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The antioxidants and secondary metabolites found in chicory (*Cichorium intybus* L.) are affected by biofertilizers, conventional fertilizers, and nano-fertilizers

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KEY WORDS:
Cichorium intybus, Antioxidant capacity, NPK, secondary metabolites, IC_{50}.

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ABSTRACT
In winter 2024, a field study was conducted on the wild chicory plant (*Cichorium intybus* L.), a medicinal species having nutritional and pharmacological properties. A completely random design with four treatments was used to determine the effect of bio-stimulant application to leaves, traditional chemical fertilizers, and NPK nano-fertilizers on secondary metabolite production and antioxidant activity in the absence of information on how modern fertilization methods affect secondary metabolite production. All data were evaluated using one-way ANOVA and LSD at $p < 0.05$. The first treatment was water spraying (T0), the second utilized conventional NPK chemical fertilizer (T1), the third used NPK nano-fertilizer (T2), and the fourth used biostimulant (T3). Wild chicory beat water-only (T0) in all variables. The biostimulant (T3) treatment outperformed the wild plants in all chemical and physiological parameters. It had the most flavonoids (61.5 mg/g), phenols (197.8 mg/g), and alkaloids (1.89%). The test group had 192.1 mg/L glutathione, up from 137.7 mg/L in the control. T3 worked best at 500 mg/L and had the lowest IC50 value of 90.9 mg/L in the DPPH antioxidant experiment, outperforming the wild plant at 104.5 mg/L and vitamin C at 140.6 mg/L. Biostimulants and nanofertilizers enhance chicory's nutritional and medicinal value by improving secondary chemicals and antioxidant activity, offering a sustainable alternative to conventional fertilizers

تأثر مضادات الأكسدة والمستقلبات الثانوية الموجودة في الهندباء البرية (*Cichorium intybus* L.) بالأسمدة الحيوية والأسمدة التقليدية والأسمدة النانوية

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الخلاصة
في شتاء عام ٢٠٢٤، أُجريت دراسة ميدانية على نبات الهندباء البرية (*Cichorium intybus* L.)، وهو نوع طبي ذو خصائص غذائية ودوائية. استُخدم تصميم عشوائي كامل بأربعة معاملات لتحديد تأثير استخدام المحفزات الحيوية على الأوراق، والأسمدة الكيميائية التقليدية، والأسمدة النانوية NPK على إنتاج المستقلبات الثانوية ونشاط مضادات الأكسدة، وذلك في ظل غياب معلومات حول تأثير طرق التسميد الحديثة على إنتاج المستقلبات الثانوية. تم تقييم جميع البيانات باستخدام تحليل التباين أحادي الاتجاه (ANOVA) واختبار LSD عند مستوى دلالة إحصائية $p < 0.05$.
كانت المعاملة الأولى هي الرش بالماء(T0) ، والثانية باستخدام الأسمدة الكيميائية التقليدية (T1) NPK، والثالثة باستخدام الأسمدة النانوية NPK (T2)، والرابعة باستخدام المحفزات الحيوية .(T3) تفوقت الهندباء البرية على المعالجة بالماء فقط (T0) في جميع المتغيرات. كما تفوقت المعالجة بالمحفزات الحيوية (T3) على النباتات البرية في جميع المعايير الكيميائية والفسيولوجية. احتوت على أعلى نسبة من الفلافونويدات (61.5 ملغم/غ)، والفينولات (197.8 ملغم/غ)، والقلويدات (1.89%). وبلغت نسبة الجلوتاثيون في المجموعة التجريبية 192.1 ملغم/لتر، مقارنةً بـ 137.7 ملغم/لتر في المجموعة الضابطة. أظهر مركب T3 أفضل النتائج عند تركيز 500 ملغم/لتر، وسجل أقل قيمة IC50بلغت 90.9 ملغم/لتر في تجربة مضادات الأكسدةDPPH ، متفوقًا بذلك على النبات البري (104.5 ملغم/لتر) وفيتامين C (140.6 ملغم/لتر). تعمل المحفزات الحيوية والأسمدة النانوية على تحسين المواد الكيميائية الثانوية ونشاط مضادات الأكسدة، مما يعزز القيمة الغذائية والطبية للهندباء في الزراعة المستدامة. كما أنها توفر بديلاً فعالاً لأساليب التسميد التقليدية.
الكلمات المفتاحية: *Cichorium intybus*، القدرة المضادة للأكسدة، NPK، المستقلبات الثانوية، IC_{50}.

INTRODUCTION

Since medicinal plant production mostly depends on collecting wild specimens at the moment, overharvesting plant material is a common concern in the field (Davis *et al*., 2025). Over eighty percent of the world's population relies on herbal remedies for health issues, and with few negative side effects, medicinal plants are in high demand as both pharmaceutical ingredients and natural flavourings and aromas (Bridges, 2016).

Cichorium intybus L., or wild chicory, is an Asteraceae family member and one of the plant species whose habitat is at risk from overharvesting and other unsustainable practices. Both Europe and Asia are rich in its distribution (Bais and Ravishankar, 2001). Alkaloids, inulin, sesquiterpene lactones, flavonoids, coumarins, vitamins, chlorophyll pigments, unsaturated sterols, saponins, tannins, and sesquiterpene lactones are some of the active substances produced by this plant, which gives it a high therapeutic potential (Atta *et al*., 2010).

Past reports have shown that *Cichorium intybus* has a hepatoprotective effect against diabetes-related liver damage due to the antioxidant and anti-inflammatory properties of the plant's active ingredients. (Hamza *et al*., 2024). Also, it had notes of its antibacterial (Al-haliem *et al*., 2025), hypoglycemic (Itrat *et al*., 2024), and anti-ulcer, gastroprotective, cardioprotective, hepatoprotective, analgesic, anti-allergic and anti-malarial actions (Pathak *et al*., 2022). It is also used to treat fever, diarrhea, jaundice, and gallstones (Abbasi *et al.*, 2009), and it possesses anti-inflammatory properties (Minaiyan *et al*., 2012). Wang *et al.* (2020) reported that there is a substantial increase in secondary plant receptors when we increase the base nutrients. Polyzos *et al*. (2022) found that using biofertilizer systems helped *Cichorium spinosum* L., a plant in the Asteraceae family, grow better and increase its antioxidant activity. Khudhair *et al*. (2024) also pointed out that

biofertilizers changed the chemical makeup of lettuce leaves (*Lactuca sativa* L.) and increased the levels of flavonoids. Al-Jibawi and Mijwel's (2025) research on biofertilizers also found that spinach leaves have a much higher total phenolic content.

This study investigates the impact of NPK, nano-NPK, and seaweed-based biostimulants on the production of secondary metabolites (phenolics, flavonoids, alkaloids, and amino acids) and antioxidant activity in chicory. Current research lacks information on how modern biofertilizer systems affect these factors, despite the established medicinal benefits of chicory. This research aims to enhance sustainable agriculture and maximize the benefits of this medicinal plant.

MATERIALS AND METHODS

1- Date and Time of the Experiment

In the winter of 2024-2025, this study was conducted at the College of Agriculture/Al-Qasim Green University. Put out 5 chicory seeds in plastic pots, which were 20 cm high by 15 cm in diameter, and they had a soil capacity of 5 kg. The pots were filled with a soil mixture consisting of German peat moss and river soil at a ratio of 2:1. The planting date was 12/12/2024, and the seedlings were fully established on 22/12/2024. After four true leaves appeared, thinning was carried out. Fertilizer was then added by spraying on 19/1/2025.

2- Fertilization Treatments

The experiment was conducted in a Completely Randomized Design (CRD) with four treatments and five replicates, using foliar sprays according to Table 1. In a random rotation, the treatments were doled out, which was a ten-day interval between each spray. A total of six sprays were conducted, concluding on March 10, 2025. Also, the plants required in terms of irrigation and weeding, which we did right through the growth period. One week after the last spray, the required measurements were taken (Zhao and Li, 2023)

Table 1: Type of Fertilization Treatments

Code	Type of Treatment	Concentration
T0	Comparison Treatment – Water Only Spray	-
T1	Balanced NPK (20:20:20 + Micronutrients)	2 $g.L^{-1}$
T2	Nano NPK (Non-Nutrient) Fertilizer	2 $g.L^{-1}$
T3	Biostimulant (Tecamin Algae, seaweed extract—Spain)	2 $ml.L^{-1}$

3- Effect of Fertilization on Metabolites

To determine the differences in chemical content, specifically flavonoids, alkaloids, and amino acids, between wild and field-grown plants treated with different types of fertilizers, 1g of plant powder was weighed for each species and treatment and placed in a clean glass beaker separately. 10 ml of concentrated organic ethanol solvent was added to the beaker, the beaker was tightly sealed, and it was left for 24 to 48 hr. at room temperature, shaking occasionally to ensure a good interaction between the solvent and the plant material. After which, the extract was filtered through clean gauze and then filter paper to obtain the filtrate, which includes the raw active ingredients. The extract was then put into storage in sterile dark-colored bottles and refrigerated till use (Harborne, 1998).

4- Preliminary (qualitative) chemical detection of active compounds in leaves

A. To detect glycosides: In a test tube, 2 mL of Benedict's reagent was combined with 1 mL of the plant extract. The mixture is thoroughly combined and then placed in a boiling water bath for 5 min. The tube was allowed to cool. Adedayo *et al*. (2001) reported that a red precipitate formed, which is a sign of glycosides.
B. To detect terpenes, combine 2 ml of chloroform, 3 ml of concentrated sulphuric acid, and 5 ml of plant extract. The appearance of the red-brown layer indicates the presence of terpenes (Ayoola *et al*. 2008).
C. To detect tannins, introduce several drops of 1% ferric chloride ($FeCl_2$) solution into a test tube containing 0.5 mL of plant extract. The appearance of the blue-green hue indicates the presence of tannins (Adedayo *et al*., 2001)
D. Flavonoid detection: combine 1 ml of plant extract with 1 ml of concentrated sulphuric acid. Al-Khazraji (1991) states that a dark yellow tint signifies the presence of flavonoids and flavanols.
E. To detect alkaloid: Marquis' reagent was prepared by mixing 1 ml of formaldehyde with 10 ml of concentrated sulphuric acid in the plant extracts. The observed turbidity signifies the existence of alkaloids (Harborne, 1984).
F. To detect phenols, combine 3 ml of plant extract with 2 ml of 1% ferric chloride solution. Harborne (1984) described a blue-green color as indicative of phenolic compounds.

5- Estimation of Total Flavonoid Content:

The aluminium chloride colorimetric approach was used to determine the total flavonoid profile of plant materials. To make the extract, 10 ml of each solution of $NaNO_2$, $AlCl_3$, and NaOH were added one by one. Following incubation, the total flavonoid content was determined by measuring the absorbance at 510 nm using a calibration curve. Per gram of dry weight, the results are expressed as milligrams of rutin (Sofiane and Wafaa, 2018).

6- Estimation of Total Phenolic Content:

Sodium carbonate was added to the ethanolic extract after its total phenolic content was assessed using the Folin-Ciocalteau reagent. The volume was then adjusted to 10 ml, incubated, and the absorbance was measured at 765 nm. To describe the results in terms of mg gallic acid equivalents/g dry weight, the data were input into a gallic acid calibration curve (Kingne *et al*., 2018).

7- Estimation of Total Alkaloid Content:

After soaking 20 g of plant material in methanol for 24 hr., it was extracted using a Soxhlet apparatus, and the extracts were diluted in hydrochloric acid and filtered after vacuum-induced evaporation had produced a dry residue. After the solutions were neutralized, the bromocresol green reagent and the phosphate buffer solution were added. The subsequent step was to transfer the mixtures to a volumetric flask and extract them using chloroform. According to Valieva and Akulov (2024), the atropine equivalents per gramme of dry weight were calculated using UV-Vis spectrophotometry at 470 nm.

8- Amino Acid Estimation:

After being dissolved in 1M HCl at 55C° for three hours, the dry powder (3 g) from each sample was filtered. It was then redissolved in a sodium citrate solution (pH 2.2). Following exposure to the ortho-phthalaldehyde (OPA) reagent, the samples were shaken. Then 100 µL of the solution was passed through an HPLC system that had a C18-NH2 column and a carrier phase composed of methanol, acetonitrile, and 5% formic acid (20:60:20), with a flow rate of 1 mL/min. Then, a fluorescence detector was calibrated to

wavelengths (Ex = 445 nm, Em = 465 nm) to identify glutathione, methionine, and cysteine, among other important amino acids (Dahl-Lassen *et al*., 2018).

9- Antioxidant Activity Test

In this study, a 400 µg. mL-1 DPPH solution in methanol was used as a positive control, and a 5000 mg. L-1 vitamin C stock solution. The sample concentrations, which ranged from 30 to 500 mg. L-1, were mixed with the solutions, allowed to sit at room temperature for 30 min., and then analyzed using a UV-VIS spectrophotometer to determine absorbance at 517 nm (Ahmad *et al*., 2013). The percentage of DPPH free radical inhibition was calculated by multiplying the following formula: Implications of DPPH elimination (%) = (A0-A1)/A0 x 100.

Increased antioxidant activity was observed at lower absorbance values, as demonstrated by the dose-response curve. Furthermore, the IC_{50} value was determined with the use of this curve.

B- Hydrogen Peroxide (H_2O_2) Method: In our experiment, samples were used at a concentration of 100 µg. ml-1, which was added to a 40 mM H_2O_2 solution at a pH of 7.4; then waited 10 min and measured the absorbance at 230 nm in comparison to the control solution. The following formula was also used to get the H_2O_2 elimination rate:

The effect of H_2O_2 removal (%) = (AC-AS)/AC x 100.

where AS is the sample's absorbance and AC is the control's absorbance (Keser *et al.*, 2012).

10- Statistical Analysis:

The results data were statistically analyzed using a complete randomized design (CRD) and analysis of variance (ANOVA). GenStat version 12.1.0.3338 (2009) was used, and the means of the coefficients were compared using the least significant difference (LSD) test at a probability level of 0.05.

RESULT AND DISCUSSION

In both wild chicory and field-treated plants, results found that five types of flavonoid chemicals (gallic acid, rutin, apigenin, kaempferol, and caffeic acid) occurred in variable amounts according to our HPLC analysis, which also determined the compounds' retention time (Rt) and area (Ar). In the case of gallic acid, we saw the highest level in the wild plant (74.08 $\mu g.ml^{-1}$), but for caffeic acid, it had the lowest (22.56 $\mu g.ml^{-1}$). Rutin, apigenin, and kaempferol had variable levels between these two values. Also, noted that plants that did not get fertilizer (T0) had a large drop in all flavonoid levels when compared to the wild plant.

Phenolic compounds, flavonoids, alkaloids, glycosides, tannins, terpenes, and amino acids are present in a large variety in *C. intybus*, as seen in early research. Each group's response to chemical reagents is as reported before, which includes the work of Riaz *et al*. (2024). These results also support what was put forth in past research: that the plant has a great many medicinal compounds in it, which include elements with antibacterial, anti-inflammatory, and antioxidant actions. This, in turn, supports the use of the plant in traditional medicine and suggests that it is time for a more in-depth study at the chemical and pharmacological levels (Al-haliem *et al*., 2025).

HPLC results also showed variations in the concentration of identified flavonoids in chicory plants under the influence of different fertilizer treatments (T1, T2, T3) compared to the control (T0) and wild-type plants. In the chemical treatment (T1), the highest

concentration of gallic acid was recorded (77.49 µg.ml^{-1}), while the lowest was caffeic acid as 26.98 µg.ml^{-1}. In the nanochemical treatment (T2), gallic acid increased to 81.00 µg.ml^{-1}, while caffeic acid remained the lowest at 30.11 µg.ml^{-1}, and the concentrations of the other compounds ranged between 60–71 µg.ml^{-1}. While the biological treatment (T3) showed clear superiority, recording the highest concentrations of all compounds, including gallic acid (88.90), rutin (79.08), apigenin (66.90), and kaempferol (69.09), caffeic acid had the lowest (33.66 µg.ml^{-1}) (Fig. 1).

When fertilizer was added from T0 to T3, the overall flavonoid level went up more than it did in the wild plant. The amounts of rutin, kaempferol, and gallic acid all went up the most, with treatment T3 having the highest levels, which were significantly higher than those found in wild plants (at $p < 0.05$). It's possible that this rise is because plants are getting more nutrients, especially nitrogen and phosphorus, which change the phenylpropanoid route that makes flavonoids.

Gallic acid, rutin, apigenin, kaempferol, and caffeic acid were all much more effective in the T3 biofertilizer-treated crop than in the control T0 treatment that did not include any additives. Also, in terms of reports of active groups and antioxidant action, this is in part due to nitrogen (N), phosphorus (P) and potassium (K), which are the main mineral players in plant growth. Nitrogen is a base element in plant structure; it plays a role in photosynthesis and respiration processes and also in the production of primary and secondary metabolites (Zhang *et al*., 2023). As the plant changed its metabolism, better mineral intake probably led to more secondary compounds being made. Also, the fact that the plants responded differently to different types of fertilizer shows that the makeup of the fertilizer is a key factor in controlling the buildup of flavonoids. These results show that using the right field management techniques can improve chicory's phytochemical makeup, making it more nutritious and useful for medicine.

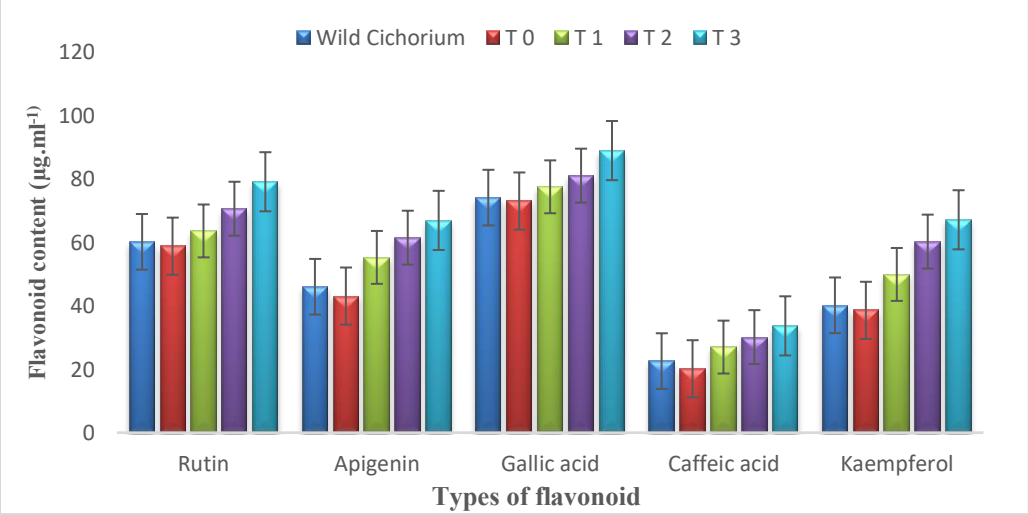

Figure 1: Comparison of flavonoid content in wild and field-treated chicory using HPLC. Where (T0) Water Only Spray; (T1) Balanced NPK; (T2) Nano NPK, and (T3) Biostimulant; (LSD at 5% probability level: Rutin = 1.18, Apigenin = 1.22, Gallic acid = 1.41, Caffeic acid = 1.21, and Kaempferol = 1.17).

In Fig. 2, the results indicate that there is a great difference between wild chicory and different field treatments in total flavonoid content. In T0, which is the control, the lowest

value was seen 30.5 mg. g^{-1}; then T1, which reported 37.9 mg. g^{-1}. Also, noted a large increase in T2, with a reported 55.7 mg. g^{-1}, and the highest in T3 at 61.5 mg. g^{-1} as compared to the wild, which reported 33.5 mg. g^{-1}. What we see from these results is that the field treatments, in particular T2 and T3, played a large role in the increase of flavonoids, which in turn reflects very well on the nutritional and medicinal value of the plant.

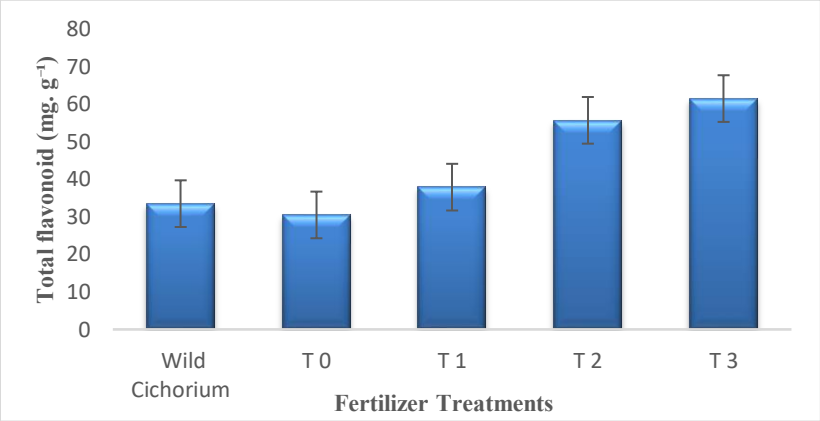

Figure 2: Comparison of total flavonoid content (mg. g^{-1}) in wild chicory and field treatments. Where (T0) Water Only Spray; (T1) Balanced NPK; (T2) Nano NPK, and (T3) Biostimulant; (LSD at 5% probability level = 1.45).

When looking at the total phenol content in wild chicory and in field-treated plants, which were exposed to four different treatments (T0, T1, T2, and T3), we saw that in treatment T0, the lowest phenol content was reported at 165.8 mg. g^{-1}, which was also followed by treatment T1 at a level of 173.8 mg. g^{-1}. In treatment T2, there was a large increase in phenol content, which went up to 188.9 mg. g^{-1}, and in treatment T3, the highest value was seen at 197.8 mg. g^{-1} as compared to the wild plant, which had a value of 174.0 mg. g^{-1} (Fig. 3).

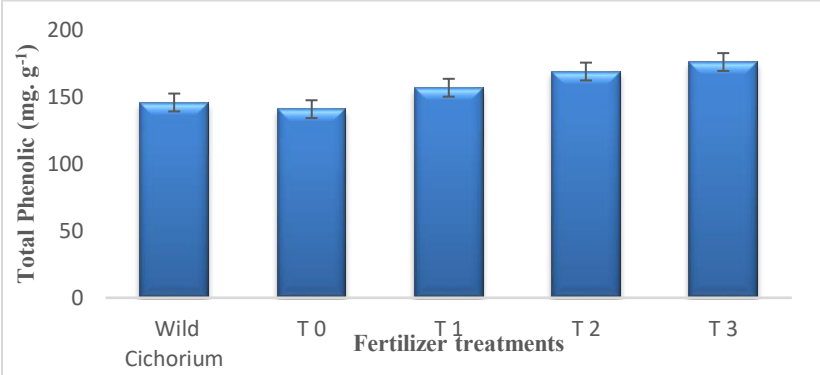

Figure 3: Comparison of total phenolic content (mg. g^{-1}) in wild chicory and field treatments. Where (T0) Water Only Spray; (T1) Balanced NPK; (T2) Nano NPK, and (T3) Biostimulant; (LSD at 5% probability level= 1.85).

Also, for alkaloids in the comparison of total content between wild chicory and field-grown plants, the results showed that the lowest content was in treatment T0 at 1.02%, which was followed by treatment T1 at 1.22%. In treatment T2, there was a large increase

in alkaloid content, which went up to 1.47%, and in treatment T3, the highest value was seen at 1.89% as compared to the wild plant, which had a content of about 1.18% (Fig. 4).

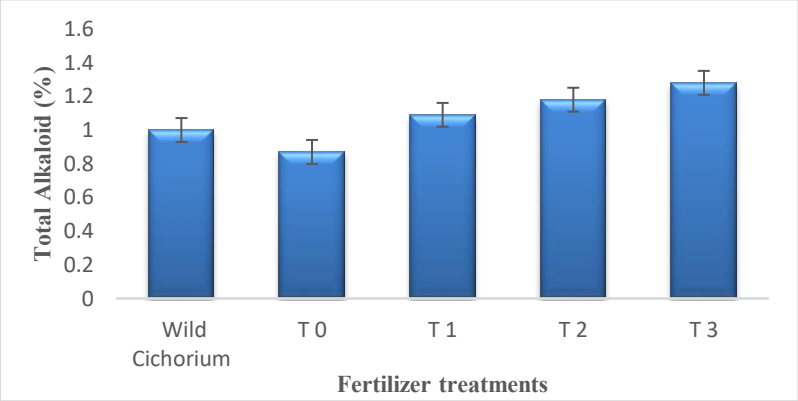

Figure 4: Comparison of the total alkaloid content as a percentage (%) in wild chicory and field-treated. Where (T0) Water Only Spray; (T1) Balanced NPK; (T2) Nano NPK, and (T3) Biostimulant; (LSD at 5% probability level = 0.09).

In field studies, T2 and T3 increase levels of phenol and alkaloid in comparison to wild and other treated plants, which means that these treatments may, in fact, improve the plant's nutritional and therapeutic value. Applying an algal extract improved the chicory's examined properties, according to the findings. The abundance of macronutrients in these plants give them with an advantage in development and supports essential biological functions such as respiration, metabolism, and photosynthesis, among others. Furthermore, these elements play a role in the formation of nucleic acids, which in turn support cell division, as well as in the synthesis of proteins, enzymes, and hormones. Of these elements, nitrogen is the most important, which promotes the growth of vegetation and also increases leaf chlorophyll content, which in turn improves the efficiency of photosynthesis and synthetic nutrient accumulation (O'Dell, 2009). Phosphorus is also very much a part of metabolic processes, secondary metabolite biosynthesis, and the regulation of antioxidant defense systems (Yan *et al*., 2021). At the same time, potassium is key in the regulation of carbohydrate and protein metabolism and supports the plant's main functions (Hassanein *et al*., 2021).

Fig. 5 showed the variation and difference in the concentration of the studied amino acids in the wild chicory plant and thus the difference between the cultivated and field-treated plants, as it was found that the amino acid methionine in wild chicory had the highest concentration of about 62.5 mg. L^{-1}, while the amino acid cysteine recorded the lowest concentration, estimated at approximately 22.5 mg. L-1, while the concentration of the GSH compound reached 141.5 mg. L^{-1}. However, when comparing these values with the field-grown plant and without the addition of any type of fertilizer (T0), we notice a clear decrease in these compounds to record 62.0, 20.1, and 137.7 mg. L^{-1} for methionine, cysteine, and GSH, respectively.

In general, it was observed that all studied active compounds gradually increased in their content from the cultivated plant to which only water was added as a control treatment (T0) to the plant to which biofertilizer was added (T3), where the plant to which biofertilizer was added (T3) represented the highest concentration of all compounds, as the concentrations of methionine, cysteine, and GSH recorded values of 86.9, 59.9, and 192.1

mg. L^{-1}, respectively (Fig. 5). The results showed a variation in the response of the active compounds to the field treatments. Methionine concentrations gradually increased until they reached their highest levels in T3, exceeding those of the wild plant. In contrast, cysteine showed a large rise in T2 and T3, getting close to the amount found in wild plants. Various treatments also gradually increased GSH levels. These findings show the importance of the field treatments that were evaluated in improving chicory plant quality, and they confirm that fertilization played a role in the increased accumulation of active chemicals, which improved their nutritional and medicinal value.

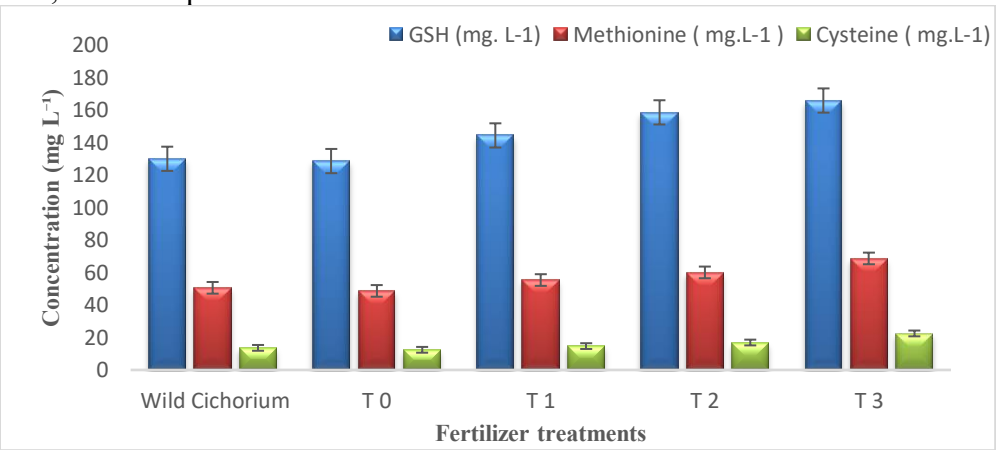

Figure 5: Comparison of the content of active compounds in wild and field-treated chicory plants. Where (T0) Water Only Spray; (T1) Balanced NPK; (T2) Nano NPK, and (T3) Biostimulant; (LSD at 5% probability level: GSH=2.7, Methionine=2.5, Cysteine=1.1).

While the plant to which chemical fertilizer was added (T1) showed the highest concentration of the amino acid methionine reaching 71.4 mg. L^{-1}, while the same plant contained the lowest concentration of the amino acid cysteine, reaching 31.6 mg. L^{-1}, while the compound glutathione (GSH) reached 165.9 mg. L^{-1}. The cultivated chicory to which nano-chemical fertilizer was added (T2) was similar in that it contained the highest concentration of the amino acid methionine, reaching 80.7 mg. L^{-1}, while the same plant contained the lowest concentration of the amino acid cysteine, reaching 44.1 mg. L^{-1}, while the concentration of the GSH compound in the same plant reached 180.7 mg. L^{-1}. While the chicory plant treated with organic fertilizers (T3) showed that it had the highest concentration of all the active compounds identified in the study, as the amino acid methionine was isolated from it at a high concentration estimated at 86.9 mg. L^{-1}, while the amino acid cysteine recorded the lowest concentration, reaching 180.7 mg. L^{-1}. 59.9 The concentration of GSH in the same plant was around 192.1 mg. L^{-1}. The results also indicated that the application of field treatments by adding different types of fertilizers (T1, T2 and T3) led to an increase in the content of active compounds in the chicory plant compared to the standard treatment (T0) and the wild plant in some compounds.

The antioxidant activity, tested via the DPPH and H_2O_2 methods, showed a significant difference between wild chicory and field-treated plants (T0, T1, T2, and T3) when compared to vitamin C (Figs. 6 and 7). At 500 mg. L-1, the plant which had been treated with organic fertilizer (T3) did the best in terms of antioxidant activity, which at that concentration reached 79.00%. Additionally, when tested at the lowest concentration

of 30 mg. L^{-1}, the antioxidant activity decreased to about 27.00%. Also, at all concentrations, the treatments T2 (nano-fertilizer) and T1 (chemical fertilizer) outperformed the control treatment T0, which did the worst. Also, what we noticed is that for the major part of the concentrations put out, the fertilizers, in particular T3, had a greater effect on growth than vitamin C. According to the IC_{50} value data, treatment T3 (90.9) was the most effective in suppressing free radicals, followed by T2 and T1, whereas vitamin C and wild chicory had higher values, at 140.6 and 104.5, respectively. The findings highlight the importance of field treatments in enhancing the plant's antioxidant properties, which in turn increases its nutritional and medicinal value.

Results showed that various field treatments had varying degrees of efficacy in lowering H_2O_2, an indicator of antioxidant activity, according to this study. Efficiency values appeared to be 3.52, 3.44, and 3.10 for treatments T3, T2, and T1, respectively; T0 had the lowest at 2.87. Furthermore, all treatments besides T0 saw an increase in antioxidant activity when compared to wild chicory, which was valued at 3.00. Furthermore, out of all the treatments, vitamin C did the worst at 2.75. What was found is that some field treatments performed better than the industrial standards, mainly T2 and T3. These results highlight the positive effect of fertilization in increasing the biological content of chicory, which enhances its health and nutritional value and supports the possibility of exploiting these treatments to improve the quality of medicinal and aromatic plants (Fig. 7).

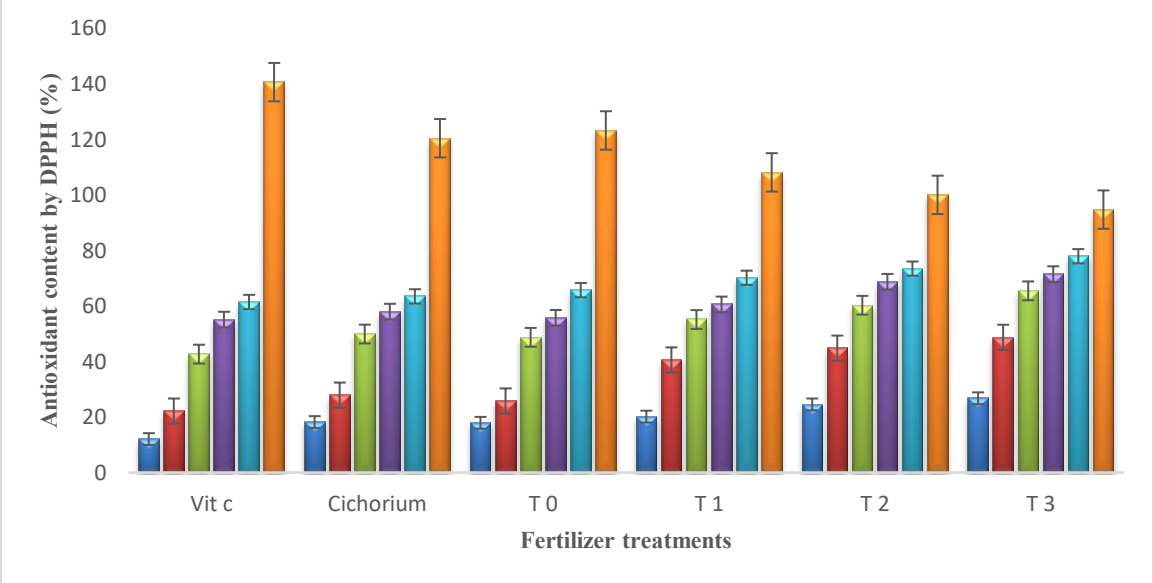

Figure 6: Comparison of antioxidant content using the DPPH% method in wild and field-treated chicory plants. Where (T0) Water Only Spray; (T1) Balanced NPK; (T2) Nano NPK, and (T3) Biostimulant; (LSD at 5% probability level = 1.27).

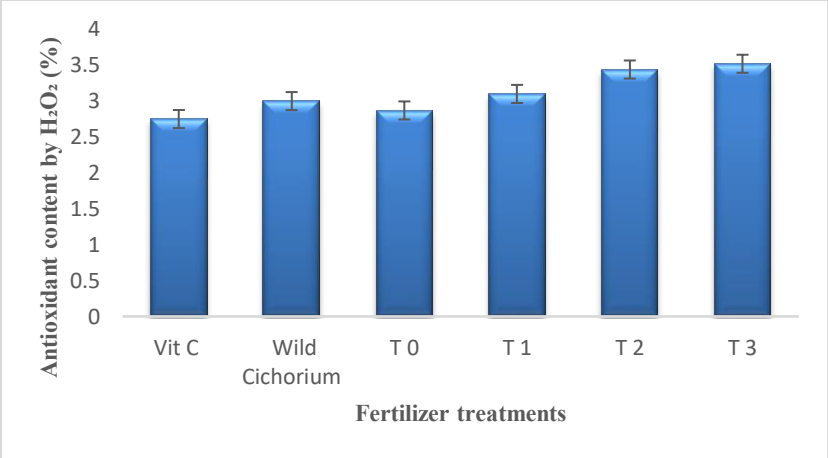

Figure 7: Comparison of antioxidant content by hydrogen peroxide reduction method (H_2O_2%) in wild and field-treated chicory plants. Where (T0) Water Only Spray; (T1) Balanced NPK; (T2) Nano NPK, and (T3) Biostimulant; (LSD at 5% probability level = 0.07).

The increased phenolics, flavonoids, and antioxidant activity in treatments T2 and T3 demonstrate that the effect is not due solely to nitrogen supply but rather to an integrated N–P–K system and biostimulant properties. In addition to nutrients, seaweed extracts provide plant hormones (auxins/cytokinins/gibberellins), polysaccharides, and trace elements, which increase chlorophyll content and photosynthetic efficiency and increase the availability of carbon skeletons for the shikimate–phenylpropanoid pathways responsible for the synthesis of phenolics and flavonoids (Talbi Zribi *et al*., 2021). Phosphorus, via ATP and phosphorylation, contributes to the activation of gene expression and defense enzymes (SOD, CAT, POD, APX) and the reduction of oxidative stress, while potassium activates several enzymes and regulates stomata and phloem transport, promoting sugar translocation and secondary metabolite accumulation (Jan *et al*., 2021). These mechanisms are consistent with the improved DPPH/H_2O_2 and decreased IC_{50}. The results also support the growth-defense balance hypothesis; very high N doses may impair the accumulation of active substances beyond a threshold of approximately 75 kg N. ha^{-1} (Mijani *et al*., 2011), which explains the superiority of T2/T3 over T0/T1.

Plant biostimulants are capable of improving product quality by modulating plant primary and secondary metabolism (Rouphael and Colla, 2020). When plants have access to abundant essential nutrients, the production of secondary metabolites is significantly enhanced (Wang *et al.*, 2020). These results are consistent with those of Al-Yasiry and Mijwel (2024) on two mint species, Al-Jibawi and Mijwel (2025) on rocket, and Choi *et al.* (2022), who confirmed that foliar spraying of seaweed extract enhanced the phenolic content and antioxidant activity of romaine lettuce and tomato.

In addition to plant hormone analogs like auxins, gibberellins, and cytokinins, seaweed extracts include micronutrients (Fe, B, Mg, and Zn) and macronutrients (N, P, and K). Amino acids, building blocks of proteins, are also included in them. These extracts improve photosynthetic efficiency, which in turn promotes vegetative growth, thickens stems, and increases root development when applied to soil or sprayed over plants. In addition, by bolstering plant cell activity, they shield plants from stress factors including drought, salt, and aging (Abdul-Hafiz, 2008). Much remains unclear about the exact pathways via which seaweed extracts induce physiological reactions.

NPK nano fertilizers suggest improving water absorption and mineral element content as their mechanism of action. This fertilizer has particles which promote the growth of vegetation, which in turn increases nutrient demand and thus supports key plant functions. Furthermore, the particles appear to bind to protein carriers, which in turn improves transport and absorption of nutrients by penetrating cell walls, which also causes root and shoot systems to activate. As a result, there are more transport channels and larger stems, which leads to improved use of potassium, phosphorus, and nitrogen (Shakir *et al*., 2019; Kamle *et al*., 2020; Adibah *et al*., 2020). Additionally, our results align with the findings reported by Mijwel and Jabbr (2019) and Singh *et al.* (2025), which also demonstrate the positive effects of nanotechnology on the improvement of medicinal plants. Also, Results showed that medicinal plants' secondary metabolism was improved by Nano NPK, as evidenced by increased nitrate reductase activity, more photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids), and better photosynthesis (Akhter et al., 2025).

Although chemical inputs are still necessary for crop productivity, using them too often might have negative consequences, such as soil erosion, acidification, and decreased nutrient efficiency (Chen *et al.*, 2018). Organic and biofertilizer solutions that may improve soil health via microbial community optimization while maintaining production have garnered attention due to this (Kour *et al*., 2020). Additionally, our findings seem to be in line with those of another research. Lots of research suggests that nanoparticles may improve plant development by influencing metabolic pathways and physiological functions. These nanoparticles regulate photosynthesis, nutrient concentrations, and enzyme activity in both aerobic and anaerobic settings, according to the scientists. Because nano-fertilizers provide the slow and continuous release of nutrients, crop plants may use them more effectively when they are sprayed to them (Abbas *et al*., 2025).

Rouphael *et al*. (2017) found that biostimulants and organic fertilizers affect the production of secondary metabolites, leading to an increase in micronutrients and antioxidant molecules. Plants depend on phenolic compounds for defence and stress response (Saini *et al.*, 2024). Romanjek *et al*. (2024) also suggest that the overall phenolic concentration stimulates the phenylpropanoid pathway. This is because they greatly influence how well phenylalanine ammonia lyase (PAL) works, which is the main enzyme needed to make polyphenols, and enzymes like PAL need amino acids to create other compounds. As a result, there is strong antioxidant activity because more phenols mean better antioxidant ability. Compounds that are involved in biological processes, like phenolic acids such as caffeine and caffeic acid, and flavonoids like rutin, are important for increasing antioxidant activity (Rouphael *et al.*, 2022).

CONCLUSION

A biostimulant derived from algae, along with a nano-fertilizer, enhances the accumulation of beneficial secondary metabolites (flavonoids, phenols, alkaloids, and amino acids) and boosts antioxidant activity in chicory plants. The bio stimulant treatment T3 exhibited the highest phenolic and flavonoid content, along with the strongest antioxidant activity. These findings suggest that nano- and bio-fertilizers offer sustainable strategies for improving the nutritional and medicinal qualities of chicory. Further research should involve multi-seasonal field trials using varying concentrations of these fertilizers. Expanded physiological and molecular analyses are needed to elucidate the underlying

mechanisms of these effects. Furthermore, heavy metal accumulation tests and environmental impact assessments are crucial to ensure the safe application of these technologies

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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