Soil Environ. 45(2): xx-xx, 2026
DOI: doi.org/10.25252/SE/2026/254073
Online ISSN: 2075-1141
Print ISSN: 2074-9546

Biostimulant mediated enhancement of photosynthetic efficiency, nutritional quality, phytochemical composition, antioxidant activity, and soil characteristics in *Thymus vulgaris*

Abida Parveen[1], Adnan Younis[2*], Farooq Ahmad[1] and Ahsan Akram[2]
[1]Department of Botany, University of Agriculture, Faisalabad, Pakistan
[2]Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan
[Received:** April 21, 2026 **Accepted:** June 10, 2026 **Published Online:** June 22, 2026**]

Abstract

Thymus vulgaris L. is an aromatic plant that improves nutrient use efficiency and plant development. Research was conducted to evaluate the effect of biostimulants on photosynthetic, nutritional, phytochemical, gas exchange, antioxidant properties of thyme and different analyses of soil. Different concentrations of moringa leaf extract (MLE) variety name Moringa oleifera, brassica water extract (BWE) variety Brassica napus, and sorghum water extract (SWE) sorghum variety locally known as jawar (1%, 2%, and 3%) of each were used. These treatments were applied three times after 15 days interval and data were collected. Randomized complete block design (RCBD) was used to organize the experiment. The physiological, biochemical, antioxidant, and soil characteristics of thyme were significantly enhanced by the application of biostimulants. Chlorophyll, carotenoids, proteins, flavonoids, anthocyanin, antioxidant enzyme activities, and soil nutrient concentrations were all significantly increased by MLE at 3% concentration, which had the most noticeable impacts of all the treatments. In the same way, as compared to the control treatment, BWE and SWE at 3% concentrations enhanced plant growth characteristics, phytochemical components, antioxidant activities, and soil nutrient parameters. Overall, the results show that natural biostimulants have a good impact on soil health and plant performance, with MLE showing the greatest efficacy. It is concluded that 3% MLE, BWE, SWE significantly enhanced the photosynthetic, nutritional, phytochemical and antioxidant properties of the Thymus vulgaris plant and improved analyses of soil. Future scope recommended exploring their potential integration into sustainable agriculture practices for enhanced crop productivity and soil fertility.

Keywords: Thyme, Biostimulants, Photosynthetic, Nutritional, Phytochemical, Antioxidants

Introduction

Thyme (*Thymus vulgaris* L.) is under the family Lamiaceae. It is a perennial aromatic plant regarded as an essential oil and a high value in the market in the medical, cosmetics, and culinary dishes. It is grown primarily in the Mediterranean and is an economically worthwhile plant whose distribution is worldwide. The increasing global demand for natural products, herbal, medicines and plant-based additives has further enhanced its economic significance, making it one of the most important aromatic and medicinal plants in international market (Hosseinzadeh *et al*., 2015). Thyme is common in the food and baking industries. It is widely applied medically because of its antiseptic, antipyretic, analgesic, bactericidal, carminative, antidepressant, and astringent effects (Zairi *et al*., 2020). Moreover, research shows that thyme essential oil can be a potentially appropriate biofuel, thus it can be possible that thyme is a source of energy vegetable (Gupta and Mishra, 2021).

In South Europe, South Asia, North Africa, and the Mediterranean, *Thymus vulgaris* L. is a perennial herb that grows well in the mint family. In South Asia, *Thymus vulgaris* is cultivated and utilized for its medicinal, culinary and essential oil applications(Gupta *et al*., 2024). Thyme herb has been categorized and labeled as a traditional herbal product by European Medicine Agency (EMA) as well as a useful raw material by European Pharmacopoeia (EP).

It has about 2.5 essential oils, which are considered to be in the top 10 in the world. According to the EP, it should

***Email:** adnanyounis@uaf.edu.pk
Cite This Paper: Parveen, A., A. Younis, F. Ahmad and A. Akram. 2026. Biostimulant mediated enhancement of photosynthetic efficiency, nutritional quality, phytochemical composition, antioxidant activity, and soil characteristics in *thymus vulgaris*. *Soil Environ*. 45(1): xx-xx.

have at least 1.2 essential oils (Kosakowska *et al*., 2021). The traditional medicine has been in use as a remedy against rheumatism, arthritis, cough, sore throat, and bronchitis (Horváth and Acs, 2015).

Due to its pleasant fragrance and the presence of important pharmacological compounds in its aerial parts, the plant is widely used in various applications, including herbal tea preparation, food flavouring, and as an active ingredient in several pharmaceutical products (Faghihi *et al*., 2025). Growing substrates are known as plant biostimulants because they have the capacity to alter physiological processes in plants that can be advantageous to their growth, development, stress response, and nutrition efficiency in order to provide organic food (Halpern *et al.*, 2015).

Moringa oleifera L. is one of the most widely used biostimulants that contains loads of vital mineral elements, antioxidants, secondary metabolites and cytokinins, making the leaves of the plant extremely therapeutic and nutritional (Elzaawely *et al*., 2017). MLE extract enhances growth, and biochemical and productivity characteristics of vegetation by enhancing the photosynthetic performance besides greater biomass deposition. MLE increased the growth and production of other crops. It caused the amplification of morphological features and cell division of plants through amplification of photosynthetic activities (Yasmeen *et al*., 2013). Moringa leaf extract (MLE) is the miracle substance that consists of zeatin as a natural growth hormone of plants, and some other micronutrients that are engaged in different important processes in plant physiology (Jahan, 2020). Along with other growth-promoting substances like ascorbates, phenolics, and minerals, MLE is also known to contain adequate concentration of cytokinin and gibberellic acid which are essential phytohormones, among others (Jahan, 2020).

Another excellent source of phytochemical with strong antioxidant potential is phytochemical compounds (Batool et al., 2022; Haider et al., 2025). Moreover, it improves the biochemical and physiological mechanisms of the plant development and facilitates secondary metabolites in plants (Ali *et al*., 2019; Hussain *et al*., 2019). It allows increased absorption of the plant through BWE and stimulation of seed germination and enzyme reactions. These enhancements enable the plants to withstand harsh climatic conditions better (Biju *et al*., 2017). This is the most abundant steroid known as brassinolide which is present in brassica (Rys *et al*., 2022). Brassinosteroids are natural plant growth regulators that can be used to improve crop yield in various rice and wheat crops (Siddiqi, 2021).

Sorghum (*Sorghum bicolor*) has natural ingredients that help in the growth of healthy and functional foods (Espitia *et al*., 2022). The leaves of sorghum contain many nutrients that supplement the biochemical activities and yield of plant (Kumari *et al*., 2021). It contains many antioxidants such as flavonoid, phenolic acid, and tannin that enhance the photosynthetic activity, biomass, chlorophyll and proline levels, and yield of various crops (Ibrahim *et al*., 2022). Although chemical fertilizer application is a critical aspect of plant production which can significantly improve plant growth and development, its input in terms of cost and environmental and human health is crucial. As a way to reduce the effects of inorganic fertilizer, it is crucial to look for safe, sustainable, and eco-friendly approaches natural biostimulants are also an alternative.

Furthermore, there is dire need to investigate biostimulants derived from plant sources which emphasize the growing imperative to reduce the adverse environmental impacts associated with chemical fertilizers**.** Biostimulants are inexpensive, environmentally friendly, and easily available.

A gap exists in understanding how biostimulants alter the physiological, nutritional and phytochemical properties of thyme. Therefore, we hypothesized that foliar application of biostimulants including moringa leaf extract, brassica water extract, and sorghum water extract can enhance the morpho-anatomical, physiological, nutritional and phytochemical properties of thyme. The main objectives of this research were to evaluate the effectiveness of MLE, BWE, and SWE as biostimulants in thyme and assess whether these plant extracts can serve as potential alternatives to synthetic fertilizers and to investigate the impact of these biostimulants on the growth, development, and overall performance of thyme plants.

Material and Methods

This field research was conducted at Gardening Wing, Lalazar Nursery, UAF which is located at 31° 25' 7.3740" N and 73° 4' 44.7924" E in Faisalabad, Pakistan. Plant nursery of thyme was acquired from a local nursery at Faisalabad. The media used in the experiment consisted of garden soil. Plant based biostimulants were prepared by using different protocols. Treatments were repeated three times at 15 days interval after transplanting of nursery plant in the field. Data was collected after 45 days of last application for determination of photosynthetic, nutritional, phytochemical, antioxidant attributes and after harvesting, soil analyses were also conducted . This experiment was conducted using randomized complete block design (RCBD) having ten treatments including control and different concentrations (1%, 2%, and 3%) of MLE, BWE and SWE. Foliar application of biostimulants was done with following treatment plan; T_0 = Control, T_1 =1% of MLE, T_2 =2% of MLE, T_3 = 3% of MLE, T_4 = 1% of BWE, T_5 = 2% of BWE, T_6 = 3% of BWE, T_7 = 1% of SWE, T_8 = 2% of SWE, T_9 = 3% of SWE.

Preparation of moringa leaf extract

Moringa leaf extract was produced by extracting fresh leaves from fully grown moringa trees. The harvested leaves were stored in an ice bath overnight before being pressed using a locally made extraction device and filtered twice with

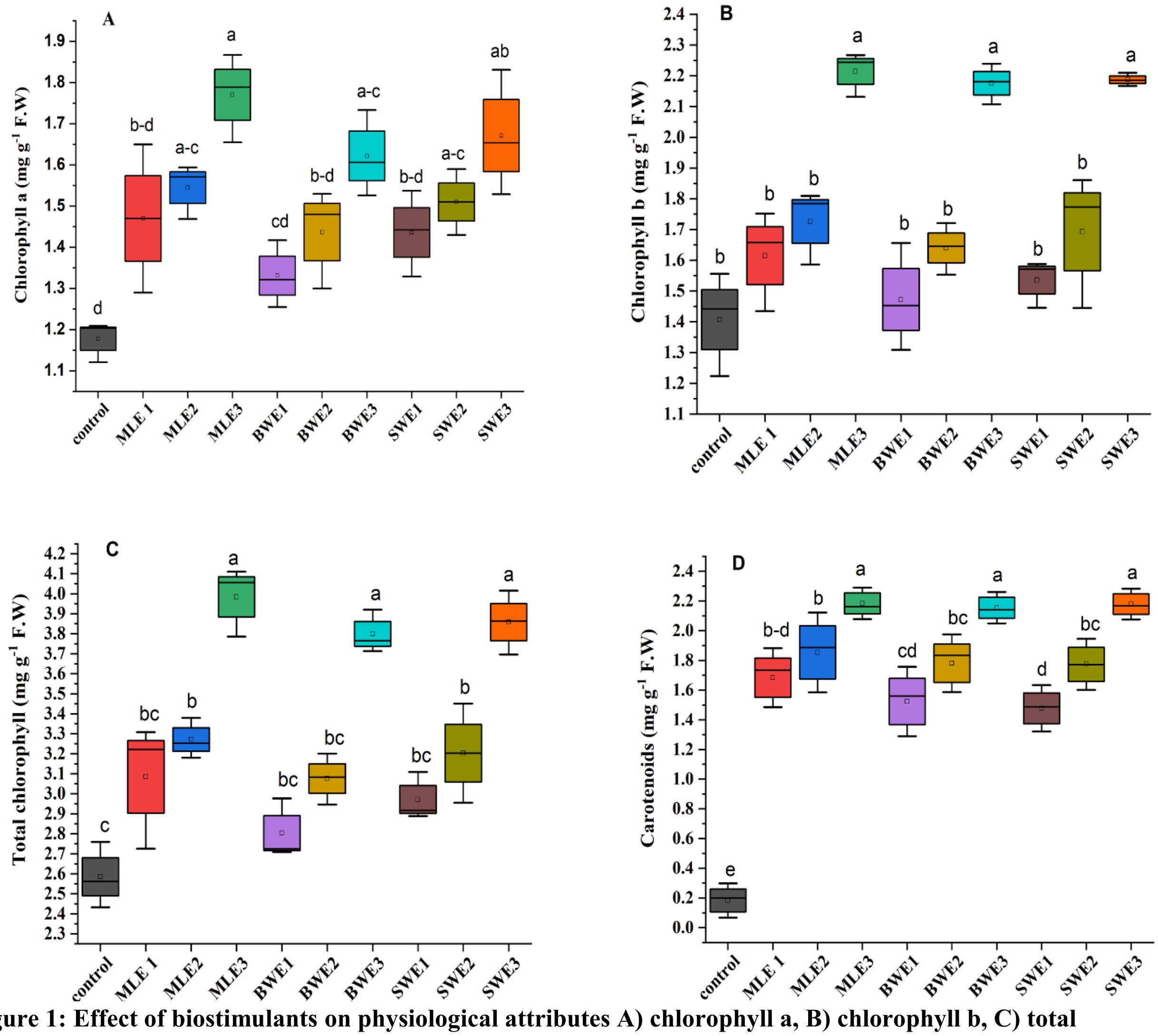

Figure 1: Effect of biostimulants on physiological attributes A) chlorophyll a, B) chlorophyll b, C) total chlorophyll and D) carotenoids content of *Thymus vulgaris.* Control (No application), moringa leaf extract (1% of MLE1), moringa leaf extract (2% of MLE2), moringa leaf extract (3% of MLE3), brassica water extract (1% of BWE1), brassica water extract (2% of BWE2), brassica water extract (3% of BWE3), sorghum water extract (1% of SWE1), sorghum water extract (2% of SWE2) and sorghum water extract (3% of SWE3)

filter paper. The resulting filtrate was then centrifuged at 8000 × g for 15 minutes, and the supernatant was collected. This was labeled as 100% MLE and stored as a stock solution for creating various concentrations (1%, 2%, and 3%) of MLE (Yasmeen *et al*., 2013).

Preparation of the brassica water extract

The extract was prepared according to the protocol described by Khaliq *et al*. (2022). Initially, mature brassica plants (leaves and herbage) were harvested, chopped into 2–3 cm pieces using a fodder cutter, and shade-dried to prevent nutrient loss through rainwater leaching. The chopped plant material was then soaked in distilled water at a ratio of 1:10 (w/v) for 24 hours at room temperature. After soaking, the mixture was boiled at 100°C until its volume was reduced by approximately 95% to facilitate handling and application. The resulting extract was filtered and stored as a 100% brassica water extract stock solution. Different concentrations (1%, 2%, and 3%) were subsequently prepared from the stock solution for the respective treatments.

Preparation of sorghum water extract

Sorghum water extract was prepared following the method of Ibrahim *et al*. (2022). Mature sorghum herbage was chopped, air-dried, and soaked in distilled water (1:10 w/v) for 24 h at room temperature. The extract was then filtered and concentrated by boiling at 100°C to obtain a stock solution, which was subsequently diluted to prepare 1%, 2%, and 3% SWE treatments.

Photosynthetic pigments

Chlorophyll content *a*, *b*, total chlorophyll and carotenoids were determined by using the Ramzan *et al*. (2023) approach. To measure chlorophyll levels, we followed this procedure: Freshly chopped leaves (0.1 g) were immersed in 5 mL of 80% acetone and allowed to stay at room temperature (25°C) overnight. A spectrophotometer (Model: WE 721 WE Lab instrument limited) was then used to take readings at wavelengths of 663, 645, and 480 nm.

Chl. *a* (mg mL^{-1}) = [12.7 (OD 663) -2.69 (OD 645)] X [V/1000] X W

Chl. *b* (mg mL^{-1}) = [22.9 (OD 645) - 4.68 (OD 663)] X [V/1000] X W

Where, V= volume of the extract (mL), W= weight of the fresh leaves of tissue (g)

Carotenoids (mg g^{-1} f.wt.) = [A car / Em 100 %] x 100

Acar = [(OD 480) +0.114 (OD 663) -0.638 (OD 645)]

E 100% = 2500

Nutritional parameters

All nutritional parameters were determined according to the protocols of Balarabe (2019).

Crude protein

Four basic steps are involved in the process described: titration, distillation, dilution, and digestion. First, a sample of thyme was crushed, mixed with sulfuric acid and a digestion combination that included potassium sulfate, copper, and ferrous sulfate, and heated gradually to 400°C. Water was used to dilute the resulting digest. The digested sample was moved to a distillation flask and distilled using a solution of sodium hydroxide and boric acid. The distillation produced a golden solution when heated. This solution's color changed from dull golden to light pink when sulfuric acid was introduced in the titration step.

Following formula was used to measure each treatment's crude protein

C.P = 21.875*1/2

Crude fiber

Two gram (2 g) thyme sample was placed in beaker. 10% (25 mL) sulfuric acid was added followed by 200 mL water and placed on hot plate for 30 min for simmering. After boiling for 30 min it was filtered with cloth and the residue was transferred again into beaker and 10% NaOH solution was added, heated on hot plate and was distilled. Residue was transferred to a crucible and sample was set in oven until material became dry. Then the material was placed in desiccator to cool the crucible. At the end the crucible was weigh. The crucible was put in Muffle Furnace for weight of ash forming.Following formula was used to measure crude fibers for each treatment:

C. F %= weight of residue after oven – wt. of ash / wt. of sample*100

- where weight of oven-dried residue refers to the residue remaining after acid and alkali digestion and subsequent drying to a constant weight in an oven (g),
- weight of ash refers to the inorganic residue obtained after incinerating the oven-dried residue in a muffle furnace (g),
- weight of sample is the initial sample weight used for analysis (g).

Total ash

The total ash was determined by using method of Harris and Marshall (2017). Thyme sample was used and placed into crucible and weighted. The crucible was put in Muffle Furnace for ash forming. W1=Weight of empty crucible:

W2=Weight of sample

W3=Weight of crucible with ash

W3-W1/W2*100

Carbohydrates

Following formula was used to measure carbohydrates for each treatment

Carbohydrate (%) = 100 – (moisture + ash + Crude Fat + Crude Protein + Crude Fiber)

Phytochemical parameters

Total phenolics content

The total phenolic compounds were determined by adhering to the protocol developed by Singleton and Rossi (1965). using an IREMCO U2020 UV-Vis spectrophotometer at 765 nm, and the data were reported as milligrams of gallic acid equivalents per gram of extract.

Total flavonoids content

The measurement of flavonoid contents was conducted using Dowd's method, adapted by Arvouet-Grand *et al.* (1994) using a UV-Vis spectrophotometer (U2020, IREMCO) at 510 nm, and the results were expressed as mg of quercetin per gram of extract.

Anthocyanin

For anthocyanin content, the method of Stark and Wray (1989) using a spectrophotometer (U2020, IREMCO) at 535 nm.

Ascorbic acid (µg g^{1} f.wt)

The ascorbic acid content was measured by using Ramzan *et al.* (2025) protocol measuring absorbance at 530 nm on a UV-Vis spectrophotometer (U2020, IREMCO).

Physiological parameters

A portable IRGA (LI-6400) was used to measure transpiration rate (E) and stomatal conductance (gs) on completely expanded leaves between 09:00 and 11:30 AM under regulated CO_2 (400 ppm), light intensity (1500 µmol m^{-2} s^{-1}), and temperature (25°C). Following normal procedures, photosynthetic pigments were extracted in 80% acetone and measured spectrophotometrically at 663, 647, and 470 nm (Peres et al., 2025).

Moisture content of thyme was measured after sun drying.

Antioxidant attributes

Superoxide dismutase (Units mg^{-1} proteins)

Superoxide dismutase (SOD) was measured in accordance with Spitz and Oberley (2001). This measurement involved determining the point at which the photochemical reduction of NBT was inhibited by 50%. The assay was performed by combining 100 µL of plant extract with 500 µL of phosphate buffer (pH 5), 200 µL of methionine, 100 µL of riboflavin, and 800 µL of distilled water. After mixing, the solution was exposed to UV light for duration of 15 minutes. Subsequently, the absorbance was analyzed at 560 nm using a UV-Vis spectrophotometer (U2020, IREMCO).

Catalase (Units mg^{-1} proteins)

For determination of catalase activity, method described by Li *et al.* (2009) was followed , that involves taking 100 µL of plant extract and mixing with 100 µL H_2O_2 having 5.9 mM concentration. Absorbance was measured at 240 nm using a UV-Vis spectrophotometer (U2020, IREMCO).

Peroxidase dismutase (Units mg^{-1} proteins)

A 100 µL sample of plant extract was combined with a reaction mixture consisting of 800 µL of phosphate buffer (50 mM, pH 5), 100 µL of H_2O_2 (40 mM), and 100 µL of 20 mM guaiacol. Using a UV-Vis spectrophotometer (U2020, IREMCO), absorbance readings were measured at 470 nm (Chance and Maehly, 1955).

Ascorbate peroxidase (Units mg^{-1})

APX activities were assayed according to Nakano and Asada (1981), with some modifications. One gram of leaf tissue was ground using liquid nitrogen. Ascorbate peroxidase (APX) was then extracted by homogenizing the sample in 5 mL of a 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA, 1 mM ascorbic acid (AsA), and 50 mg PVP-10. The resulting extract was filtered through two layers of cheesecloth and centrifuged at 12,000 × g for 15 minutes. APX activity was subsequently measured spectrophotometrically at 290 nm by monitoring the hydrogen peroxide-dependent oxidation of ascorbic acid.

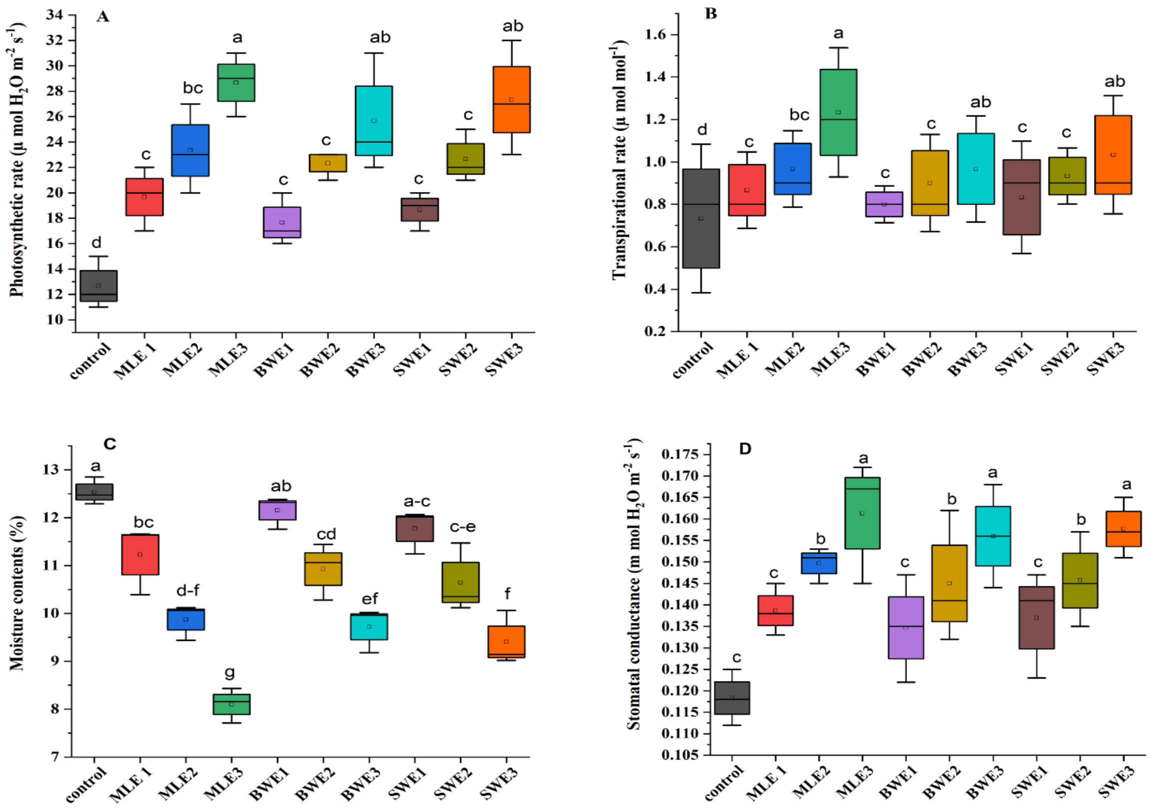

Figure 2: Effect of biostimulants on A) photosynthetic rate, B) transpiration rate, C) moisture contents and D) stomatal conductance of *Thymus vulgaris*. Control (No application), moringa leaf extract (1% of MLE1), moringa leaf extract (2% of MLE2), moringa leaf extract (3% of MLE3), brassica water extract (1% of BWE1), brassica water extract (2% of BWE2), brassica water extract (3% of BWE3), sorghum water extract (1% of SWE1), sorghum water extract (2% of SWE2) and sorghum water extract (3% of SWE3).

Soil analyses

After harvesting *Thymus vulgaris*, soil samples were taken from each plot at a depth of 0 to 15 cm, air-dried, powdered, and sieved (2 mm). EC and pH meters were used to measure the electrical conductivity (EC) and pH of a 1:5 soil water extract, respectively. The Olsen method was used to assess the amount of accessible phosphorus. Ammonium acetate extraction, flame photometry, and atomic absorption spectrophotometry were used to assess exchangeable potassium, calcium, and magnesium.

Nitrogen

The Kjeldahl digestion method was used to measure total nitrogen. Concentrated sulfuric acid is used to digest one gram of soil in the presence of a catalyst mixture until a clear digest is produced. Following digestion, the sample is diluted and moved to a distillation machine, where alkali is added to release ammonia. To determine the nitrogen concentration, the released ammonia is trapped in a boric acid solution and titrated with standard acid. The nitrogen is measured into mg g^{-1} soil Goyal *et al*., 2022).

Phosphorus

The Olsen method was used to assess the amount of accessible phosphorus. The AB-DTPA solution is frequently used to extract available phosphorus, and it works especially well in Pakistan's calcareous soils. Ten grams of soil and 20 mL of extractant solution are combined, agitated for fifteen minutes, and then filtered. A spectrophotometer (give make a model) was used to determine the phosphorus concentration when the filtrate reacts with a color-developing reagent. When determining plant-available phosphorus in agricultural soils, this technique is quite sensitive (Koralage *et al*., 2015).

Table 1: Effect of biostimulants on phytochemical attributes; anthocyanin, flavonoids and total phenolics, ascorbic acid and hydrogen peroxide (H_2O_2) of *Thymus vulgaris*

Treatment	Anthocyanin	Flavonoids	Total phenolics	Ascorbic acid
Control	0.149±0.014c	0.24±0.032e	0.042±0.008d	13±1.156d
MLE 1	0.177±0.01c	0.451±0.038cd	0.077±0.006cd	19.67±1.455c
MLE2	0.276±0.008b	0.614±0.04b	0.134±0.008ab	23.33±2.03bc
MLE3	0.361±0.008a	0.9±0.03a	0.17±0.007a	28.67±1.455a
BWE1	0.148±0.01c	0.367±0.026de	0.044±0.006d	17.67±1.203c
BWE2	0.245±0.009b	0.563±0.035bc	0.116±0.006bc	22.33±0.667c
BWE3	0.353±0.009a	0.865±0.02a	0.143±0.012ab	25.67±2.732ab
SWE1	0.157±0.009c	0.384±0.022de	0.053±0.011d	18.67±0.883c
SWE2	0.259±0.013b	0.582±0.026bc	0.128±0.01ab	22.67±1.203c
SWE3	0.334±0.007a	0.894±0.024a	0.155±0.009ab	27.33±2.607ab

Values (mean ± standard error), HSD = honestly significant difference; values sharing same case letter or without lettering for a parameter do not differ significantly ($p \leq 0.05$) by the HSD. Control (No application), moringa leaf extract (1% of MLE1), moringa leaf extract (2% of MLE2), moringa leaf extract (3% of MLE3), brassica water extract (1% of BWE1), brassica water extract (2% of BWE2), brassica water extract (3% of BWE3), sorghum water extract (1% of SWE1), sorghum water extract (2% of SWE2) and sorghum water extract (3% of SWE3)

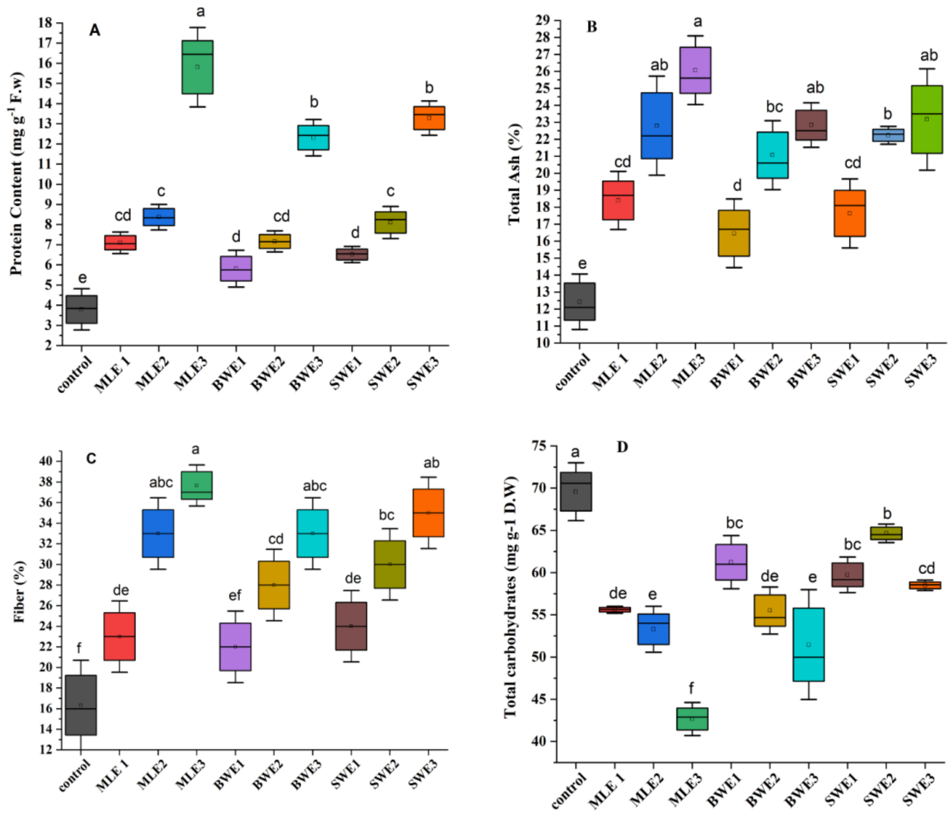

Figure 3: Effect of biostimulants on nutritional attributes A) crude protein, B) total ash C) crude fibers and D) carbohydrates of *Thymus vulgaris.* Control (No application), moringa leaf extract (1% of MLE1), moringa leaf extract (2% of MLE2), moringa leaf extract (3% of MLE3), brassica water extract (1% of BWE1), brassica water extract (2% of BWE2), brassica water extract (3% of BWE3), sorghum water extract (1% of SWE1), sorghum water extract (2% of SWE2) and sorghum water extract (3% of SWE3)

Potassium, Calcium, Magnesium and Sodium

Ammonium acetate extraction, flame photometry, and atomic absorption spectrophotometry were used to assess exchangeable potassium, calcium, and magnesium. The same AB-DTPA extract used for phosphorus analysis is typically used to calculate available potassium. A flame photometer is used to immediately analyze the filtered extract, measuring potassium concentration based on flame emission intensity. Depending on the standards for experimental concentrations reporting into mg g^{-1} soil. Ammonium acetate solution is used to remove soil for use to determine calcium, magnesium, and sodium levels. The flame photometry is used to examine the extract. While sodium is primarily measured to detect salt accumulation and ionic balance in treated soils, calcium and magnesium are significant secondary elements that affect membrane integrity and metabolic activity (Angelova *et al.*, 2021).

Electrical conductivity (EC) and pH

The same soil-water extract used to determine pH is also used to assess electrical conductivity (EC). The EC electrode is submerged in the clear solution when it has properly settled, and a conductivity meter is used to measure the result, which is expressed as dS m^{-1}. Lower EC values often mean better soil conditions for thyme root growth and nutrient uptake. Electrical conductivity is a measure of the quantity of soluble salt in the soil (Monteleone *et al.*, 2016).

Statistical analysis

This study employed a rRandomized cComplete bBlock dDesign (RCBD) with ten treatments. The data collected was statistically analyzed using Statistix 8.1 software, and R software was used to create a heatmap. Fisher's analysis of variance (ANOVA) was performed, and treatment means were compared using Tukey's test at a 5% probability level (Steel *et al.,* 1960).

Results better to rewrite the whole section

Photosynthetic parameters

Mean data showed that biostimultants have positive impact on the photosynthetic pigments of thyme as compared to control conditions. However, foliar treatments with MLE, BWE and SWE significantly enhanced photosynthetic pigments, including chlorophyll a, chlorophyll b, total cholorophyll and carotenoids content compared to control. Notably, significant results were observed in thyme for photosynthetic pigments. The application of different treatments significantly enhanced the photosynthetic pigments of thyme compared to the control (T0). Among all treatments, T3 proved to be the most effective, resulting in the highest increase in chlorophyll a (50%), chlorophyll b (57%), total chlorophyll (54%), and carotenoid content (52%). Similar positive effects were also observed in T9 and T6, which consistently showed substantial improvements in all pigment attributes. Moderate increases were recorded in T2, T8, and T5, whereas comparatively lower enhancements were observed in T1, T7, and T4.

Nutritional attributes

Data revealed that biostimultants have positive impact on the nutritional properties of thyme as compared to control conditions. However, foliar treatment with MLE, BWE and SWE significantly enhanced nutritional properties, including carbohydrates, crude protein, crude fiber, fat, fibers, and ash content compared to control. Significant results were observed in thyme for nutitional properties. Among the treatments, T3 produced the highest significant increases in crude protein (316%), crude fiber (130%), total ash (109%), and carbohydrates (38%) compared with the control (T0). T9 and T6 also showed substantial improvements, ranking second and third for most nutritional parameters. Overall, T3 was the most effective treatment in enhancing the nutritional composition of *Thymus vulgaris.*

Phytochemical attributes

Biostimulant application exerted a positive influence on the phytochemical attributes of thyme compared with the control treatment. Foliar sprays of MLE, BWE, and SWE significantly enhanced total phenolic content, total flavonoid content, anthocyanin concentration, and ascorbic acid levels. Among all treatments, T3 consistently produced the highest improvements in phytochemical traits, recording increases of 308% in total phenolics, 27.4% in flavonoids, 14.3% in anthocyanins, and 15.7% in ascorbic acid relative to the control (T0). Treatments T9 and T6 also demonstrated significant enhancements and ranked among the most effective treatments across all measured phytochemical parameters. Moderate but significant improvements were observed under T2, T8, and T5, whereas T1, T7, and T4 exhibited comparatively lower responses. Overall, the results indicate that biostimulant treatments, particularly T3, followed by T9 and T6, were most effective in improving the phytochemical quality of thyme plants compared with the untreated control.

Physiological parameters

The results demonstrated that, in comparison to control conditions, biostimulants significantly affect the gas exchange characteristics of thyme. In contrast to control, foliar treatment with MLE, BWE, and SWE markedly improved gas exchange characteristics, such as photosynthetic rate, transpiration rate, and stomatal conductance. Significant findings regarding the antioxidant properties of thyme were noted. Treatment T3 exhibited the highest significant enhancement in physiological attributes, including photosynthetic rate (126%), transpiration rate (68%), and stomatal conductance (36.3%), compared with the control (T0). Similar positive effects were observed in T9 and T6, which consistently ranked among the top-performing treatments. Likewise, T3 resulted in the greatest increase in moisture content (35%), followed by T9 and T6, indicating improved plant water status and physiological performance.

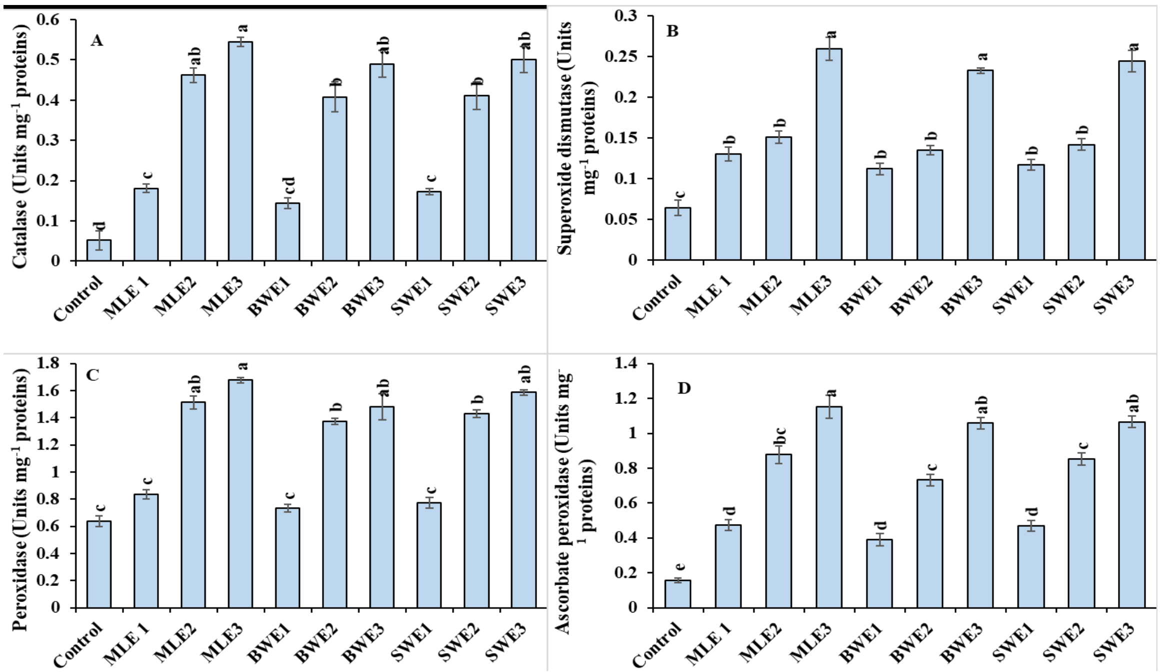

Figure 4: Effect of biostimulants on antioxidant properties A) catalase, B) superoxide dismutase, C) peroxidase and D) ascorbate peroxidase in *Thymus vulgaris.* Control (No application), moringa leaf extract (1% of MLE1), moringa leaf extract (2% of MLE2), moringa leaf extract (3% of MLE3), brassica water extract (1% of BWE1), brassica water extract (2% of BWE2), brassica water extract (3% of BWE3), sorghum water extract (1% of SWE1), sorghum water extract (2% of SWE2) and sorghum water extract (3% of SWE3)

Table 2: Effect of biostimulants on Magnesium, Nitrogen, Phosphorus, Potassium, Calcium, Sodium, pH and Electrical Conductivity of *Thymus vulgaris* soil

Treatments	Mg (mg g^{-1})	Soil N (%)	Soil P (mg g^{-1})	Soil K^+ (mg g^{-1})	Soil Ca^{2+} (mg g^{-1})	Na^+ (mg g^{-1})	pH	EC (dS m^{-1})
Control	0.041±0.0012f	0.42±0.0016g	0.0073±0.0002g	0.14±0.002d	0.081±0.0011g	0.031±0.0008a	7.72±0.021a	1.13±0.024a
MLE 1	0.049±0.0014e	0.46±0.0014f	0.0088±0.0004f	0.15±0.002cd	0.089±0.0013f	0.028±0.0004b	7.58±0.024b	1.016±0.023
MLE2	0.057±0.0016c	0.56±0.0013c	0.0101±0.0005c	0.16±0.002bc	0.097±0.0017c	0.024±0.0003d	7.46±0.025d	0.92±0.026d
MLE3	0.066±0.0014a	0.63±0.0014a	0.0116±0.0002a	0.18±0.002a	0.108±0.0015a	0.021±0.0005f	7.32±0.026f	0.82±0.025f
BWE1	0.046±0.0012e	0.54±0.0012d	0.0081±0.0003f	0.14±0.002d	0.085±0.0016fg	0.028±0.0006b	7.6±0.02ab	1.03±0.022b
BWE2	0.053±0.0013d	0.52±0.0015e	0.0094±0.0004db	0.16±0.002bc	0.093±0.0014d	0.025±0.0007c	7.53±0.025c	0.96±0.023c
BWE3	0.061±0.0015b	0.58±0.0014b	0.011±0.0004e	0.17±0.002ab	0.104±0.0015b	0.022±0.0004ef	7.38±0.024e	0.86±0.025e
SWE1	0.047±0.0014e	0.55±0.0015cd	0.0085±0.0006f	0.15±0.002cd	0.087±0.0012f	0.027±0.0007bc	7.61±0.025b	1.02±0.021b
SWE2	0.055±0.0012cd	0.53±0.0013de	0.0099±0.0003c	0.16±0.0023bc	0.096±0.0013c	0.024±0.0002d	7.48±0.021d	0.91±0.024d
SWE3	0.064±0.0016ab	0.62±0.0013a	0.0114±0.0004a	0.17±0.0024ab	0.105±0.0017ab	0.021±0.0004f	7.34±0.023f	0.83±0.023f

Values (mean ± standard error), HSD = honestly significant difference; values sharing same case letter or without lettering for a parameter do not differ significantly ($p \leq 0.05$) by the HSD test. Control (No application), moringa leaf extract (1% of MLE1), moringa leaf extract (2% of MLE2), moringa leaf extract (3% of MLE3), brassica water extract (1% of BWE1), brassica water extract (2% of BWE2), brassica water extract (3% of BWE3), sorghum water extract (1% of SWE1), sorghum water extract (2% of SWE2) and sorghum water extract (3% of SWE3).

Enzymatic antioxidant attributes

The results demonstrated that biostimulant application significantly improved the antioxidant defense system of thyme compared with the untreated control. Foliar application of MLE, BWE, and SWE enhanced the activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX). Among all treatments, T3 consistently exhibited the highest antioxidant enzyme activities, recording increases of 303% in SOD, 948% in CAT, 163% in POD, and 640% in APX relative to the control. Treatments T9 and T6 also produced substantial and statistically significant enhancements in all antioxidant parameters and ranked among the most effective treatments. Moderate but significant improvements were observed under T2, T8, and T5, whereas T1, T7, and T4 showed comparatively lower responses. Overall, T3 proved to be the most effective treatment for strengthening the antioxidant capacity of thyme, followed by T9 and T6, indicating the superior role of these biostimulant treatments in enhancing enzymatic antioxidant defenses.

Soil analysis

The results demonstrated that, in comparison to control conditions, biostimulants significantly affect the soil analysis concentrations. However, foliar treatment with

MLE, BWE and SWE significantly enhanced soil analysis including N, P, K^+, Ca^{2+}, Mg, Na^+, pH and electrical conductivity of soil as compared to control. Significant

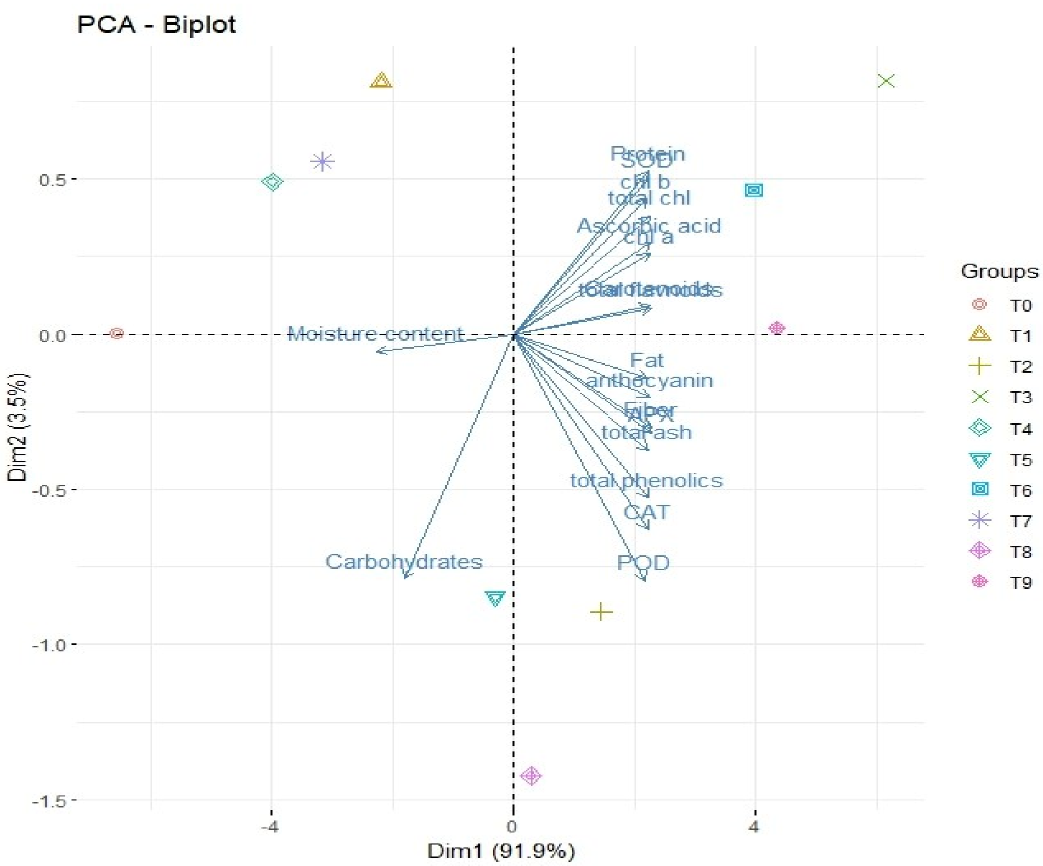

Figure 5: PCA analysis showed the effect of biostimulants on phtosynthetic, nutritional, phytochemical, gas exchange and antioxidant Properties of *Thymus vulgaris,* Control (No application), moringa leaf extract (1% of MLE1), moringa leaf extract (2% of MLE2), moringa leaf extract (3% of MLE3), brassica water extract (1% of BWE1), brassica water extract (2% of BWE2), brassica water extract (3% of BWE3), sorghum water extract (1% of SWE1), sorghum water extract (2% of SWE2) and sorghum water extract (3% of SWE3)

improvements in soil chemical properties were recorded under T3, which consistently showed the highest increases in N, P, K^+, Ca^{2+}, Mg, Na^+, pH, and EC compared to the control (T0). Treatments T9 and T6 also showed notable and consistent enhancements across most parameters, ranking as the second and third most effective treatments, respectively. Overall, T3 was the most effective treatment in improving soil nutrient availability and chemical properties.

PCA and heatmap analysis

To investigate the effect of biostimulants on several thymus vulgaris findings, a two-way heatmap with a tree diagram was created. Based on similarities found at different stages of the treatment, the observations were divided into groups, and the relationships between the groups were shown by colored squares. For a number of data, the color navy blue had a significant positive correlation and the color maroon had a strong negative relationship when biostimulants treatment was taken into account. Four groups have been formed from the heatmap. Moisture and carbohydrate contents were grouped together in the first group. These measures have a modestly favorable correlation at the T4 (1% of BWE) level and a substantial positive correlation with T0 (control), when biostimulants treatment is absent. At T3, there was a significant negative correlation between these qualities (3% of MLE). According to this group, the best outcomes were obtained when T3 (3% of MLE) was applied. The second group's content of ascorbic acid, fat, fiber, APX, total ash, and chlorophyll was significantly impacted by the biostimulants. When no biostimulants were used, there was a substantial negative correlation under control conditions and a strong positive connection under T3 (3% of MLE) and T6 (3% of BWE). Enzymatic antioxidants (POD, CAT) and total phenolics were present in the third group. These characteristics showed a strong negative correlation at T0 (Control), but a strong positive correlation under T3 (3% of MLE), T6 (3% of BWE), and T9 (3% of SWE). Total chlorophyll, chlorophyll b, carotenoids, SOD, protein, flavonoids, and anthocyanins were collected in the fourth group. At T3 (3% of MLE), T6 (3% of BWE), and T9 (3% of SWE), these measurements showed a substantial positive correlation, however at T0 = Control and T1 = 1% of MLE, they showed a negative correlation. According to these readings, the application of a biostimulant improved the thymus's photosynthetic pigments and antioxidant activities while lowering oxidative damage.

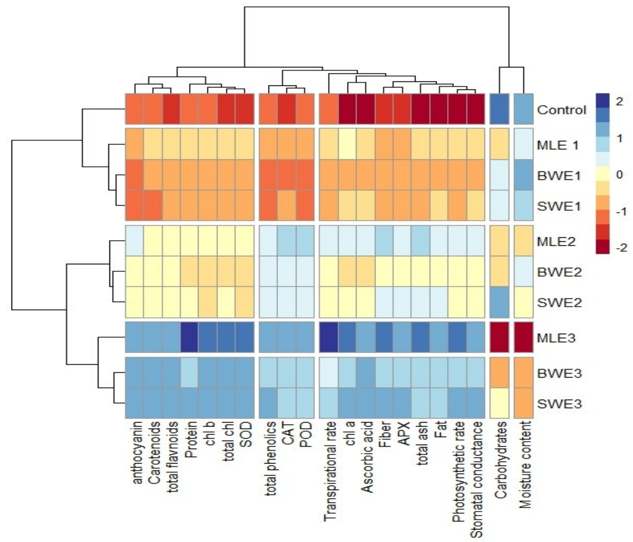

Fig.6: Heatmap analysis showed effect of biostimulants on phtosynthetic, nutritional, phytochemical, gas exchange and antioxidant properties of *Thymus vulgaris.* Control (No application), moringa leaf extract (1% of MLE1), moringa leaf extract (2% of MLE2), moringa leaf extract (3% of MLE3), brassica water extract (1% of BWE1), brassica water extract (2% of BWE2), brassica water extract (3% of BWE3), sorghum water extract (1% of SWE1), sorghum water extract (2% of SWE2) and sorghum water extract (3% of SWE3)

Discussion

MLE, BWE and SWE are plant biostimulants that influence the plant photosynthetic, phytochemical, nutritational and antioxidant properties (Iqbal, 2014). The current research was done to investigate the effects of foliar application of biostimulants in promoting the growth and physiological, nutritional, phytochemical and antioxident properties of thyme. The findings of the present research confirm our hypothesis, according to which the biostimulant influenced the thyme plants and application of the biostimulant had a significant impact on the photosynthetic activity and biochemical and nutritional activity. Under MLE, SWE and BWE treatments carotenoids content was higher as compared to the control treatment in thyme chlorophyll content. The use of biostimulants improves the nutriotional and biochemical properties of the plants and is associated with the growth and development of the plant (Yuniati *et al*., 2022). The use of biostimulants leads to enhanced productivity in thyme, which is consistent with the fact that moringa leaves contain high levels of growth promoting compounds, including amino acids, calcium, potassium, iron, and ascorbate (Yasmeen *et al.*, 2011).

It has been found that the MLE, BWE application, and SWE application have improved the parameters of growth in thyme plants. It was also found that the biostimulants considerably increased the plant growth which can be attributed to the fact that the plant physiological characteristics can be accredited to the strong plant hormones that are capable of controlling all the growth stages and the progression of plant growth. A key cytokinin used by plants is zeatin, which is commonly found in plants, especially in the moringa leaves (Taiwo *et al.,* 2022). The moringa leaves are a good source of zeatin that can be used as an organic cytokinin source and as a source of antioxidants, phenolics, ascorbic acid, vitamins, proteins, minerals, and other nutrients. These nutritional characteristics do not only aid growth, but are biostimulants (Irshad *et al*., 2024). This rise in the nutritional, phytochemical values of thyme is consistent with the fact that the moringa leaves are a good source of growth promoting factors, amino acids, calcium, potassium, and iron, ascorbate. Our results are consistent with those of Hoque *et al*. (2020), who noted that MLE has a strong impact on physiological and nutritional parameters. Moreover, our findings corroborate the findings of Khan *et al*. (2021) who depicted increment of photosynthetic pigments, protein components, and other crucial aspects in rice using MLE foliar application. Sorghum is a good source of various phenolic compounds including phenolic acids, flavonoids, tannins, 3-deoxyanthocyanidins and anthocyanins (Hong *et al*., 2020). The use of extract made out of moringa leaves enhanced the content of a and b chlorophyll on the leaves, reported by Amirjani *et al*. (2012).

They also added that cytokinin which is the major component of MLE stimulates chlorophyll synthesis. The addition of MLE with zeatin led to the pigment alterations of the wheat leaves. To avoid premature leaf senescence, the production of cytokinin biosynthesis through zeatin-rich MLE is required to increase the level of chlorophyll and make the photosynthetic dynamic leaf area more available (Ibrahim *et al*., 2023; Khursheed *et al*., 2024).

SWE was also used as foliar reagent in our study, where it increased physiological, biochemical antioxidant, and nutritional characteristics of thyme since the SWE had a high concentration of phenolics as a reducing agent, hydrogen donor, and singlet oxygen quencher in the plants (Ibrahim *et al.,* 2022). The use of phenolics is crucial in relieving the abiotic stresses like nutrient deficiency, low temperature, and light and increases the antioxidant reactions and inhibits the activity of numerous oxidizing enzymes and removes ROS (Shelembe *et al*., 2014). The presence of the moringa leaf extract instigated antioxidant enzymes (catalases and peroxidases). Moringa leaf extract further showed the scavenging activity of the antioxidative enzymes in eliminating the Cd stress in wheat. In another study, the use of MLE in wheat increased the secondary metabolites. The carboxyl and hydroxyl groups of such compounds protect against oxidative damage (Lu *et al*., 2021). However, AsA is an antioxidant enzyme found in MLE that ensures that oxidative damage is reduced and a variety of cell developmental processes, such as differentiation, division, and senescence, are regulated (Alamri *et al*., 2018). Further, morphine leaf extract was used to raise the content of wheat in terms of TSP and amino acids, which reduce oxidative stress (Basu *et al*., 2022; Khursheed *et al*., 2024;). Proline, an aliphatic amino acid helped the thyme to overcome oxidative destruction. The usage of MLE was helpful according to a recent study because it possesses proteins and flavonoids that are required to build up osmolytes and cause its growth in plants (Farhat *et al*., 2022; Khursheed *et al*., 2024). These findings are consistent with ours (Figure 3).

The results of the present study indicated that sorghum water extract (SWE) at 2% and 3% concentrations significantly enhanced the photosynthetic performance, phytochemical composition, nutritional quality, and antioxidant properties of the plants (Ghadas *et al*., 2021). Foliar treated SWE stimulated the action of antioxidants SOD, POD and CAT and decreased the harmful influence of the reactive oxygen species on cellular organelles. The activity of antioxidants was much better in foliar applied (Hasanuzzaman *et al*., 2021). The BWE usage facilitates physiological activity and antioxidant activity. The category of plant steroid hormones known as brassinosteroids may play a role in the mediation of different processes in plants (Bhanu *et al*., 2019). The findings of the current research also indicate that BWE various concentrations, were able to enhanced the physiological attributes in the thyme plant because BWE involved to increased the cell division in plants (Zhou *et al*., 2024). Further, in current research the evidence indicated that brassica water extracts repressed physiological activities by improving activity of photosynthetic pigments in thyme.

The foliar BWE has boosted the physiological processes, which is due to the enhancement of the leaf water status, photosynthetic activity, and antioxidant defense system through the action of 24-eppibrassinolide (Khaliq *et al*., 2022). The damaging effect of the ROS was decreased by BWE, which enhanced the activity of antioxidant enzymes, such as SOD, POD and CAT. Equally, BWE application to foliage has been reported to increase the activities of antioxidant enzymes in barley largely because of scavenging activity of 24-eppibrassinolide by BR and alleviated oxidative stress, owing to the use of the BWE (Gill *et al*., 2017). The finding of this study was that MLE, BWE, and SWE treatment could have led to an overall improvement of physiological conditions of thyme plants due to increased activity of antioxidative enzymes. The application of BWE to foliage increased the antioxidant enzyme activities in plants but decreased the antioxidant enzyme activities in the control treatment without BWE application in wheat implied a lower ability of the wheat plants to fight against oxidative damages (Shahzad *et al*., 2018). This research showed that better antioxidant activities minimized under the application of BWE. With the presence of BWE application, the greater efficacy of biochemical properties was identified. These results are congruent with the report in the past who demonstrated that the use of BWE improved the activity of antioxidant enzymes and alleviated oxidative damage in plants. (Iqbal, 2014).

The current study's soil analysis results clearly showed that, in comparison to control conditions, foliar application of biostimulants (MLE, BWE, and SWE) greatly enhanced the nutritional status and physicochemical characteristics of the soil. According to previous research, the presence of bioactive substances, organic acids, and micronutrients in biostimulants that promote microbial activity and nutrient mineralization may be responsible for this improvement (Yasmeen *et al*., 2013; Halpern *et al*., 2015). The crucial role of biostimulants in enhancing soil fertility and ionic balance both of which are critical for plant metabolic processes is further supported by the rise in secondary nutrients (Ca^{2+} and Mg). Taiwo *et al*. (2022) reported similar improvements in soil nutrient dynamics after applying plant-based extracts, noting increased nutrient availability as a result of organic inputs. Additionally, this study's minor increase in soil pH and EC indicates enhanced soil buffering ability and soluble nutrient content that showed same results with the finding of Monteleone *et al*. (2016).

This study found a strong correlation between improved soil nutrient status and improved plant characteristics, such as greater antioxidant activity, stronger photosynthetic pigments, and nutritional content. Improved phosphorus and potassium levels enhanced energy transfer and metabolic activities, which ultimately led to growth and biochemical performance, while higher nitrogen and magnesium availability probably contributed to increased chlorophyll production. Because MLE contains hormones (zeatin), vitamins, and minerals that not only affect plant physiology but also indirectly improve soil quality, it may be more successful than BWE and SWE at improving soil parameters. Khan *et al.* (2021) and Ibrahim *et al*. (2022) have observed similar relationships between enhanced soil fertility and plant biochemical characteristics.

Overall, the results demonstrate biostimulants as environmentally beneficial soil stimulants that improve soil health and nutrient availability, which in turn favorably influences plant physiological, nutritional, and phytochemical characteristics. The potential of biostimulants as sustainable alternative for chemical fertilizers to enhance crop performance and soil quality is highlighted by this integrated response. There is a need to study further the exact mechanisms through which the biostimulants influence metabolism, stress tolerance and overall performance of plants. However, field experiments and long-term studies are required to analyse the practical implications of the supplementation of thyme with biostimulants in different environmental conditions.

Conclusion

This study highlights how natural biostimulants can be used as sustainable agricultural practices to improve crop quality and reduce dependency on synthetic fertilizers. According to the findings, applying 3% MLE, BWE, or SWE may be a practical way to raise the nutritional and economic worth of thyme, with further implications for sustainable farming methods. This study offers insightful information about how MLE, BWE, and SWE treatments may improve plant growth, stress tolerance, and general performance. In light of growing environmental concerns, the results highlight the significance of additional study to clarify the molecular mechanisms, improve application techniques, and evaluate the long-term effects of supplementing crops with biostimulants for sustainable production. Further research is needed to evaluate their long-term effects and field-scale applications under diverse environmental conditions.

Acknowledgments

The authors are thankful to Dr. Adnan Younis and Ahsan Akram from the Institute of Horticultural Sciences for support and are also grateful to Dr. Farooq Ahmad Department of Botany, Faculty of Sciences, University of Agriculture Faisalabad.

Author contribution

Conceptualization (Adnan Younis); methodology (Abida Parveen, M. Ahsan); software and formal analysis (Abida Parveen); Investigation (Dr Farooq Ahmad). All authors reviewed the manuscript.

Funding

This research received no external funding.

Data Availability

The data was taken from a study conducted under my Ph.D. Research.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Ali, S., M. Rizwan, S. Noureen, S. Anwar, B. Ali, M. Naveed and P. Ahmad 2019. Combined use of biochar and zinc oxide nanoparticle foliar spray improved the plant growth and decreased the cadmium accumulation in rice (*Oryza sativa* L.) plant. *Environmental Science and Pollution Research* 26:11288-11299.

Amirjani, M. 2012. Effects of cadmium on wheat growth and some physiological factors. *International Journal of Forest Soil and Erosion* (IJFSE) 2:50-58.

Angelova, L., N. Genova, S. Stoyanova, O. Surleva, L.H. Nekov, D. Ilieva and A. Surleva 2021. Comparative study of soil test methods for determination of plant available potassium in Bulgarian arable soils. *Аналитика и контроль* 25:182-192.

Arvouet-Grand, A., B. Vennat, A. Pourrat and P. Legret 1994. Standardization of propolis extract and identification of principal constituents. *Journal de pharmacie de Belgique* 49:462-468.

Balarabe, M.A 2019. Nutritional analysis of cooked and dried leaves of *Moringa oleifera. Plant* 7:71-75.

Basu, S., A.A. Prabhakar, S. Kumari, R.R. Aabha Kumar, S. Shekhar and R. Prasad 2023. Micronutrient and redox homeostasis contribute to *Moringa oleifera*-regulated drought tolerance in wheat. *Plant Growth Regulation* 100:467-478.

Batool, M., A.M. El-Badri, M.U. Hassan, Y. Haiyun, W. Chunyun, Y. Zhenkun and G. Zhou 2023. Drought stress in *Brassica napus*: Effects, tolerance mechanisms, and management strategies. *Journal of Plant Growth Regulation* 42:21-45.

Bhanu, A. N. 2019. Brassinosteroids: Relevance in biological activities of plants and agriculture. *Journal of Plant Science and Research* 35:1-15.

Chance, B. and A. Maehly 1955. Assay of catalases and peroxidases. Incomplete

Elzaawely, A.A., M.E. Ahmed, H.F. Maswada and T.D. Xuan. 2017. Enhancing growth, yield, biochemical, and hormonal contents of snap bean (*Phaseolus vulgaris* L.) sprayed with moringa leaf extract. *Archives of Agronomy and Soil Science* 63:5687-699.

Espitia-Hernández, P., M.L. Chavez Gonzalez, J.A. Ascacio-Valdés, D. Dávila-Medina, A. Flores-Naveda, T. Silva and L. Sepúlveda. 2022. Sorghum (*Sorghum bicolor* L.) as a potential source of bioactive substances and their biological properties. *Critical Reviews in Food Science and Nutrition* 62:2269-2280.

Faghihi, E., F. Aghamir, M. Mohammadi and G. Eghlima 2025. Kaolin application improved growth performances, essential oil percentage, and phenolic compound of *Thymus vulgaris* L. under drought stress. *BMC Plant Biology* 25: 892.

Farhat, F., M. Arfan, X. Wang, A. Tariq, M. Kamran, H.N. Tabassum and A.M. El-Sabrout 2022. The impact of bio-stimulants on Cd-stressed wheat (*Triticum aestivum* L.): Insights into growth, chlorophyll fluorescence, Cd accumulation, and osmolyte regulation. *Frontiers in Plant Science* 13:850567.

Gill, M.B., K. Cai, G. Zhang and F. Zeng 2017. Brassinolide alleviates the drought-induced adverse effects in barley by modulation of enzymatic antioxidants and ultrastructure. *Plant Growth Regulation* 82:447-455.

Goyal, K., N. Singh, S. Jindal, R. Kaur, A. Goya and R. Awasthi. 2022. Kjeldahl method. *Advanced techniques of analytical chemistry* 1:105.

Gupta, E. and P. Mishra 2021. Functional food with some health benefits, so called superfood: A review. *Current Nutrition & Food Science* 17:144-166.

Gupta, N., S. Bhattacharya, A. Dutta, J. Tauchen, P. Landa, K. Urbanová and O. Leuner. 2024. Synthetic polyploidization induces enhanced phytochemical profile and biological activities in *Thymus vulgaris* L. essential oil. *Scientific Reports* 14:5608.

Haider, A., E.A. Waraich, S. Hussain and M. Shahbaz. 2026. Characterization of morpho-physiological, biochemical, ionic, and root responses of *Camelina sativa* genotypes under drought stress by corn-cob-based biodegradable super-absorbent polymer. *New Zealand Journal of Crop and Horticultural Science* 54:70054.

Halpern, M., A. Bar-Tal, M. Ofek, D. Minz, T. Muller and U. Yermiyahu. 2015. The use of biostimulants for enhancing nutrient uptake. *Advances in Agronomy* 130:141-174.

Hong, S., P. Pangloli, R. Perumal, S. Cox, L.E. Noronha, V.P. Dia and D. Smolensky 2020. A comparative study on phenolic content, antioxidant activity and anti-inflammatory capacity of aqueous and ethanolic extracts of sorghum in lipopolysaccharide-induced RAW 264.7 macrophages. *Antioxidants* 9:1297.

Hoque, T.S., M.S. Rana, S.A. Zahan, I. Jahan and M.A. Abedin. 2020. Moringa leaf extract as a bio-stimulant on growth, yield and nutritional improvement in cabbage. *Asian Journal of Medical and Biological Research* 6:196-203.

Horváth, G. and K. Ács. 2015. Essential oils in the treatment of respiratory tract diseases highlighting their role in bacterial infections and their anti-inflammatory action: A review. *Flavour and Fragrance Journal* 30:331-341.

Hosseinzadeh, S., A. Jafarikukhdan, A. Hosseini and R. Armand. 2015. The application of medicinal plants in traditional and modern medicine: A review of *Thymus vulgaris. International Journal of Clinical Medicine* 6:635-642.

Hussain, A., M. Rizwan, Q. Ali and S. Ali 2019. Seed priming with silicon nanoparticles improved the biomass and yield while reduced the oxidative stress and cadmium concentration in wheat grains. *Environmental Science and Pollution Research* 26: 7579-7588.

Ibrahim, M.U., A. Khaliq, S. Hussain and G. Murtaza. 2022. Sorghum water extract application mediates antioxidant defense and confers drought stress tolerance in wheat. *Journal of Plant Growth Regulation* 41:863-874.

Iqbal, M.A. 2014. Improving the growth and yield of canola (*Brassica napus* L.) with seed treatment and foliar sprays of brassica (*Brassica naups* L.) and moringa (*Moringa oleifera* L.) leaf extracts. *American-Eurasian Journal of Agricultural and Environmental Sciences* 14:1067-1073.

Irshad, S., A. Matloob, A. Ghaffar, M.B. Hussain, and M.H. N.Tahir. 2025. Agronomic and biochemical aspects of moringa dried leaf extract mediated growth and yield improvements in soybean. *New Zealand Journal of Crop and Horticultural Science* 53:1510-1529.

Jahan, I. 2020. Foliar application of moringa leaf extract as a bio-stimulant on growth, yield and nutritional quality of brinjal. *Journal of Agriculture, Food and Environment* 2708-5694, 1:94-99.

Khan, S., A.Basit, M.B. Hafeez, S. Irshad, S. Bashir, S. Bashir, M.M. Maqbool, M.S. Saddiq, Z. Hasnain, B.S. Aljuaid and A.M. El-Shehawi. 2021. Moringa leaf extract improves biochemical attributes, yield and grain quality of rice (*Oryza sativa* L.) under drought stress. *Plos One* 16:0254452.

Khursheed, M.H.u.R., M. Shahbaz, T. Ramzan, A. Haider, M.F. Maqsood, A. Khanand A.A. Al-Ghamdi. 2024. Enhancing wheat tolerance to cadmium stress through moringa leaf extract foliar application. *Scientifica* 2024:2919557.

Koralage, I., N. Silva and C. De Silva 2015. The determination of available phosphorus in soil: A quick and simple method. Incomplete reference

Kosakowska, O., Z. Węglarz and K. Bączek. 2021. The effect of open field and foil tunnel on yield and quality of the common thyme (*Thymus vulgaris* L.) in organic farming. *Agronomy* 11:197.

Kumari, P., V. Kumar, R. Kumar and S.K. Pahuja 2021. RETRACTED ARTICLE: Sorghum polyphenols: plant stress, human health benefits, and industrial applications. *Planta* 254:47. must be deleted from text and list

Li, Y. 2009. Physiological responses of tomato seedlings (*Lycopersicon esculentum*) to salt stress. *Modern Applied Sciences* 3*:*171-176.

López-Bascón, M. and M.L. De Castro. 2020. Soxhlet extraction. *In Liquid-phase extraction* (327-354): Elsevier. Incomlete and porr formating

Monteleone, M., G. Lacolla, G. Caranfa and G. Cucci 2016. Indirect measurement of electrical conductivity and exchangeable cations on soil water extracts: Assessing the precision of the estimates. *Soil Science* 181: 465-471.

Nakano, Y. and K. Asada 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology* 22:867-880.

Peres, M. Z., J.E.M. Pezzopane, G.F. da Silva, P. Gádoros, J.V. Toledo, A. Barócsi and S. Lenk. 2025. Impact of different levels of water deficit on physiological and fluorescence responses in *Eucalyptus urophylla*× *Eucalyptus grandis*. *Canadian Journal of Forest Research* 55:1-15.

Ramzan, T., M. Shahbaz, F. Ahmad and E.A. Waraich. 2025. Changes in the Morpho-physiological, and biochemical attributes of canola (*Brassica napus* L.) varieties caused by seed priming with melatonin and ascorbic acid under salinity stress. *Pakistan Journal of Agricultural Sciences* 62. pages?

Ramzan, T., M. Shahbaz, M.F. Maqsood, U. Zulfiqar, R.U. Saman, N. Lili andB. Shahzad. 2023. Phenylalanine supply alleviates the drought stress in mustard (*Brassica campestris*) by modulating plant growth, photosynthesis, and antioxidant defense system. *Plant Physiology and Biochemistry* 20:107828.

Rys, M., D. Saja-Garbarz and A. Skoczowski. 2022. Phytotoxic effects of selected herbal extracts on the germination, growth and metabolism of mustard and oilseed rape. *Agronomy* 12:110.

Shahzad, B., S. Cheema, M. Farooq, Z. Cheema, A. Rehman and T. Abbas 2018. Growth stimulating influence of foliage applied brassica water extracts on morphological and yield attributes of bread wheat under different fertilizer regimes. *Planta Daninha* 36:018178331.

Shelembe, J.S., D. Cromarty, M. Bester, A. Minnaar and K.G. Duodu. 2014. Effect of acidic condition on phenolic composition and antioxidant potential of aqueous extracts from sorghum (*Sorghum bicolor*) bran. *Journal of Food Biochemistry* 38:110-118.

Siddiqi, K.S. and A. Husen. 2021. Significance of brassinosteroids and their derivatives in the development and protection of plants under abiotic stress. *Biologia* 76:2837-2857.

Singleton, V. L. and J.A. Rossi Jr. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16:144-158.

Spitz, D.R. and L.W. Oberley. 2001. Redox state in cancer and apoptosis. *Molecular Aspects of Medicine* 22:225-233.

Starck D and V. Wray. 1989. Anthocyanins, in; Methods in Plant Biochemistry, Vol. 1, Edited by J.B. Horborne, *Academic Press Ltd* 326. poor formating

Steel, R.G D., and J.H. Torrie 1960. *Principles and Procedures of Statistics.* better to replacevwith latest edition

Taiwo, A. M., O.R. Oladotun, A.M. Gbadebo, W.O. Alegbeleye and T.M. Hassan 2022. Nutrient enhancement potentials of moringa (*Moringa oleifera*), neem (*Azadirachta indica*), and pawpaw (*Carica papaya*) fortified composts in contaminated soils. *Environmental Monitoring and Assessment* 194:237.

Yasmeen, A. 2011. Exploring the potential of moringa (*Moringa oleifera*) leaf extract as natural plant growth enhancer. PhD thesis. Department of Agronomy, Faculty of Agriculture, University Of Agriculture, Faisalabad, Pakistan.

Yasmeen, A., S. Basra, M. Farooq, H. Rehman, N. Hussain and H.U.R. Athar. 2013. Exogenous application of moringa leaf extract modulates the antioxidant enzyme system to improve wheat performance under saline conditions. *Plant Growth Regulation* 69: 225-233.

Yuniati, N.and K. Kusumiyati, S. Mubarok and B. Nurhadi 2022. The role of moringa leaf extract as a plant biostimulant in improving the quality of agricultural products. *Plants* 1: 2186.

Zaïri, A., S. Nouir, M.A. Khalifa, B. Ouni, H. Haddad, A. Khélifa and M. Trabelsi. 2020. Phytochemical analysis and assessment of biological properties of essential oils obtained from Thyme and Rosmarinus species. *Current Pharmaceutical Biotechnology* 21:414-424.

Zhou, F., B. Hu, J. Li, H. Yan, Q. Liu, B. Zeng and C. Fan 2024. Exogenous applications of brassinosteroids promote secondary xylem differentiation in *Eucalyptus grandis. PeerJ.* 1:6250.