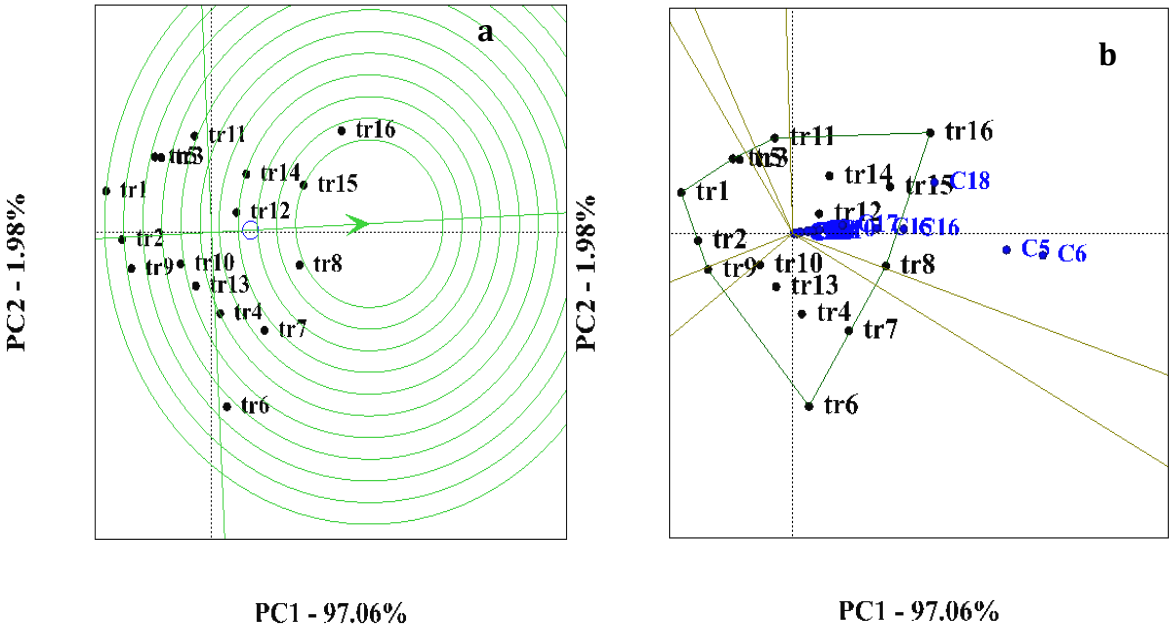


Fig. 3. Graphical analysis for selecting the most suitable treatment based on evaluated traits in the experiment: a) Biplot Polygon, b) Ideal Treatment Selection Plot.
(C1: Artemisinin – pre flowering, C2: Artemisinin – post flowering, C3: Leaf water content - before flowering, C4: Leaf Water Content – Post flowering, C5: Leaf Number – Pre flowering, C6: Leaf Number – Post flowering, C7: Fresh Leaf Weight – Pre flowering, C8: Fresh Leaf Weight – Post flowering, C9: Dry Leaf Weight – Pre flowering, C10: Dry Leaf Weight – Post flowering, C11: Protein, C12: Chlorophyll a, C13: Chlorophyll b, C14: Total Chlorophyll, C15: Stem Height – Pre flowering, C16: Stem Height – Post flowering, C17: Number of Lateral Stems - Pre flowering, C18: Number of Lateral Stems - Post flowering, C19: Stem Water Content – Pre flowering, C20: Stem Water Content – Post flowering, C21: Fresh Stem Weight – Pre flowering, C22: Fresh Stem Weight – Post flowering, C23: Dry Stem Weight – Pre flowering, C24: Dry Stem Weight – Post flowering.

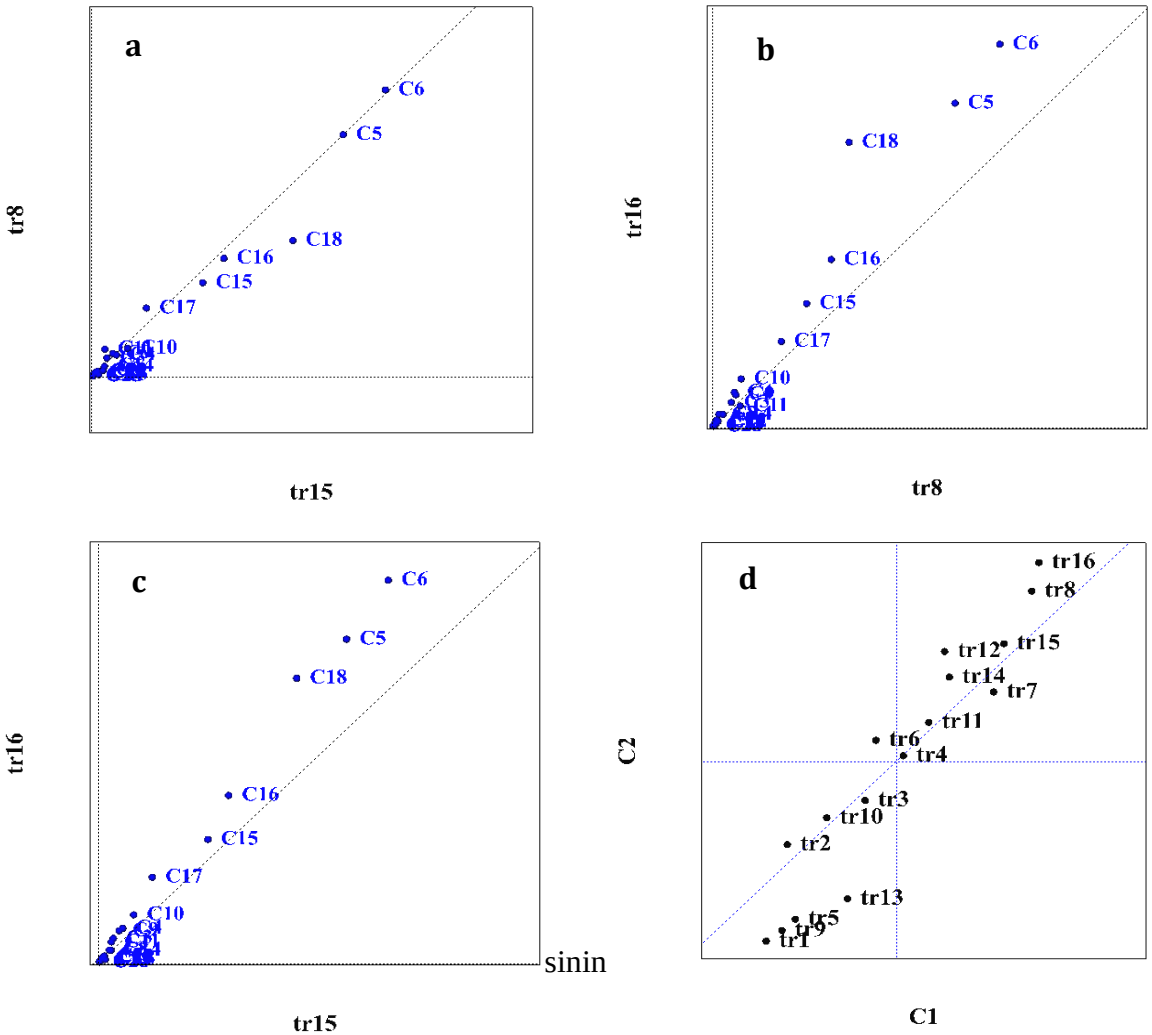
Based on the ideal treatment method the ranking plot connects a line from the origin of the plot to the mean point and extends it in both directions. The best treatment is the one that tends towards the positive end and has a smaller vertical distance from this line. In this figure, the best point is the center of the concentric circles, marked with an arrow, and the other treatments are ranked based on this point (Shojaei et al., 2022). According to this plot, the most desirable treatments based on the ideal treatment were tr15, tr8, tr16, tr14, and tr12 compared to the other treatments.

Treatments tr1, tr2, and tr9 were identified as undesirable treatments. The order of treatments from desirable to undesirable is as follows (Fig. 3b):

$tr_{15} > tr_8 > tr_{16} > tr_{14} > tr_{12} > tr_7 > tr_4 > tr_{13} > tr_{10} > tr_{11} > tr_{15} > tr_3 > tr_5 > tr_6 > tr_9 > tr_2 > tr_1$.

Based on the graphical analysis (Fig. 3), treatments tr15, tr8, and tr16 were identified as desirable treatments. Regarding the results obtained from this analysis, it showed complete agreement with the interaction effect chart (Fig. 1a and 1b), and in terms of the tr16 treatment, a high degree of similarity was also observed with the interaction effect mean comparison analysis (Table 4).

Various researchers have used treatment stability charts and the assessment of treatments based on the ideal treatment to investigate the stability of their genotypes and treatments. This includes research conducted on wheat (Omrani et al., 2024), maize (Khatibi et al., 2023; Shojaei et al., 2023a), and oilseed plants like canola (Shojaei et al., 2023b).



sinin

Fig. 4. Scatter Plot Analysis of Superior Treatments and Traits. a: Comparison of Treatments tr15 and tr8, b: Comparison of Treatments tr8 and tr16. c: Comparison of Treatments tr15 and tr16, d: Comparison of Traits C1 (Artemisinin (pre-flowering)) and. C2 (Artemisinin (post-flowering)). (tr_1: Control; tr_2: $N_{40}P_{40}$; tr_3: $N_{80}P_{40}$; tr_4: $N_{80}P_{80}$; tr_5: Nitroxin; tr_6: Nitroxin + $N_{40}P_{40}$; tr_7: Nitroxin + $N_{80}P_{40}$; tr_8: Nitroxin + $N_{80}P_{80}$; tr_9: Biophosphorus; tr_{10}: Biophosphorus + $N_{40}P_{40}$; tr_{11}: Biophosphorus + $N_{80}P_{40}$; tr_{12}: Biophosphorus + $N_{80}P_{80}$; tr_{13}: Vermicompost; tr_{14}: Vermicompost + $N_{40}P_{40}$; tr_{15}: Vermicompost + $N_{80}P_{40}$; tr_{16}: Vermicompost + $N_{80}P_{80}$)

Additionally, considering traits Artemisinin (pre-flowering) and Artemisinin (post-flowering) as the most critical traits in this experiment, with their enhancement being of paramount importance, scatter plot analysis was employed to investigate the separation of traits based on superior treatments and the separation of treatments based on key traits (Fig. 4).

According to the separation of traits based on treatments tr15 and tr8, traits stem height (pre-flowering), stem height (post-flowering), and number of lateral stems (post flowering) exhibited superior performance in treatment tr15, while the remaining traits demonstrated higher performance in treatment tr8 (Fig. 4a).

In the comparison of treatments tr8 and tr16, traits protein and dry stem weight (pre-flowering) showed superior performance in treatment tr8, while the remaining traits displayed higher performance in treatment tr16 (Fig. 4b). Furthermore, when comparing treatments tr16 and tr15, approximately 99% of the traits exhibited higher performance and desirability in treatment tr16 (Fig. 4c).

Upon examining the scatter plot comparing the two key traits artemisinin (pre-flowering) and artemisinin (post-flowering), treatments tr16, tr8, tr15, tr12, tr11, tr6, tr10, and tr2 exhibited superior desirability for trait artemisinin (post-flowering), while the remaining treatments demonstrated high performance and desirability for trait artemisinin (pre-flowering). Treatment tr4, located on the average axis of the plot, was used as the intermediate treatment (Fig. 4d).

Based on the evaluation of the treatments, it can be concluded that the application of N40P40 and vermicompost can significantly enhance trait artemisinin (post-flowering) performance, while the effects of nitroxin can be highly effective in increasing trait Artemisinin (pre-flowering) performance.

In the comparison between tr16 and tr15 treatments, it is noteworthy that 99% of the traits exhibit suitable performance efficiency in the tr16 treatment, which indicates the superiority of the tr16 treatment.

CONCLUSION

Artemisinin, a vital metabolite of *Artemisia annua* L., plays a crucial role in malaria treatment. Consequently, enhancing both the quantity and quality of this compound through optimized agricultural practices is of paramount importance for cultivating this valuable medicinal plant. This study aimed to investigate the effects of biological and chemical fertilizers on the growth and artemisinin content of *Artemisia annua* L. The results demonstrated that the interaction between different fertilizer treatments significantly affected most of the evaluated traits. Mean comparison analysis of the interaction effects using the LSD test identified treatment tr16 as the superior treatment. This treatment, along with tr8, emerged as the most favorable options for increasing artemisinin levels, exhibiting a strong correlation among the assessed traits. Notably, a comparison between treatments tr16 and tr15 revealed that 99% of the traits in tr16 exhibited higher performance efficiency, clearly confirming the superiority of tr16. Therefore, it can be concluded that treatment tr16, as the most effective treatment for enhancing both growth and artemisinin content in *Artemisia annua* L., can be recommended for implementation in cultivation programs of this medicinal plant.

Conflict of interest

The authors declare no competing interests.

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