# Effect of Pyruvate and Magnetic Nanoparticle Treatments on Growth, Bioactive Components and Biological Activity of *Stevia Rebaudiana*

Hamdi M. Ibrahima[1], Asmaa A. Mahdi[1], Emad S. Hamed[2] and Manal M. Adel[3]*

[1]*Plant Genetic Resources Department, Desert Research Center, Cairo, Egypt*
[2]*Medicinal and Aromatic Plants Department, Desert Research Center, Cairo, Egypt*
[3]*Pests and Plant Protection Department, National Research Center, Cairo, Egypt*

## Abstract

This trial had been carried out in Maryout region, Alexandria Governorate. The study explored the impact of utilizing nanoparticles of magnetite (NPs) and calcium pyruvate as a source of pyruvate alone or in association with magnetic NPs at various concentrations on stevia plants. The investigation's treatments were control, pyruvate 100 mg/L, pyruvate 200 mg/L, magnetic NPs 0.5 mg/L, magnetic NPs 1.0 mg/L, pyruvate 100 mg/L@ magnetic NPs 0.5 mg/L, pyruvate 100 mg/L@ magnetic NPs 1.0 mg/L, pyruvate 200 mg/L@ magnetic NPs 0.5 mg/L, and pyruvate 200 mg/L@ magnetic NPs 1.0 mg/L. The results proved that all treatments, including the application of nanoparticles of magnetite and pyruvate alone or in combination, were superior to the control treatment regarding different quantitative and qualitative parameters. Spraying with pyruvate at 100 mg/L gave the significantly maximum weights of fresh and dry herbs, the highest concentrations of stevioside and rebaudioside A, and the highest anti-diabetic effects. It was noticed that spraying with magnetic nanoparticles improved the product's antimicrobial and antitumor activities. In this concern, pyruvate 200 mg/L@ magnetic NPs 1.0 mg/L recorded the best traits.

**Keywords:** *Stevia rebaudiana*; Pyruvate; Magnetite nanoparticles; Stevioside; Rebaudioside A

## Introduction

As the Egyptian market's need for stevia products increased, it became necessary to investigate agricultural applications to achieve the highest possible yield with rich secondary metabolites.

Stevia is an economic plant because it serves to make sugar. It can be farmed instead of sugar cane, particularly in Egypt, as sugar cane requires a lot of water. Since the country has water problem and insufficient water to grow sugar cane, stevia plants are a great alternative. Demand for stevia sugar has increased recently, especially among diabetics and overweight people. Stevia, or *Stevia rebaudiana*, belongs to the Asteraceae family. Commercial cultivation occurs in Brazil, China, Thailand, Paraguay, India, and other places. It is broadly applied in food, beverages, cosmetics, medicine, and other food-related industries (Stoyanova et al., 2011) [1].

Stevia leaves contain a complicated blend of eight tasty diterpene glycosides with no calories. These include dulcoside a, isosteviol, rebaudiosides, steviolbioside, and stevioside (Rajasekaran et al., 2008; Goyal et al., 2010). The beverage sector is interested in steviol glycosides (SGs), especially stevioside and rebaudioside A. This has led to more people growing stevia. These glycosides provide *S. rebaudiana* with a superior taste to sucrose (Tavakoli et al. 2019). The high amount of stevioside in stevia, a sweetener, has made it more critical for the nutrition sector to grow stevia (Kovačević et al., 2018). Stevia leaves have been extensively studied as a source of high potency that tastes sweet. Stevioside and rebaudioside A are two tetracyclic diterpene steviol glycosides that are active, sweet-tasting, and non-toxic [2].

Stevia is antibacterial, antifungal, anti-inflammatory, antimicrobial, antiviral, anti-yeast, cardiotonic, diuretic, hypoglycemic, lowers arterial pressure, and is a vasodilator (Goyal et al., 2010). The antioxidant, antiparasitic, and antiproliferative effects of stevia extracts and isolated chemicals are mentioned (Borgo et al., 2021). The water-based extract is used to get sweet parts out of the plant and to sweeten products. Preclinical and clinical experiments have also demonstrated some physiological functions, such as anticarcinogenic and antihypertensive activities (Ruiz-Ruiz et al., 2017) [3, 4].

*Stevia rebaudiana* is an essential plant for making high-quality sugar substitutes with no calories that can help people with diabetes. Diabetes and other metabolic illnesses have been treated and managed with their leaves (Jan et al., 2021). Type 2 diabetes is a common metabolic and endocrine condition caused by insulin deficiency (Gaudel et al., 2013). The WHO estimates that numerous individuals worldwide had diabetes in 2000. Diabetes will almost certainly affect more people worldwide by 2030 (Hossain et al., 2013). In 2019, 463 million individuals worldwide were diagnosed with diabetes. The population is predicted to increase to 578 million over the following decade and 700 million by 2045 (Saeedi et al., 2019) [5, 6].

Tumors are the second-leading cause of mortality worldwide. The most common cancers in 2017 were lung, colorectal, prostate, and breast (Ferlay et al., 2018). Cancer, which will claim more than 10 million lives globally in 2020, will be the top reason for mortality (Ferlay et al., 2021).

Cancer incidence will increase globally, with cases predicted by 2040 compared to 2020 (Sung et al., 2021). Steviol inhibits the growth of six different types of gastrointestinal cancer cells in humans (Chen et al., 2018).

In this research, nanomaterials were used to increase sugar components in stevia. Nanoparticles have recently aroused much

interest due to their low size-to-volume ratio compared to their bulk states (Nagajyothi et al., 2014). Metallic oxide nanoparticles have been deemed the safest due to their stability and other differentiating features (Pandurangan and Kim 2015; Javed et al. 2017). Iron oxide nanoparticles (IO-NPs) have drawn particular interest in research and development due to their distinctive properties, making them suitable for abundant usage in different fields (Ali et al., 2016). Due to their specific qualities, such as their tiny size, sizeable external part-to-volume ratio, surface modifiability, improved magnetic properties, and superior biocompatibility, nanoparticles containing iron oxide have received much attention in environmental protection technology (Xu et al. 2012). About El-Nasr et al. (2015) observed that magnetic nanoparticles have physiochemical and superparamagnetism, which change biomass and biochemical properties and, in turn, plants. Yew et al. (2016) found that biocompatibility, biodegradability, and ease of encapsulation are all good things about magnetite nanoparticles (MNPs) [7-10].

Generating materials at the nanoscale, like magnetite $Fe_3O_4$, with unique properties like biocompatibility, physiological environment stability, superparamagnetism, and low cytotoxicity, opens the door for various uses, particularly the enhancement of bioactive compound accumulation in stevia (Chin et al., 2011).

Pyruvic acid, a crossover molecule, provides cells energy when oxygen is required (aerobic respiration) (Krebs cycle). When supply is insufficient, it ferments to form lactate (lactic acid) (anaerobic respiration) (Ranamukhaarachchi et al. 2000). ''The'' As the Egyptian market's desire for stevia products grew, it became necessary to research the agricultural processes to produce the highest possible yield with rich secondary metabolites. Our research aimed to see how pyruvate and magnetic nanoparticles, alone or together, affected herbage yield, stevioside, rebaudioside A, and other anti-diabetic, anti-cancer, and antimicrobial properties [11].

## Materials and Methods

### Agriculture procedures and treatments:

The research was executed at the Station of the Desert Research Center in the Maryout district (30˚ 59′ 57.12″ N and 29˚ 46′ 59.16″ E), Alexandria Governorate. The work was conducted throughout the two consecutive seasons of 2020 and 2021. The physical characteristics of the soil were sand = 59.00%, silt = 27.00%, clay = 14.00%, and soil texture = sandy loam. The soil's chemical characteristics were as follows: pH = 8.28, organic matter = 0.19%, E.C. = 2101 ppm, and $CaCO_3$ = 25.09%. The E.C. of irrigation water was 390 ppm.

Seedlings of *Stevia rebaudiana*, measuring 20 cm in height, were obtained from the Institute of Sugar Crops Research. On June 30th, the transplants were cultivated in a greenhouse with black seran sheets (63% shade). During soil preparation, compost manure was put at 2 kg m⁻². The stevia seedlings were cultivated at a spacing of 20 cm between plants; the distance between rows was kept at 25 cm, and the density was set at 20 plants per square meter. Chemical fertilization was added, as mentioned by El-Sirafy et al., 2015. All production processes were carried out following good agricultural practices [12, 13].

The research design employed was a randomized complete block with three replicates. It consisted of nine treatments. Spraying the subsequent magnetite nanoparticles and chemical inducers was done one month and a half after transplanting and repeated after the first cut. Calcium pyruvate was the source of pyruvate [14]. Spraying on the leaves was done until runoff. The following were the investigation's treatments:

- Control (only distilled water was sprayed).
- Pyruvate 100 mg/L.
- Pyruvate 200 mg/L.
- Magnetic NPs 0.5 mg/L.
- Magnetic NPs 1.0 mg/L.
- Pyruvate 100 mg/L@ magnetic NPs 0.5 mg/L.
- Pyruvate 100 mg/L@ magnetic NPs 1.0 mg/L.
- Pyruvate 200 mg/L@ magnetic NPs 0.5 mg/L.
- Pyruvate 200 mg/L@ magnetic NPs 1.0 mg/L.

Each season, two cuts were taken at flowering by harvesting the aerial parts of plants 10 cm above the soil surface in mid-September and late November. Plant height and fresh and dry herb weights per square meter (g) were measured. The harvested herb was cleaned of any foreign substances that might have been present. The dry weight was estimated by drying the samples at 70°C. The sampling method ensured that it represented raw material [15].

### Nano materials

The magnetic nanoparticles were acquired from Sigma Aldrich at a size <50 nm. A known weight of pyruvate was loaded after sonication of magnetite in distilled water that had been deionized for one hour.

### Characterization of magnetic loaded pyruvate:

The properties of loaded magnetite were detected by particle size analyzer, scanning electronic microscope and Fourier Transform-infra red [16].

### Biochemical studies

**Antioxidant capacity:** The DPPH test for radical scavenging by Desmarchelier et al. (1997) was employed to determine the extracts' capacity to scavenge free radicals.

**Hydrogen peroxide content:** Fresh stevia leaves were directly homogenized with 1 ml of a solution containing 0.25 ml TCA (0.01% (w:v), 0.50 ml KI (1 M), and 0.25 ml potassium phosphate buffer (10 mM) at 4 °C for 10 min. The resulting mixture underwent centrifugation at 12,000 g for 15 min at 4°C. Each tube was filled with 200 μL of supernatant, which was incubated at 20-22°C for 20 mins before being read at 350 nm for absorbance (Velikova et al. 2000) [17, 18].

**Lipid peroxidation product:** A well-known technique for assessing lipid peroxidation is the TBARS (Thiobarbituric Acid Reactive Substances) assay. The rate of peroxidation of lipid in stevia callus plants was assessed using a modified protocol of Heath and Packer's (1968) method for estimating the end product, malondialdehyde. A 0.5 g sample of fresh stevia leaves was homogenized in 2.5 mL of trichloroacetic acid at 0.1% (w/v) (TCA). For 15 minutes, the mixture underwent centrifugation at 14.000 rpm. 0.5 mL of the liquid supernatant from the leaf sample was applied to 2.0 mL of 5% thiobarbituric acid (TBA) and 20% TCA solution. The combination had been placed inside the bath of water for 30 mins at 95°C, followed by 5 minutes of incubation in an ice bath. The product was read at two wave lengths 532 and 600 nm for non-specific turbidity. The concentration of MDA-TBA adduct was calculated from MDA standard curve and converted to nmol g-1 fresh weight [19, 20].

**Identification of bioactive compounds by HPLC:** According

to Bondarev et al. (2001) and Brandle (1998), a known weight of dry matter was mixed in methanol before being filtered, and the filtrate was then stored until HPLC analysis. The stevioside extracted from methanol was examined using HPLC-DAD. Operating at 25°C was the thermo-hypersil reversed phase C18 column 2.530 cm. Distilled water and methanol, in a 37:63 isocratic ratio, make up the mobile phase. The separation temperature was 25°C. Both the standards' and the samples' UV absorption spectra were noted in the 219 nm range. The mobile phase and standards solutions were degassed and filtrated with a membrane filter with a thickness of 0.45 m. and an injection volume of 10 l (Millipore). The identification of the compounds was done through the comparison of their retention times and UV absorption spectra by computer software [21].

## Biological investigations

### Antidiabetic studies

α-Amylase inhibitor screening: α-Amylases inhibitor screening was estimated according to www.biovision.com

α-Glucosidase Inhibitor Screening Kit (Colorimetric): α-Glucosidase inhibitor screening was conducted according to www.biovision.com

### Evaluation of cell toxicity properties

**Mammalian cell lines:** MCF-7 cells (human breast cancer cell line), HepG-2 cells (human hepatocellular carcinoma), and CACO$_2$ (intestinal carcinoma) were obtained from the VACSERA Tissue Culture Unit.

**Chemicals used:** Sigma (St. Louis, Mo., USA) provided the dimethyl sulfoxide (DMSO), crystal violet, and trypan blue dyes.

Lonza provided fetal bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin, and 0.25% Trypsin-EDTA.

**Crystal violet stain (1%):** This stain is made out of 0.5% (w/v) crystal violet and 50% methanol, which is then mixed with respect to volume with ddH$_2$O and filtered via a Whatmann No. 1 filter paper.

**Cell line propagation:** Dulbecco's modified Eagle's medium (DMEM) supplied with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer, and 50 g/ml gentamycin was used to grow the cells. All cells were cultivated twice a week and kept at 37°C in an atmosphere with humidity and 5% CO$_2$ [22].

**Cytotoxicity evaluation using viability assay:** For the cytotoxicity assay, the cells were seeded in a 96-well plate at a cell concentration of $1\times10^4$ cells per well in 100μl of growth medium. After 24 hours of seeding, fresh medium containing varied quantities of the test sample was added. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO$_2$ for a period of 24 h. Three wells were used for each concentration of the test sample. Control cells were incubated without the test sample and with or without DMSO. The small percentage of DMSO present in the wells (a maximum of 0.1%) was found not to affect the experiment. After incubation of the cells at 37°C for 24 h, the viable cell yield was determined by a colorimetric method [23].

After the incubation period was completed, the media were aspirated, and the crystal violet solution (1%) was applied to each well for at least 30 minutes. The stain was removed, and the plates were cleaned with tap water to eliminate any remaining discoloration. Glacial acetic acid (30%) was added to all wells and properly mixed, and the absorbance of the plates was measured using a microplate reader (TECAN, Inc.) after gently shaking with a test wavelength of 490 nm. Background absorbance detected in wells without additional stain was adjusted for in all results. In the presence of the tested substances, treatments were matched with the cell control. All steps were conducted in triplicate. The cell-toxicity properties of each tested compound were calculated. To determine the number of viable cells, the optical density was measured with a microplate reader (SunRise, TECAN, Inc., USA), and the percentage of viability was calculated as [(ODt/ODc)]x100%, where ODt is the average optical density of wells that were treated with the tested sample and ODc is the average optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC$_{50}$), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using the Prism program from Graphpad (San Diego, CA, USA) [24-26].

### Antimicrobial activity assay

The antiseptic properties of the synthesized compounds were investigated in order to increase the selectivity of these derivatives towards test microorganisms. All microbial strains were provided from the culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The antiseptic profile was evaluated against gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), gram-negative species (*Escherichia coli* and *Proteus vulgaris*), and fungi, including one filamentous fungus (*Aspergillus fumigatus*) and one yeast species (*Candida albicans*), using a modified well diffusion method. The plates were then incubated at 37°C for 24-48 hours (for bacterium and yeast) and 28°C for 48 hours (for filamentous fungi). After incubation, the microorganism's growth was observed. The resulting inhibition zone widths were measured in millimeters and used as a criterion for antimicrobial activity. If an organism is put on the agar, it will not grow around the well if it is susceptible to the chemical. This area of no growth is known as a "zone of inhibition" or "clear zone". The dimension of the clear zone is proportional to the inhibitory action of the constituent under investigation. As negative controls, solvent controls (DMSO) have been utilized in all experiments. The tested substance was dissolved in DMSO, which revealed no inhibitory zones, indicating that it had no effect on the growth of the examined microorganisms. Positive controls were also performed using gentamycin as a standard antibacterial drug and ketoconazole as a standard antifungal drug. All the biologically active samples were subjected to determination of the MIC by the broth microdilution method. After incubation, the minimum rate showing complete inhibition of growth was documented as the MIC of the respective sample.

Statistical analysis: An analysis of variance (ANOVA) was performed on the means, and Duncan's multiple range tests was used to detect differences at the 0.05 probability level. SPSS program version 16 (Richmond, USA) was used, as mentioned by Dytham (1999) [27-31].

## Results and Discussion

### Characterization of nanoparticles:

In (Figure 1), (Figure 1A) introduces the infrared spectrum for magnetite nanoparticles loaded with calcium pyruvate. The functioning
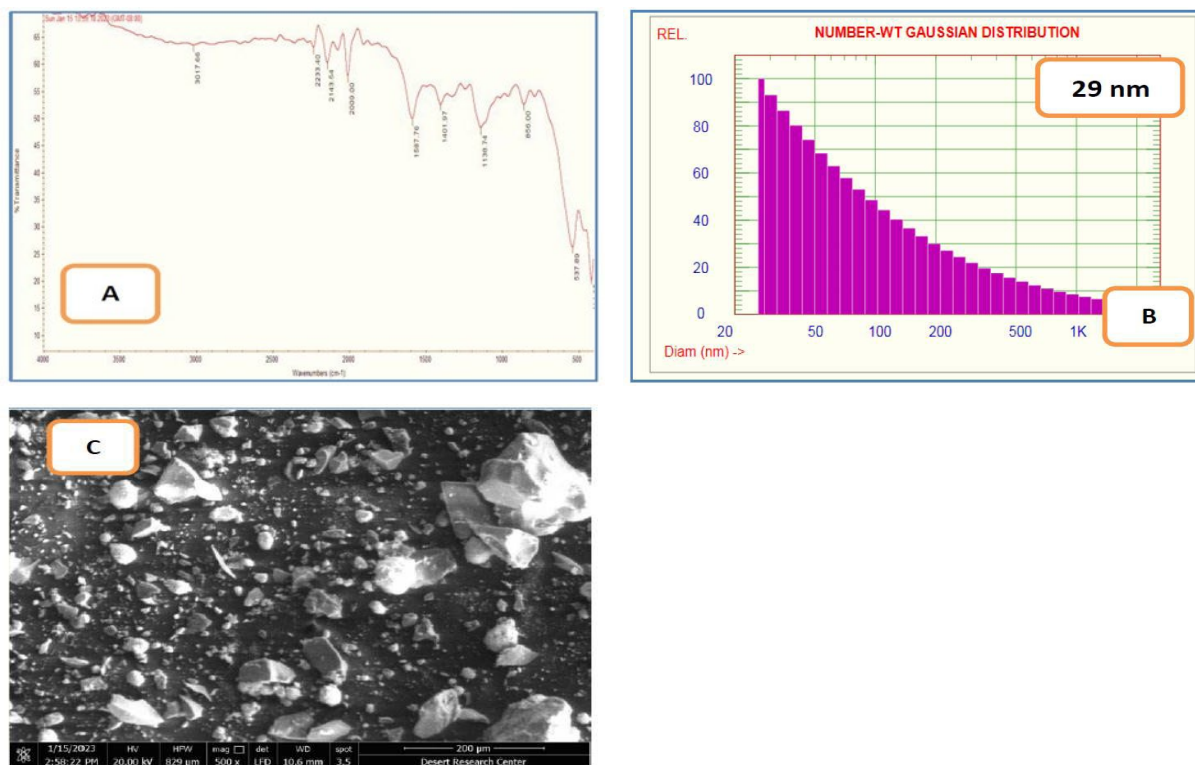
**Figure 1:** Classified to (A): FTIR spectrum of functionalized magnetic nanoparticles, (B): Dynamic light scattering and (C): Scanning electronic microscope

nanoparticles of the magnetic material spectrum gave the function groups: 3017, 2233, 2143, 2009, 1587, 1401, 1138, 856, and 537 λ-1. Concerning dynamic light scattering in (Figure 1B), the dimensions of loaded nanoparticles became 29 nm. In SEM graph (Figure 1C), the small particles exhibited magnetic nanoparticles, but the large white particles were referred to as pyruvate bulky compounds, and this was compatible with previous investigations by (El- Saber et al. 2021; Mahdi et al. 2022) [32,33].

**Plant growth parameters:**

The findings about plant growth characteristics are displayed in (Tables 1-3), which show how spraying with magnetite nanoparticles and chemical inducers affected plant development characteristics. Based on the findings in this work, the treatments that included pyruvate, magnetic nanoparticles, and pyruvate conjugated with nanoparticles that were magnetic at various concentrations significantly improved these attributes [34].

Regarding plants' height, top values for the first cut were recorded at pyruvate 200 mg/L@ magnetic NPs 1.0 mg/L (44.10 cm). Following this, pyruvate 100 mg/L@ magnetic NPs 1.0 mg/L (43.00 cm) and then magnetic NPs 1.0 mg/L (42.78 cm) with no significant differences. The second harvest revealed non-significant variations between treatments except for the control, which had the lowest height [35].

Concerning the weight of fresh herb produced per square meter, in both cuts, the significantly highest values were found when the herb was sprayed with pyruvate 100 mg/L, followed by pyruvate 200 mg/L@ magnetic NPs 1.0 mg/L. In the first cut, these records were 80.33 and 69.46 g, and in the second cut, these values were 180.73 and 134.33 g, respectively. In the first cut, the differences in treatments between pyruvate 100 mg/L and pyruvate 200 mg/L@ magnetic NPs 1.0 mg/L

**Table 1:** Effect of magnetite nanoparticles and some chemical inducers on plant height (cm) of *Stevia rebaudiana* (mean values of the two successive seasons).

| Treatments | 1st cut | 2nd cut |
|---|---|---|
| Control | 31.20 c | 15.33 b |
| Pyruvate 100 mg/L | 37.63 b | 23.00 a |
| Pyruvate 200 mg/L | 42.51 a | 26.00 a |
| Magnetic NPs 0.5 mg/L | 40.45 b | 22.00 a |
| Magnetic NPs 1.0 mg/L | 42.78 a | 24.00 a |
| Pyruvate 100 mg/L@ magnetic NPs 0.5 mg/L | 38.81 b | 24.67 a |
| Pyruvate 100 mg/L@ magnetic NPs 1.0 mg/L | 43.00 a | 27.00 a |
| Pyruvate 200 mg/L@ magnetic NPs 0.5 mg/L | 41.81 a | 24.00 a |
| Pyruvate 200 mg/L@ magnetic NPs 1.0 mg/L | 44.10 a | 24.00 a |
| Means with the same letter are not significantly different at 5% level of probability | | |

**Table 2:** Effect of magnetite nanoparticles and some chemical inducers on fresh weight of herb per square meter (g) of *Stevia rebaudiana* (mean values of the two successive seasons).

| Treatments | 1st cut | 2nd cut |
|---|---|---|
| Control | 40.00 d | 90.00 d |
| Pyruvate 100 mg/L | 80.33 a | 180.73 a |
| Pyruvate 200 mg/L | 50.00 cd | 113.67 bc |
| Magnetic NPs 0.5 mg/L | 53.33 cd | 105.00 bc |
| Magnetic NPs 1.0 mg/L | 51.33 cd | 110.00 bc |
| Pyruvate 100 mg/L@ magnetic NPs 0.5 mg/L | 42.67 ed | 81.67 d |
| Pyruvate 100 mg/L@ magnetic NPs 1.0 mg/L | 62.40 bc | 124.67 bc |
| Pyruvate 200 mg/L@ magnetic NPs 0.5 mg/L | 48.20 d | 100.00 cd |
| Pyruvate 200 mg/L@ magnetic NPs 1.0 mg/L | 69.47 ab | 134.33 b |
| Means with the same letter are not significantly different at 5% level of probability | | |

did not reach statistical significance [36].

The weight of dry herb produced per square meter exhibited the same pattern as the fresh herb. In both cuts, the significant maximum

**Table 3:** Effect of magnetite nanoparticles and some chemical inducers on dry weight of herb per square meter (g) of *Stevia rebaudiana* (mean values of the two successive seasons).

| Treatments | 1st cut | 2nd cut |
|---|---|---|
| Control | 13.21 d | 46.67 d |
| Pyruvate 100 mg/L | 26.53 a | 91.67 a |
| Pyruvate 200 mg/L | 16.11 cd | 60.67 bc |
| Magnetic NPs 0.5 mg/L | 17.33 cd | 56.67 bc |
| Magnetic NPs 1.0 mg/L | 16.67 cd | 60.33 bc |
| Pyruvate 100 mg/L@ magnetic NPs 0.5 mg/L | 14.33 ed | 44.33 d |
| Pyruvate 100 mg/L@ magnetic NPs 1.0 mg/L | 20.50 bc | 68.33 bc |
| Pyruvate 200 mg/L@ magnetic NPs 0.5 mg/L | 16.08 d | 54.65 cd |
| Pyruvate 200 mg/L@ magnetic NPs 1.0 mg/L | 22.73 ab | 71.40 b |
| Means with the same letter are not significantly different at 5% level of probability | | |

**Table 4:** Effect of magnetite nanoparticles and some chemical inducers on biochemical markers DPPH, hydrogen peroxide, malondialdehyde content of *Stevia rebaudiana*.

| Chemical inducers and nanoparticles (mg/L) | DPPH% | Malondialdehyde (nmol/gm fresh weight) | Hydrogen peroxide (μg/gm fresh weight) |
|---|---|---|---|
| Control | 75.31 d | 16.65 d | 18.94 g |
| Pyruvate 100 | 81.36 b | 19.38 de | 22.27 f |
| Pyruvate 200 | 73.62 e | 25.47 b | 42.17 b |
| Magnetic NPs 0.5 | 84.83 a | 5.95 e | 15.30 h |
| Magnetic NPs 1.0 | 80.01 c | 22.46 bc | 28.38 e |
| Pyruvate100@ magnetic NPs 0.5 | 80.88 bc | 19.43 de | 32.70 d |
| Pyruvate100@ magnetic NPs 1.0 | 75.56 d | 33.16 a | 42.51 b |
| Pyruvate200@ magnetic NPs 0.5 | 81.74 b | 16.28 d | 39.03 c |
| Pyruvate200@magnetic NPs 1.0 | 81.50 b | 18.47 b | 55.05 a |

values were revealed when the vegetation was sprayed with pyruvate 100 mg/L, which was then followed by pyruvate 200 mg/L@ magnetic NPs 1.0 mg/L. These weights, in order, were 26.53 and 22.73 g in the first cut, and they were 91.67 and 71.40 g in the second cut, respectively. In the first cut, non-significant differences existed between the treatments of pyruvate 100 mg/L and pyruvate 200 mg/L@ magnetic NPs 1.0 mg/L.

Pyruvate spray on plants at a level of 100 mg/L may be beneficial for several physiological reasons, which account for improved weights. Pyruvate is essential to the completion of some steps in biochemistry. When oxygen is present, it generates energy for the cell via the Krebs cycle (aerobic respiration). However, fermentation occurs in the presence of oxygen, and lactate is produced (anaerobic respiration). The addition of pyruvate from the outside could help the energy mechanisms. Other studies also discovered that applying pyruvate to plants through their leaves could improve their resistance to abiotic stresses (Jain, 2017; Silva et al., 2022) [37].

Iron, important for many enzymes, is a necessary element for plant development, including chlorophyll synthesis and chloroplast development. It has a vital function in the reactions during photosynthesis and is an essential element. Iron is necessary for RNA production and boosts photosystem efficiency in plants. Because these nanoparticles contain magnetic nanomaterials, they can move across biomembranes without stopping. The superparamagnetic characteristics of these ions may affect the ion fluxes that occur across the membrane. Therefore, they enhance the growth features and production of the plant. These results agreed with the literature (Khater, 2015; Seghatoleslami et al., 2015; Bhandari et al., 2022; El-Sonbaty et al., 2022) [38].

## Biochemical studies:

### Oxidative stress biomarkers:

The influence of pyruvate and its combination with magnetic NPs on DPPH in stevia was introduced in (Table 4) and (Figure 2). It was detected that the top DPPH (Figure 2A) activity (84.83%) was observed after application of magnetic NP (0.5 mg/L), subsequently by a DPPH activity of 81.50% in stevia supplemented with pyruvate 200@magnetic NPs1.0 mg/L, and a DPPH activity of 81.36% obtained with pyruvate 100 mg/L. The results agree with Khan et al. (2020), who observed that treatment with iron NP led to increased activity of DPPH in stevia. Also, according to Mahdi et al. (2021), magnetite NP positively affected the action of DPPH in stevia callus. Among the different ratios used, the lowest value of 73.62% was got with pyruvate at 200 mg/L. It was concluded that the top concentration of treatment leads to decreasing DPPH activity compared with the minimum concentration of

treatment, probably because of the reduction in phenolic compounds. The addition of higher concentrations of magnetic NPs reduced DPPH activity in *Matricaria chamomilla*, according to a study by Rastegaran et al. (2022). Iron is a vital element for many plant-growing processes, including the synthesis of chlorophyll and the formation of chloroplasts (Mamatha, 2007) [39, 40].

It is noticeable that the best treatment for reducing MDA and $H_2O_2$ was magnetic NPs 0.5 mg/L, where the values of MDA and $H_2O_2$ were recorded as 5.295 nmol/g and 15.30 μg/g, respectively. This finding agrees with El-Saber et al. (2021), who reported that employing magnetite NPs reduced MDA in wheat leaves at salinity. In addition, Tawfik et al. (2021) discovered that spraying *Moringa oleifera* with micromagnetite NPs in a saline environment lowered MDA and $H_2O_2$ levels (Figure 2C). The MDA is a biomarker for calculating the rate of lipid peroxidation and harm to organelle membranes, including the plasmalemma, caused by ROS damage from environmental stimuli (Ozkur et al. 2009). Iron is an assistant for many enzymes that catalyze different metabolic reactions in plants. According to Soliman et al. (2015), the rising impact of these enzymes' activities is being attributed to magnetite NPs, a crucial element for plants that works as a functional structure or cofactor for protein production and is a metal constituent of many enzymes (Marschner, 1995). High concentrations of nanomaterials induce oxidative stress and prevent plant cell activity; they prompt the activation of plant defense mechanisms to combat damage (Gupta et al., 2018). We found that when the concentration of the treatments increased, the values of MDA and $H_2O_2$ increased. Finally, there is an obvious link within the treatment concentration and malonaldehyde and hydrogen peroxide levels (Figure 2B) [41, 42].

### Bioactive compounds in *Stevia rebaudiana* leaves:

Our investigation explained that stevia cultivation in the presence of pyruvate (100 mg/L) and pyruvate 200 mg/L loaded with magnetic NP 1 mg/L showed the highest contents of stevioside and rebaudioside A (103.77 mg/g DW and 79.79 mg/g DW) and (51.99 mg/g DW and 34.30 mg/g DW), respectively. On the contrary, the lowest content of stevioside (4.1 mg/g DW) was obtained after applying pyruvate 100 mg/L loaded with magnetic NP 1.0 mg/L. The minimum value of Rebaudioside A (1.41 mg/g DW) was obtained after magnetic treatment at NP 0.5 mg/L [43]. As shown in (Table 5), exposure to higher pyruvate concentrations (200 mg/L) showed a reduction in stevioside content (6.258 mg/g DW) compared with the control. As a result of these findings, we have observed that plants cultivated in the presence of magnetic NP (0.5 mg/L) positively affected the accumulation of stevioside, which was recorded at 36.67 mg/g DW compared with
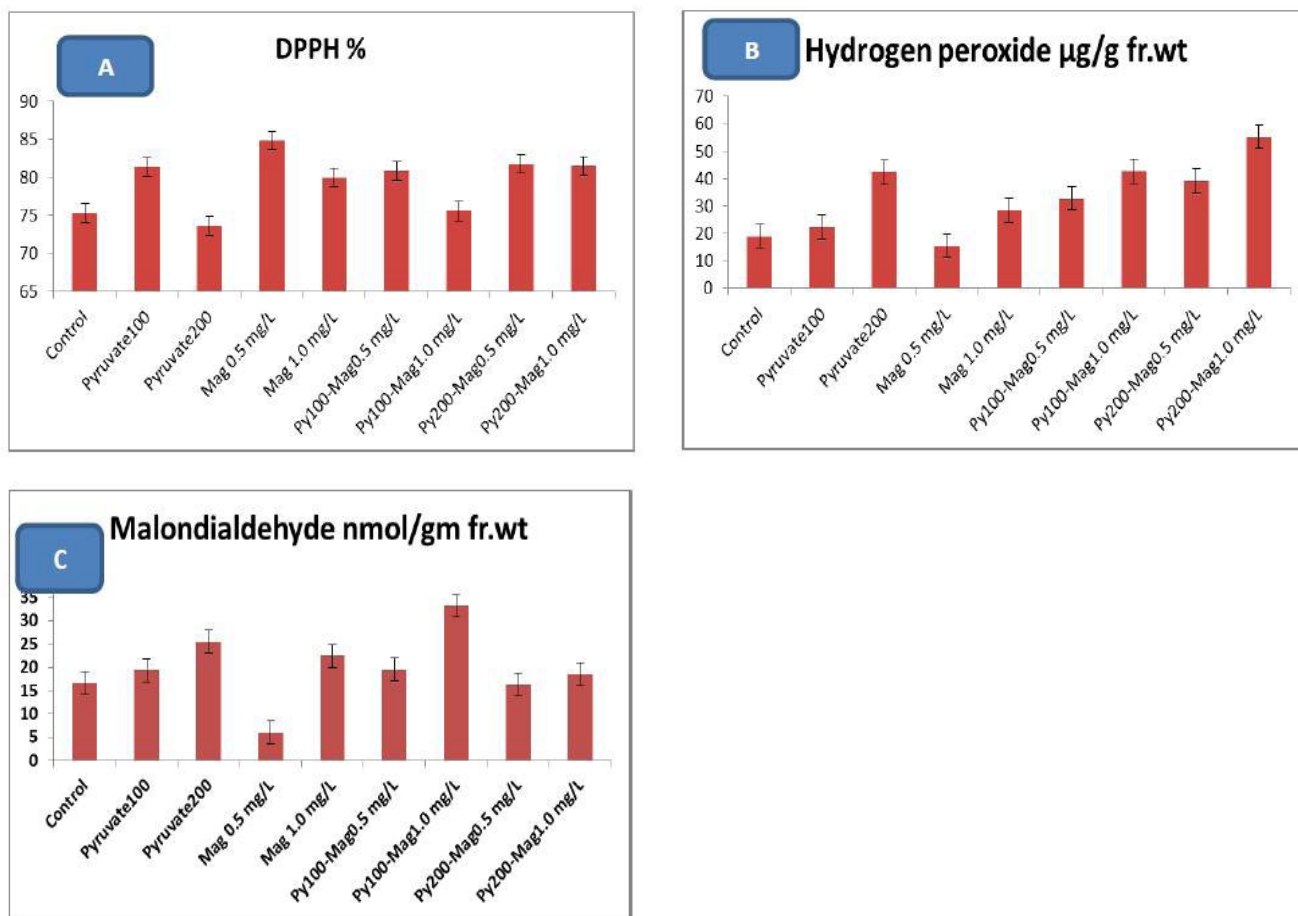
**Figure 2:** Effect of magnetite nanoparticles and some chemical inducers on biochemical markers (A): DPPH, (B): hydrogen peroxide, (C): malondialdehyde content of *Stevia rebaudiana*

**Table 5:** Effect of magnetite nanoparticles and some chemical inducers on the content of stevioside and rebaudioside A in *Stevia rebaudiana*.

| Treatments | Active constituents content | |
| --- | --- | --- |
| | (mg/g dry weight) | |
| | Stevioside | Rebaudioside |
| Control | 10.32 | 3.59 |
| Pyruvate 100 mg/L | 103.77 | 79.79 |
| Pyruvate 200 mg/L | 6.25 | 3.63 |
| Magnetic NPs 0.5 mg/L | 36.67 | 1.41 |
| Magnetic NPs 1.0 mg/L | 5.18 | 3.44 |
| Pyruvate 100 mg/L@ magnetic NPs 0.5 mg/L | 7.45 | 2.27 |
| Pyruvate 100 mg/L@ magnetic NPs 1.0 mg/L | 4.1 | 2.82 |
| Pyruvate 200 mg/L@ magnetic NPs 0.5 mg/L | 7.64 | 3.48 |
| Pyruvate 200 mg/L@ magnetic NPs 1.0 mg/L | 51.99 | 34.30 |

**Table 6:** Effect of magnetite nanoparticles and some chemical inducers on the productivity per square meter of stevioside and rebaudioside A in *Stevia rebaudiana*.

| Treatments | Active constituents content | | | |
| --- | --- | --- | --- | --- |
| | (g/m²) | | | |
| | Stevioside | | Rebaudioside | |
| | 1st cut | 2nd cut | 1st cut | 2nd cut |
| Control | 0.14 d | 0.48 c | 0.05 c | 0.17 c |
| Pyruvate 100 mg/L | 2.75 a | 9.51 a | 2.12 a | 7.31 a |
| Pyruvate 200 mg/L | 0.10 d | 0.38 c | 0.06 c | 0.22 c |
| Magnetic NPs 0.5 mg/L | 0.64 c | 2.08 b | 0.02 c | 0.08 c |
| Magnetic NPs 1.0 mg/L | 0.09 d | 0.31 c | 0.06 c | 0.21 c |
| Pyruvate 100 mg/L@ magnetic NPs 0.5 mg/L | 0.11 d | 0.33 c | 0.03 c | 0.10 c |
| Pyruvate 100 mg/L@ magnetic NPs 1.0 mg/L | 0.08 d | 0.28 c | 0.06 c | 0.19 c |
| Pyruvate 200 mg/L@ magnetic NPs 0.5 mg/L | 0.12 d | 0.42 c | 0.06 c | 0.19 c |
| Pyruvate 200 mg/L@ magnetic NPs 1.0 mg/L | 1.18 b | 3.71 b | 0.78 b | 2.45 b |

untreated plants. On the contrary, exposure to a greater concentration of magnetic NP (1.0 mg/L) showed a reduction in stevioside content compared with the control. The output per square meter of sugar components followed the similar general pattern as previously stated. The significant top values were given by spraying with pyruvate at 100 mg/L, followed by pyruvate at 200 mg/L@ magnetic NPs 1.0 mg/L (Table 6) [44].

These results were confirmed by Khan et al. (2020), who found that low-dose iron nanoparticles exhibited the maximum amount of stevioside compared to high doses. On the contrary, Hendawey et al. (2015) showed that a low concentration of iron nanoparticles led to increased stevioside and rebaudioside content in stevia callus. The synthesis of steviol glycosides is regulated by the action of a diverse group of enzymes, which regulate how sugar residue is shifted from an activated donor to an acceptor molecule (Vazquez-Hernandez et al. 2019). Iron is minimal for optimal growth and output; its absence may cause considerable disturbance in the plant's physiological and metabolic processes, according to Brittenham (1994) [45]. Iron is helpful for many enzymes that catalyze specific biological reactions. In this regard, iron plays various critical roles in plant physiology and development (Miller et al., 1995; Sheikhbaglu et al., 2014). Iron oxide

magnetic nanoparticles have been detected for their role in iron release in plant cells and acceleration of the Fenton reaction, which results in the creation of free radicals (Taghizadeh et al. 2019). While exposure to magnetic NP (0.5 and 1.0 mg/L) showed a reduction in Rebaudioside A content. In this work, all pyruvate treatments loaded with magnetic nanoparticles had a negative effect on stevioside and rebaudioside, except for the pyruvate (200 mg/L) loaded with magnetic nanoparticles (1.0 mg/L), which increased the values of stevioside and rebaudioside compared with the control [46].

## Biological investigations:

### Inhibitory effects of stevia extract against α-amylase and α-glucosidase:

The results showed pyruvate at 100 ppm and pyruvate at 200 ppm combined with magnetic NP at 1.0 ppm. Pyruvate 100 ppm was demonstrated to have an advantage over other treatments in enhancing the anti-diabetic effects of α-amylase and α-glucosidase. The ethanol extract's half maximum inhibitory concentration ($IC_{50}$) was determined to be 4.642 mg/ml and 0.753 mg/ml, respectively, in the α-amylase glucosidase assays. The test solution of various doses of extract from the pyruvate treatment demonstrated a higher percent inhibition than other treatments going in the similar direction [47]. This is because the concentrations of stevioside and rebaudioside A are at their highest. The use of 100 ppm pyruvate resulted in an accumulation compared to the other treatment (Table 7).

These findings were supported by Zaidan et al. (2018), who discovered that this plant extract positively impacted anti-diabetic amylase activity. The principal steviol glucosidase inhibitors (stevioside and rebaudioside A) in stevia extract highly inhibited α-amylase and α-glucosidase activity (Table 8). At various stevia concentrations, it has an antihyperglycemic action (Chowdhury et al., 2022) [48]. Stevia leaf enhanced the insulinogenic index, which decreased blood glucose and regulated glucagon in diabetic individuals. Stevia reduces nuclear factor k-light chain enhancer activity in activated cells, which promotes insulin to raise insulin sensitivity and glucose infusion levels and lower blood sugar levels (Yang et al., 2012). The strongest α-amylase inhibitory action is seen in stevia extract, which has a great affinity for α-amylase, suggesting that *S. rebaudiana* can be exploited in the creation of medicinal medicines for the management of diabetes (Singla et al., 2019) [49].

### Anticancer activity:

In this work, we have prepared and analyzed an ethanolic extract tested for antiproliferative activities (hepatocellular carcinoma, intestinal carcinoma, and breast cancer). The cell viability and cytotoxic impact of ethanolic extract with pyruvate 100 ppm and pyruvate 200@magnetic NPs 1.0 ppm were observed against hepatocellular

carcinoma, intestinal carcinoma, and breast cancer cells. The data show that pyruvate 200@ magnetic NPs 1.0 ppm treatment inhibited cell line proliferation more than pyruvate 100 ppm treatment. These findings may be explained by higher doses of magnetic NPs, which result in excessive production of ROS-mediated oxidative stress in the cell and, as a result, DNA damage, as previously reported (Kamaraj et al., 2019) [50]. Because iron-based nanoparticles are a potent inducer of ROS, a sufficient quantity can selectively destroy tumor cells while inhibiting their growth. Because cancer cells produce more ROS and have more oxidative DNA damage than normal cells in tissues, nanoparticles can be utilized to cure cancer. The cytotoxic influence of pyruvate 200 @ magnetic NPs 1.0 ppm against hepatocellular carcinoma, intestinal carcinoma, and breast cancer cells showed a higher antiproliferative effect ($IC_{50}$ 49.12, 86.57, and 86.11μg/ ml) than pyruvate 100 ppm (Table 9). This finding was confirmed by Alshawwa et al. (2022), who discovered that using this plant extract biosynthesize nanoscale alpha hematite ($-Fe_2O_3$) nanoparticles (NPs) had superior activity against cancer cell lines such as lung tumors [51,52]. Nanoparticles outperform standard antioxidant delivery strategies in many ways, including better absorption and environmental safeguarding of bioactive components, targeted antioxidant distribution, and regulated freeing at the site of action (Verma, 2014). In this trial, we discovered an adverse link among the concentration of active constituents in the stevia plant, such as stevioside and Rebaudioside A, and cytotoxicity in tumor cells and cell growth inhibition on Hep G2 cell lines, CAco2, and MCF-7, with lower levels of active materials inhibiting cells more than higher contents of active constituents (Table 10). The presence of other materials in stevia

**Table 8:** α-glucosidase inhibitory activity of pyruvate 100 ppm and pyruvate 200 ppm + magnetic NP 1.0 ppm extract.

| Percent inhibition | | | |
|---|---|---|---|
| Concentration | Pyruvate (100 ppm) | Pyruvate 200 ppm + magnetic NP 1.0 ppm | Acarbose |
| 0.1 µg/ml | 8.62 | 5.08 | 16.5 |
| 1.0 µg/ml | 27.3 | 22.7 | 37.2 |
| 10 µg/ml | 54.1 | 41.6 | 63.5 |
| 100 µg/ml | 80.6 | 64.8 | 86.4 |
| 1000 µg/ml | 93 | 82.7 | 95.00 |

**Table 9:** α-amylase IC50 (mg/ml) and α-glucsidase IC50 (mg/ml) of pyruvate 100 ppm and pyruvate 200 ppm + magnetic NP 1.0 ppm extract.

| α-amylase IC50 (mg/ml) | | |
|---|---|---|
| Pyruvate (100 ppm) | Pyruvate 200 ppm + magnetic NP 1.0 ppm | Acarbose |
| 4.64 | 7.62 | - |
| **α-glucsidase  IC50 (mg/ml)** | | |
| Pyruvate (100 ppm) | Pyruvate 200 ppm + magnetic NP 1.0 ppm | Acarbose |
| 0.75 | 0.75 | 0.34 |

**Table 10:** Cytotoxicity against HEPG-2 cell line of pyruvate 100 ppm and pyruvate 200 ppm + magnetic NP 1.0 ppm extract.

| Concentration | Percent cell viability HEPG-2 cell line | |
|---|---|---|
| | Pyruvate  (100 ppm) | Pyruvate 200 ppm + magnetic NP 1.0 ppm |
| 3.9 µg/ml | 100 | 100 |
| 7.8 µg/ml | 100 | 97.43 |
| 15.6 µg/ml | 98.15 | 89.54 |
| 31.25 µg/ml | 90.67 | 65.08 |
| 62.5 µg/ml | 69.54 | 38.71 |
| 125 µg/ml | 43.18 | 29.46 |
| 250 µg/ml | 23.72 | 12.88 |
| 500 µg/ml | 9.85 | 5.74 |

**Table 7:** α-amylase inhibitory activity of pyruvate 100 ppm and pyruvate 200 ppm + magnetic NP 1.0 ppm extract.

| Percent inhibition | | | |
|---|---|---|---|
| Concentration | Pyruvate (100 ppm) | Pyruvate 200 ppm + magnetic NP 1.0 ppm | Acarbose |
| 0.1 µg/ml | 2.15 | 1.99 | 17.00 |
| 1.0 µg/ml | 8.25 | 7.41 | 35.00 |
| 10 µg/ml | 20.4 | 16.3 | 54.90 |
| 100 µg/ml | 60.1 | 53.4 | 81.80 |
| 1000 µg/ml | 85.9 | 81 | 93.50 |

leaves, such as polyphenols, chlorogenic acid derivatives, and flavonoid glycosides, is increasing inhibition (Karaköse et al., 2015). The current findings are in harmony with Gupta et al. (2017), who found that steviol from stevia has a cytotoxic effect on breast (MCF-7) cells. Similarly, Jayaraman et al. (2008) discovered that *Stevia rebaudiana* is safe for normal cells while also having anticancer and antiproliferative properties against cancerous HEp2 cells (Table 11) [53-55].

### Antimicrobial activity:

The efficiency of *Stevia rebaudiana* extract against pathogenic bacterial and fungal strains with ethanol extract is shown in (Tables 12, 13). Ethanol extract with two treatments (pyruvate 100 ppm and pyruvate 200 ppm combined magnetic NP 1.0 ppm) showed activity against pathogenic microorganisms like *B. subtilis, S. aureus, Escherichia coli*, and *Proteus vulgaris*. Pyruvate 200 ppm combined with magnetic NP 1.0 ppm against bacteria was more effective than pyruvate

**Table 11:** Cytotoxicity against HEPG-2 cell line of pyruvate 100 ppm and pyruvate 200 ppm + magnetic NP 1.0 ppm extract.

| Concentration | Percent cell inhibition HEPG-2 cell line | |
|---|---|---|
| | Pyruvate (100 ppm) | Pyruvate 200 ppm + magnetic NP 1.0 ppm |
| 3.9 µg/ml | 0 | 0.00 |
| 7.8 µg/ml | 0 | 2.57 |
| 15.6 µg/ml | 1.85 | 10.46 |
| 31.25 µg/ml | 9.33 | 34.92 |
| 62.5 µg/ml | 30.46 | 61.29 |
| 125 µg/ml | 56.82 | 70.54 |
| 250 µg/ml | 76.28 | 87.12 |
| 500 µg/ml | 90.15 | 94.26 |

**Table 12:** Antimicrobial activity of the extracts of *Stevia rebaudiana*.

| Zone of inhibition (mm) | | | |
|---|---|---|---|
| Test organism | Pyruvate 100 ppm | Pyruvate 200 ppm + magnetic NP 1.0 ppm | Control |
| Fungi | | | |
| *Aspergillus fumigatus* | NA | NA | 17.00 |
| *Candida albicans* | NA | NA | 20.00 |
| Bacteria | | | |
| *Staphylococcus aureus* | 23 | 27 | 26.00 |
| *Bacillus subtillis* | 25 | 28 | 29.00 |
| *Escherichia colli* | NA | 8 | 31.00 |
| *Proteus vulgaris* | 25 | 31 | 30.00 |

**Table 13:** Minimal inhibitory concentration (MIC) of the extracts of *Stevia rebaudiana*.

| MIC (µg/ml) | | | |
|---|---|---|---|
| Test organism | Pyruvate 100 ppm | Pyruvate 200 ppm + magnetic NP 1.0 ppm | Control |
| Fungi | | | |
| *Aspergillus fumigatus* | NA | NA | 156.25 |
| *Candida albicans* | NA | NA | 312.50 |
| Bacteria | | | |
| *Staphylococcus aureus* | 156.25 | 78.13 | 9.70 |
| *Bacillus subtillis* | 78.13 | 78.13 | 4.80 |
| *Escherichia colli* | NA | 5000 | 4.80 |
| *Proteus vulgaris* | 156.25 | 39.06 | 4.80 |

100 ppm [56]. These results confirmed those of Abdel-Rahman et al. (2015), who found that ethanol stevia extract has antimicrobial action against *Bacillus subtilis* [57]. Also, Debnath (2008) found that *S. rebaudiana* leaves have been shown to have antibacterial efficiency for *Streptococcus mutants, Escherichia coli, Staphylococcus aureus*, and *Bacillus subtilis* [58]. Stevia has the natural potency to produce principles like phenols and their oxygen-substituted derivatives. This protects the plants from microbial infections and prevents them from dying (Jayaraman et al., 2008) [59]. Similarly, Bibi et al. (2021) indicated that the ethanolic extract of leaves showed the maximum inhibition against *Staphylococcus aureus* and *E. coli* [60]. Iron nanoparticles have antibacterial properties for their nanoscale size, which allows them to accumulate and deposit on bacteria during testing (Sandhya et al., 2020). Magnetic NPs' effectiveness against pathogenic bacteria can be explained by several mechanisms, including stability in an ambient environment, the generation of ROS (superoxide radical anions ($O_2$), hydroxyl radicals (OH), etc.), oxidative stress, and the release of ions by nanoparticles reacting with the bacteria's thiol groups (-SH), which can alter the cellular structure of microorganisms, thus interfering with DNA reproduction and inhibiting protein production (Arakha et al., 2015). Meanwhile, ethanol stevia extract with two treatments is most effective in inhibiting the growth of *Aspergillus fumigatus* and *Candida albicans*. They concluded stevia might have a function in being utilized as a pharmaceutical and/or preservative. The acetone extract showed excellent activity against Epidermophyton species and *Candida albicans* (Jayaraman et al. 2008). Barba et al. (2014) reported that the application of *Listeria monocytogenes* for 5 min with 2.5% (w/v) of stevia extract succeeded in activating over 5 log cycles of the bacteria. In this investigation, MIC determination with pyruvate 200 ppm combined with magnetic NP1.0 ppm achieved lower MIC values than pyruvate 100 ppm against B*acillus subtilis, Staphylococcus aureus, Escherichia coli*, and *Proteus vulgaris* [61-67].

## Conclusion

Based on the results of this study on *Stevia rebaudiana* plants, endorse spraying vegetation with pyruvate at 100 mg/L to maximize fresh and dry herb weights, sugar component concentration (stevioside and rebaudioside A), and anti-diabetic effectiveness.

Spraying with pyruvate 200@magnetic NPs 1.0 ppm is recommended to produce promising natural sources of anticancer drugs and antimicrobial medicines from stevia.

### References

1. Abdel-Rahman, Abdelwahed T, Elsaid MA, MAEE, Ei-Beih, et al. (2015) Free calorie sweetness and antimicrobial properties in *Stevia rebaudiana*. Res J Pharm Biol Chem Sci 6: 669-679.

2. Abou El-Nasr, El-Hennawy MK, El-Kereamy HM, Abou El-Yazied AMH, Salah Eldin A, et al. (2015) Effect of magnetite nanoparticles ($Fe_3O_4$) as nutritive supplement on pear saplings. Middle East J Appl Sci 5: 777-785.

3. Ali, Zafar A, Zia H, Phull, Ali AR, et al. (2016) Synthesis, characterization, applications, and challenges of iron oxide nanoparticles. Nanotechnol Sci Appl 9: 49-67.

4. Alshawwa, Mohammed SZ, Hashim EJ, Sharaf N, Selim M, et al (2022) In situ biosynthesis of reduced alpha hematite ($\alpha$-$Fe_2O_3$) nanoparticles by *Stevia rebaudiana* L. leaf extract: insights into antioxidant, antimicrobial, and anticancer properties. Antibiotics 11: 1252.

5. Arakha, Pal M, Samantarrai S, Panigrahi D, Mallick TK, et al. (2015) Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface. Scientific reports 5: 14813.

6. Bhandari, Srivastava NS, Tarakeshwari RK, Chand S (2022) Effect of nano and macro iron sprays on growth, flowering, seed and oil yielding attributes in calendula (*Calendula officinalis* L.). J Hortic Sci 17:1-10.

7. Bibi, Sarwar S, Sabir S, Nisa M, Khan, et al. (2021) Antimicrobial potential of Stevia rebaudiana leaves extract against selected bacterial and fungal strains. J Adv Nutr Sci Tech1: 157-163.

8. Bondarev N, Reshetnyak O, Nosov A (2001) Peculiarities of diterpenoid steviol glycoside production in vitro cultures of Stevia rebaudiana Bertoni . Plant Sci 161: 155-63.

9. Borgo, Laurella J, Martini LC, Catalán F, Sülsen, et al. (2021) Stevia genus: phytochemistry and biological activities update. Molecules 2733: 1-45.

10. Brandle, Starratt JE, Gijzen (1998) Stevia rebaudiana: Its agricultural, biological, and chemical properties. Can J Plant Sci78: 527-536.

11. Brittenham, GÁ (1994) New advances in iron metabolism, iron deficiency, and iron overload. Cur Opi Hem 1: 101-106.

12. Chen, Xia J, Sui Y, Peng X, Zhang Q, et al. (2018) Steviol, a natural product inhibits proliferation of the gastrointestinal cancer cells intensively. Oncotarget 9: 26299-26308.

13. Chin, Pang SF, Tan (2011) Green synthesis of magnetite NPs (via thermal decomposition method) with controllable sizeand shape. J Mater Env Sci 2: 299-302.

14. Chowdhury, Rahanur Alam AI, Raihan M, Rahman MM, Islam T, et al. (2022) Effect of stevia leaves (*Stevia rebaudiana* Bertoni) on diabetes: A systematic review and meta-analysis of preclinical studies. Food Sci Nutr 10: 2868-2878.

15. Debnath, M (2008) Clonal propagation and antimicrobial activity of an endemic medicinal plant *Stevia rebaudiana*. J Med Plants Res: 45-51.

16. Desmarchelier, Bermudez C, Coussio MJN, Ciccia J, Boveris, et al (1997) Antioxidant and prooxidant activities in aqueous extract of Argentine plants. International J Phar 1: 116-20.

17. Dytham C (1999) In: Choosing and Using Statistics: A Biologist's Guide Blackwell Science Ltd., London UK.

18. El-Saber, Mahdi MM, Hassan AA, Farroh AH, Osman A, et al. (2021) Effects of magnetite nanoparticles on physiological processes to alleviate salinity induced oxidative damage in wheat. J Sci Food Agri 101: 5550-5562.

19. El-Sirafy, Hassan Z, El-Shazly R, Gad M (2015) Role of bio and organic fertilizers in reducing some chemical fertilizers doses on yield of stevia plants under some different soil types. J Soil Sci Agri Eng 6: 829-844.

20. El-Sonbaty, Farouk AE, Al-Yasi, Ali HM, Abdel-Kader EF, et al. (2022) Enhancement of rose scented geranium plant growth, secondary metabolites, and essential oil components through foliar applications of iron (nano, sulfur and chelate) in alkaline soils. Agronomy 2164: 1-20.

21. Ferlay, Colombet J, Soerjomataram M, Parkin I, Piñeros DM, et al. (2021) Cancer statistics for the year 2020: An overview. International J Can 149: 778-789.

22. Ferlay, Colombet J, Soerjomataram M, Dyba I, Randi T, et al. (2018) Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. Euro J Can 103: 356-87.

23. Gaudel, Nongonierma C, Maher AB, Flynn S, Krause S (2013) A whey protein hydrolysate promotes insulinotropic activity in a clonal pancreatic β-cell line and enhances glycemic function in ob/ob mice. The J Nutr 143: 1109-1114.

24. Goyal, Samsher SK, Goyal RK (2010) Stevia (*Stevia rebaudiana*) a bio-sweetener: a review. International J Food Sci Nutr 61: 1-10.

25. Gupta, Agarwal SD, Pradhan S (2018) Phytostimulatory effect of silver nanoparticles (AgNPs) on rice seedling growth: An insight from antioxidative enzyme activities and gene expression patterns. Ecoto Env Safety 161: 624-633.

26. Heath, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives Bioch Bio 125: 189-198.

27. Hendawey, FADL MH, El-Din TS (2015) Biochemical role of some nanoparticles in the production of active constituents in *Stevia rebaudiana* L. callus Life Sci 12: 144-156.

28. Hossain, Shoaib MM, Mallick SM, Hore R, Tanmy TT, et al. (2013) An assessment of present status and related complications of Diabetes Mellitus (DM) at port city Chittagong in Bangladesh. Standard Res J Medi Medical Sci 1: 6-11.

29. Jain, VK (2017) Fundamentals of Plant Physiology. S. Chand Publishing.

30. Jan, Habib SA, Shinwari N, Ali ZK, Ali M, et al. (2021) Fundamentals of Plant Physiology. S. Chand Publishing. SN Applied Sciences 3:1-6.

31. Javed, Yucesan R, Zia, Gurel E (2017) Differential effects of plant growth regulators on physiology, steviol glycosides content, and antioxidant capacity in micropropagated tissues of *Stevia rebaudiana*. Biologia 72: 1156-1165.

32. Jayaraman, Manoharan S, Illanchezian S (2008) In-vitro antimicrobial and antitumor activities of *Stevia rebaudiana* (Asteraceae) leaf extracts. Tropical J Pharm Res 7: 1143-1149.

33. Kamaraj, Kidane M, Muluken T, Aravind J (2019) Biofabrication of iron oxide nanoparticles as a potential photocatalyst for dye degradation with antimicrobial activity. International J Env Sci Tech 16: 8305-8314.

34. Karaköse, Müller H, Kuhnert N (2015) Profiling and quantification of phenolics in *Stevia rebaudiana* leaves. J Agr Food Chem 63: 9188-9198.

35. Khan, Ali MA, Mohammad A, Ali S, Khan H, et al. (2020) Iron nano modulated growth and biosynthesis of steviol glycosides in *Stevia rebaudiana*. Plant Cell Tissue and Organ Culture (PCTOC) 143:121-130.

36. Khater MS (2015) Magnetite-nanoparticles effects on growth and essential oil of peppermint. Cur Sci Inter 4: 140-144.

37. Kovačević, Barba DB, Granato FJ, Galanakis D, Herceg CM, et al. (2018) Pressurized hot water extraction (PHWE) for the green recovery of bioactive compounds and steviol glycosides from *Stevia rebaudiana* Bertoni leaves. Food Chem 254: 150-157.

38. Mahdi, Suliman AA, Farroh MN, Ghaly KY, Hamed O, et al. (2022) Chitosan and amino acids-coated nanoparticles positively altered atropine accumulation, the gene expression of tropinone reductase 1 and the yield of *Datura innoxia* Mill. Plant Cell Biot Mol Bio 23: 26-50.

39. Mamatha N (2007) Effect of sulphur and micronutrients (iron and zinc) on yield and quality of cotton in a vertisol. Master Sci (Agriculture) col agri Dharwad University of Agri Sci India.

40. Marschner H (1995) Mineral nutrition of higher plants. Academic Press, London.

41. Miller, Huang GW, Welkie IJ, Pushmik JC (1995) Function of iron in plants with special emphasis on chloroplasts and photosynthetic activity. In: Abadia, J., (Ed.), Iron nutrition in soils and Plants. Kluwer Academic Publishers Dordecht 19-28.

42. Nagajyothi, Sreekanth PC, Tettey TVM, Jun CO, Mook SH, et al. (2014) Characterization, antibacterial, antioxidant, and cytotoxic activities of ZnO nanoparticles using Coptidis Rhizoma. Bio Medic Che Let 24: 4298-4303.

43. Ozkur, Ozdemir O, Bor F, Turkan I (2009) Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovata* Desf. To drought. Env Exp Botany 66: 487-492.

44. Pandurangan, Kim DH (2015) ZnO nanoparticles augment ALT, AST, ALP and LDH expressions in C2C12 cells. Saudi J Bio Sci 22: 679-684.

45. Rajasekaran, Ramakrishna T, Udaya Sankar A, Giridhar K, Ravishankar GA, et al. (2008) Analysis of predominant steviosides in *Stevia rebaudiana* Bertoni by liquid chromatography/electrospray ionization-mass spectrometry. Food Bio 22: 179-188.

46. Ranamukhaarachchi, Kan DG, Guy M.E, Li QB (2000) Modified AFLP technique for rapid genetic characterization in plants. Biotechniques 29: 858-866.

47. Rastegaran, Hassanpour MM, Ziyadi H (2022) Synthesized $Fe_3O_4$ nanoparticles induced antioxidant activity and total phenolic and flavonoid content in *Matricaria chamomilla* seedlings. Iranian Journal of Plant Physiology 12: 4003-4011.

48. Ruiz-Ruiz, Moguel-Ordoñez JC, Segura-Campos MR (2017) Biological activity of *Stevia rebaudiana* Bertoni and their relationship to health. Critical Reviews in Food Sci Nut 57: 2680-2690.

49. Saeedi, Petersohn P, Salpea I, Malanda P, Karuranga B, et al. (2019) Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. Diabetes Res Cli Practice 157: 107843.

50. Sandhya, Kalaiselvam S (2020) Biogenic synthesis of magnetic iron oxide nanoparticles using inedible borassus flabellifer seed coat: Characterization, antimicrobial, antioxidant activity and in vitro cytotoxicity analysis. Materials Res Exp 7: 015045.

51. Seghatoleslami, Feizi M, Mousavi H, Berahmand A (2015) Effect of magnetic field and silver nanoparticles on yield and water use efficiency of *Carum copticum* under water stress conditions. Polish J Che Tech 17: 110-4.

52. Sheikhbaglu, Sedghi R, Salehian M, Rahimzadeh S (2014) Spraying effect of maternal plants with nano-iron oxide on germination indices and electrical conductivity of produced soybean seeds. International J Bio 5: 22-27.

53. Silva, Dias FDAD, Fernandes MDS, Lacerda PD, Marcelino, et al. (2022) Calcium pyruvate as a salt stress mitigator in yellow passion fruit seedlings. Revista Brasileira de Engenharia Agrícolae Ambiental 8: 763-70.

54. Singla, Singla R, Jaitak V (2019) *Stevia rebaudiana* targeting α-amylase: An in-vitro and in-silico mechanistic study. Nat Product Res 33: 548-552.

55. Soliman, El-feky AS, Darwish E (2015) Alleviation of salt stress on *Moringa peregrina* using foliar application of nanofertilizers. J Horti Forestry 7: 36-47.

56. Stoyanova, Geuns S, Hideg J, Van Den Ende W (2011) The food additives inulin and stevioside counteract oxidative stress. International J Food Sci Nutr 62: 207-214.

57. Sung, Ferlay H, Siegel J, Laversanne RL, Soerjomataram M, et al. (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer J Cli 71: 209-49.

58. Taghizadeh, Nasibi M, Kalantari F, Ghanati F (2019) Evaluation of secondary metabolites and antioxidant activity in *Dracocephalum polychaetum* Bornm. Cell suspension culture under magnetite nanoparticles and static magnetic field elicitation. Plant Cell Tissue Org Cul136: 489-498.

59. Tavakoli, Tavakoli H, Moradi F (2019) The effect of the elicitors on the steviol glycosides biosynthesis pathway in *Stevia rebaudiana*. Functional Plant Bio 46: 787-795.

60. Tawfik, Mohamed MM, Sadak MH, Thalooth AT (2021) Iron oxide nanoparticles effect on growth, physiological traits and nutritional contents of *Moringa oleifera* grown in saline environment. Bulletin of National Res Centre 45: 1-9.

61. Vazquez-Hernandez, Feregrino-Perez C, Perez-Ramirez AA, Ocampo-Velazquez I, Rico-García RV, et al. (2019) Controlled elicitation increases steviol glycosides (SGs) content and gene expression-associated to biosynthesis of SGs in *Stevia rebaudiana* B. cv. Morita II. Industrial Crops and Products 139: 111479.

62. Velikova, Yordanov V, Edreva AJPS (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Science 151: 59-66.

63. Verma AK (2014) Anti-oxidant activities of biopolymeric nanoparticles: Boon or bane. J Phar Res 8: 871-876.

64. Xu, Zeng P, Huang GM, Feng DL, Huang Z, et al. (2012) Use of iron oxide nanomaterials in wastewater treatment: a review. Sci Total Env 424: 1-10.

65. Yang, Chen Q, Ren G, Wang H, Yu C, et al. (2012) Analysis of the Chemical constituents of *Stevia rebaudiana* and its sweetness. J Beijing University of Chem Tech 39: 28.

66. Yew, Shameli YP, Miyake K, Kuwano, Bt Mohamad NB, et al. (2016) Green synthesis of magnetite ($Fe_3O_4$) nanoparticles using seaweed (*Kappaphycus alvarezii*) extract. Nanoscale Res Letters 11: 276.

67. Zaidan, Hamid UH, Yusof SNM, Ahmad MFM, Abd Ghani S, et al. (2018) Chemical evaluation and antioxidant properties of extracts and essential oil from *Stevia rebaudiana* leaves. Malaysian Appl Bio 47: 15-23.