



## Article

# Interactive Effects of Arbuscular Mycorrhizal Inoculation with Nano Boron, Zinc, and Molybdenum Fertilization on Stevioside Contents of Stevia (*Stevia rebaudiana*, L.) Plants

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**Citation:** Zewail, R.M.Y.; Ali, M.; El-Gamal, I.S.H.; Al-Maracy, S.H.A.; Islam, K.R.; Elsadek, M.; Azab, E.; Gobouri, A.A.; ElNahhas, N.; Mohamed, M.H.M.; et al. Interactive Effects of Arbuscular Mycorrhizal Inoculation with Nano Boron, Zinc, and Molybdenum Fertilization on Stevioside Contents of Stevia (*Stevia rebaudiana*, L.) Plants. *Horticulturae* **2021**, *7*, 260. <https://doi.org/10.3390/horticulturae7080260>

Academic Editor: Moreno Toselli

Received: 30 June 2021

Accepted: 19 August 2021

Published: 22 August 2021

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**Abstract:** Stevia (*Stevia rebaudiana*, L.) is receiving increasing global interest as a diabetes-focused herb associated with zero-calorie stevioside sweetener glycoside production. This study was conducted to determine whether the arbuscular mycorrhiza (AM), as a biofertilizer integrated with nano boron (B), zinc (Zn), and molybdenum (Mo), would improve stevia growth and stevioside content. A factorial experiment with four replicates was conducted to evaluate the effect of AM at 0, 150, and 300 spore/g soil and three nano microelements B at 100 mg/L, Zn at 100 mg/L, and Mo at 40 mg/L on growth performance, stevioside, mineral contents, and biochemical contents of stevia. Results indicated that the combination of AM at 150 and B at 100 mg/L significantly increased plant height, number of leaves, fresh and dry-stem, and herbal g/plant during the 2019 and 2020 growing seasons. Chlorophyll content was increased by the combination between AM at 150 spore/g soil and B at 100 mg/L during both seasons. Stevioside content in leaves was increased by AM at 150 spore/g soil and B at 100 mg/L during the second season. In addition, N, P, K, Zn, and B in the leaf were increased by applying the combination of AM and nano microelements. Leaf bio constituent contents were increased with AM at 150 spore/g soil and B at 100 mg/L during both seasons. The application of AM and nano B can be exploited for high growth, mineral, and stevioside contents as a low-calorie sweetener product in stevia.

**Keywords:** chlorophyll; stevioside; sweetener; biofertilizer; diabetes; herbal

## 1. Introduction

Stevia is a perennial herb, whose co-products are widely used as a natural low-calorie sugar supplement for diabetic patients [1,2]. Its leaves are used to make sauces, herbal teas, soups, color enhancers, salads, fruit, and coffee, among others [3] it has a sweet taste due to the accumulation of di-terpene stevioside glycoside derivative with 300 times the

sweetening capacity of saccharine [4]. Stevia helps to adjust blood pressure, fight cavities, increase insulin production in the pancreas, and acts as a bactericidal mediator without any potential side effects [5,6].

Stevia is a versatile plant that can be grown in a variety of sites and environments [3]. Stevia is very adaptable. Nevertheless, under present management techniques, the production of stevia leaf biomass is both economically and environmentally incompatible. Sustainable stevia production, using and renewing underused soils and reducing the impacts of climate change, is critical for economic growth based on new and comprehensive techniques.

It is reported that inoculation of stevia by AM improves plant growth by influencing water and nutrient uptakes [7]. The most important impact of AM is the capability to scavenge P and micronutrients in nutrient-poor soils through its hyphae and make them available to growing plants [8,9]. Numerous studies found that using AM improved plant growth and enhanced the active components of medicinal substances, such as carotenoids, which boost public health through their antioxidants: sulphides, polyphenols, phepotos-terols, stilbenes, vitamins, lignans, and terpenoids [10–12]. According to previous studies, the AM inoculum provided C energy to support soil microbial diversity and functions [13], improved plant enzymatic functions [14], increased photosynthesis [15], improved biological N fixation [13], and enhanced plants response to root pathogens [16,17]. AMF promotes plant development and the absorption of several essential nutrients, including nitrogen and phosphorus, under adverse situations. This promotion of growth is linked to the AMF spread throughout the coat system. The areas of depletion of rhizosphere nutrients allow a higher volume of soil. Furthermore, fungal hyphae penetrate small holes and consume more nutrients than the root [18].

Nanotechnology has recently been investigated in agriculture to minimize the use and loss of reactive chemicals and nutrients while improving crop growth [19]. Nanoparticles are commonly less than 100 nm in size in the conversion zone between separate molecules and the consistent majority materials, where they exert mutually helpful and harmful effects on living cells [15]. Untreated release of nano-elements into the soil or growth medium is anticipated to fertilize the soil or growing media and prevent eutrophication and pollution of freshwater resources [20]. Crop productivity has increased significantly as a result of the use of chemical nutrition. However, the soil nutrient imbalance is prone, and the soil health and overall ecosystems are extinct, which in the longer run are serious impairs. It is also relevant to develop smart ingredients that can release elements to beleaguered areas and contribute to keeping the environment clean [21]. Boron is a vital element for plant growth, as it aids in the allocation of sugars and nutrients from leaves to the storage part (fruit) as well as for specific functions in plant production by improving pollination and seed quality. Boron is a significant micronutrient for root growth, cell division, and fruit production. Nano form becomes more efficient for elements' role through plant growth and development. On the other hand, zinc is a microelement involved in the production of tryptophan, which is the precursor of indole acetic acid (IAA), which is responsible for growth stimulation [22], and plays a vital role in the synthesis of carbonic anhydrase enzyme, which helps transport CO<sub>2</sub> during photosynthesis [23,24]. Molybdenum (Mo) is an erratic conversion element that has long been known as a critical microelement for plants [25]. The status of Mo for plants was first reported by Arnon, and Stout [26]. In almost all plant tissues, Mo is the smallest abundant essential microelement [27]. The requirements of plants for Mo are less than any other microelement [28]. Mo is inactive in biological systems until it becomes part of organic pterin molecules called Mo co-factor [29]. Plants, like other organisms, use Mo in specific enzymes (nitrate reductase, xanthine dehydrogenase, aldehyde oxidase, and sulfite oxidase) for redox reactions in specific processes involving the nitrogen metabolism and biosynthesis of phytohormones and indole-3 butyric acid [27]. The metabolism of nitrogen is partly through Mo, which acts directly on the reductase enzyme, which is a biological indication by Mo and ne in plants is the effect of this enzyme [30,31].

The bio assimilation of stevioside in stevia plants is affected by the environment and agronomic managing. Since *S. rebaudiana* comes from semi-humid and subtropic area, soil water content is one of the main measurable ecological aspects influencing plant morphology, physiology, and biochemistry [32]. Soil moisture content is one of the main measurable ecological aspects, affecting many characteristics of morphology, physiology, and biochemistry in plants, *Stevia rebaudiana* originated in a semi-humid and subtropic area [33–35].

While the leaf biomass is the main source of stevioside, the lateral growth of shoot production by means of stevioside is a serious issue in its production and development [35]. The growing demand for stevia sweeteners in the nutrition and pharmacological industries has led to its marketable manufacturing around the world. In Egypt, this plant has recently been introduced as a new marketable crop. This study aims to determine the role of AM as a biofertilizer in improving plant growth and expanding the active ingredients of medicinal compounds of stevia, as well as to evaluate the role of B, Zn, and Mo as nano-form on growth aspects, chemical composition, and stevioside content in stevia plants.

## 2. Materials and Methods

### 2.1. Experimental Site Characteristics

A field experiment was established at the Sugar Crops Research Institute, Kafr Elsheikh Governorate, Egypt (31°09' N latitude and 30°94' E longitude at 13 m above mean sea level), during two successive growing seasons in 2019 and 2020. The area is mainly characterized as an arid climate, with mean annual temperatures ranging between 12.5 °C and 27 °C. There is an enormous difference between the average annual evaporation (2750–2800 mm) and the mean annual rainfall (70–80 mm), meaning irrigation is a chief requirement for stevia production in this zone. Soil is loamy with sand 26.6%, silt 31.9%, clay 41.5%, pH 7.2, organic matter 20.2 g/kg, total nitrogen 0.23 g/kg, total phosphorus 0.83 g/kg, exchangeable Na 17.5 mg/kg, K 4.7 mg/kg, Ca 234.2 mg/kg, Mg 20.8 mg/kg, Cl 15.9 mg/kg, and SO<sub>4</sub> 8.2 mg/kg.

### 2.2. Experimental Design and Cultural Practices

A split-plot experiment was laid out with AM inoculum as the main plot and nano fertilizers as a sub-plot, replicated three times in 3 m × 3.5 m plots with a 1m buffer between plots. AM was applied at 0, 150, and 300 spore/g soil, and the nano B, Zn, and Mo were applied at 100, 100, and 40 mg/L, respectively. The AM inoculum was obtained from the Soil, Water, and Microbiology Institute of the Agriculture Research Center in Egypt. The nano fertilizers were prepared at the Lazier Institute of Cairo University, Egypt.

Stevia seedlings were collected from the Sugar Crops Research Institute of Egypt. The seedlings were approximately 15 to 17 cm tall with 6 to 7 leaves. The seedlings were growing under partial shade for five days before transplanting in the field during the first week of May for both growing seasons. The seedlings were planted in rows 60 cm apart and 35 cm between plants within rows (a total of 47,000 seedlings/ha).

The AM inoculum was made up of *Glomus mosseae*-NRC31 and *Glomus fasciculata*-NRC15, which were initially isolated from Egyptian soils; it was allowed to grow on sterilized peat-vermiculite-perlite mixtures and added at 0, 150, and 300 spore/g to the soil pits immediately before planting of stevia and it was repeated twice with irrigation for each cut. The nano-fertilizers were exposed to the high-power laser ray and applied as foliar application four times at 20-d intervals for each cut. During the 2019 and 2020 seasons, all other activities were performed in accordance with standard cultural practices.

### 2.3. Data Collection, Processing, and Chemical Analyses

Ten random plants were selected from each treatment to measure plant height (cm), number of leaves/plants, number of main branch/plant, fresh and dry weight of leaf biomass (g/plant), fresh and dry weights of herb yield (g/plant), fresh and dry weights of stems (g/plant), and branches on the main stem (after the first and second cuts in both

seasons) were recorded and collected. The fresh weight of different plant organs (i.e., stem, branches, and leaves) was air-dried under shade for one week, before being oven dried at 45 °C for 48 h to achieve a constant weight. The dry biomass samples were ground with a porcelain mortar and pestle followed by sieving with a 125 µm mesh and storing in an airtight container prior to chemical analysis.

Stevioside content in processed leaves was extracted and analyzed by following the method described by [36], which included two steps: solvent removal and isocratic HPLC analysis. The sample, 1 g of dried *S. rebaudiana* leaves, was crushed and solvent was extracted by shaking for 30 min. in a water bath at 70 °C with 100 mL EtOH 70% (*w/w*) in 250% Erlenmeyer flasks. Once the extract was cooled, it was filtered, then 5 µL HPLC examined to the solution in the flask. Quantitative results for each analysis were obtained from standard solutions of pure stevioside and rebaudioside A, using an external standard calibration curve. Chlorophyll A, B, and carotenoids were analyzed according to the procedure of [37]. Nitrogen, phosphorus, and potassium in leaf concentration were determined by [37]. The micronutrients B, Zn, and Mo in leaves were also measured [38]. Total and reducing sugars, as well as total carbohydrates were determined according to the procedure described by [37]. The non-reducing sugar was calculated as total sugars (%)—[reducing sugars (%) × 0.95].

#### 2.4. Statistical Analyses

Multivariate statistics were performed to evaluate the predictor variables (AM inoculum and nano fertilization) on dependent variables of stevia using analysis of variance procedure of the CROPSTAT® 2007.2 program. Both AM inoculum and nano fertilizer were considered as fixed variables, the main plot, sub-plot, and their interaction were means separated by F-protected Duncan Multiple Range Test (DMRT) at  $p \leq 0.05$  unless otherwise mentioned.

### 3. Results

#### 3.1. Vegetative Growth

Arbuscular mycorrhiza (AM) significantly increased plant growth when applied at 150 and 300 spore/g alone or in combination with foliar fertilization of B, Zn, and Mo at 100, 100, and 40 mg/L, respectively (Table 1). At 70 days after transplanting (DAT), plant height, number of branches, number of leaves per plant, and dry weight of leaves were compared with untreated control. The superior treatment was the combination between AM at 150 spore/g and foliar spraying with nano B at 100 mg/L during the first and second seasons, followed by the combination between AM at 150 spore/g and nano Mo at 40 mg/L during both seasons. Meanwhile, applying AM at 300 spore/g alone or in combination with nano B at 100 mg/L, Zn at 100 mg/L, and Mo at 40 mg/L significantly increased vegetative growth traits compared to untreated control but not to the level of 150 spore/g soil with nano Br At 100 mg/L. Moreover, foliar spraying of B, Zn, and Mo increased plant height, number of branches, number of leaves per plant, and dry weight of leaves compared to the control but did not give the highest level with these traits.

Fresh weight (f.w.) of stem g/plant, dry weight of stem, fresh weight of herb/plant, and dry weight of herb were significantly increased by AM. The two rates (i.e., 150 and 300 spore/g) foliar spraying with nano micronutrients (i.e., B at 100 mg/L, Zn at 100 mg/L, and Mo. at 40 mg/L) and the interaction between them at 70 DAT during the 2019 and 2020 seasons are presented in Table 2. The highest value of these traits was obtained by combining AM at 150 spore/g and B at 100 mg/L, yielding 128%, 81%, 30%, and 37% more than the control (untreated treatment) for stem dry weight and herbal dry weight during first and second seasons. The second increase of these traits was the interaction between AM at 300 spore/g and Mo at 40 mg/L, which gave 118%, 71%, 26%, and 27% more than the control for stem dry weight and herbal dry weight during the 2019 and 2020 seasons.

**Table 1.** Effect of arbuscular mycorrhiza (AM) and nano B, Zn, and Mo microelements on growth characteristics of stevia plants during 2019 and 2020 seasons.

Main-Plot Sub-Plot		Plant ht. (cm)		No. of Branches/Plant		Leaf Number/Plants		Leaf Dry Weight (g/plant)	
AM (g)	Nano Fertilizer (mg/L)	2019	2020	2019	2020	2019	2020	2019	2020
Main-plot effect									
	0 (control)	66.7 ± 1.0 C	55.2 ± 0.8 C	8.7 ± 0.2 C	7.2 ± 0.1 C	456.7 ± 4.2 C	579.7 ± 6.5 C	52.5 ± 1.3 C	66.8 ± 1.6 C
	150 AM spore/g soil	71.6 ± 3.2 D	56.2 ± 0.7 C	11.1 ± 1.1 A	7.8 ± 0.5 B	741.3 ± 6.8 A	716.7 ± 6.3 A	57.4 ± 3.5 B	79.6 ± 4.1 A
	300 AM spore/g soil	67.7 ± 5.5 C	57.3 ± 2.6 C	10.8 ± 1.8 AB	7.5 ± 0.9 C	523.7 ± 8.9 AB	508.4 ± 8.2 C	54.4 ± 5.6 B	73.5 ± 6.7 AB
Sub-plot effect									
	Control	66.7 ± 0.2 C	55.2 ± 0.1 C	5.5 ± 0.5 D	6.1 ± 0.2 C	373.5 ± 3.9 D	283.3 ± 2.7 C	36.9 ± 2.1 C	49.8 ± 3.2 C
	B-100 mg/L	74.8 ± 0.5 B	68.2 ± 0.4 B	10.5 ± 0.8 A	8.1 ± 0.4 A	505.7 ± 12.9 A	733.3 ± 8.5 A	58.2 ± 6.9 A	67.2 ± 7.8 A
	Zn-100 mg/L	78.8 ± 0.9 A	71.1 ± 0.6 A	10.3 ± 0.9 A	7.3 ± 0.3 AB	507.9 ± 20.3 A	525.1 ± 18.6 A	56.8 ± 7.3 A	87.1 ± 8.9 A
	Mo-40 mg/L	73.7 ± 0.9 B	68.8 ± 0.6 C	8.6 ± 1.0 AB	7.3 ± 0.5 AB	439.6 ± 23.9 B	777.1 ± 28.6 A	57.9 ± 4.2 A	62.9 ± 4.8 A
Main-plot x sub-plot									
0 (control)	B 100 mg/L	73.7 ± 1.1 b	68.7 ± 1.0 b	8.9 ± 1.0 ab	7.3 ± 0.5 ab	439.6 ± 20.3 b	777.1 ± 26.7 a	57.9 ± 11.6 a	62.9 ± 10.1 a
	Zn 100 mg/L	74.8 ± 3.2 b	68.2 ± 3.0 b	10.5 ± 1.8 a	8.0 ± 0.9 a	505.7 ± 7.0 a	733.3 ± 22.4 a	58.2 ± 7.1 a	67.2 ± 7.6 a
	Mo 40 mg/L	78.9 ± 5.5 a	71.1 ± 4.1 a	10.3 ± 3.8 a	7.3 ± 2.1 ab	507.9 ± 7.6 a	525.1 ± 7.9 a	56.8 ± 2.9 a	87.1 ± 4.1 a
	control	66.7 ± 3.7 c	55.2 ± 2.6 c	5.5 ± 0.9 d	6.1 ± 0.5 c	373.5 ± 34.7 d	283.3 ± 23.6 c	36.0 ± 7.0 c	49.9 ± 8.2 c
Mean		73.53	65.9	8.72	7.2	456.7	579.7	52.5	66.7
Mycorrhiza at 150 spore/g soil	B 100 mg/L	81.6 ± 0.5 a	79.3 ± 0.4 a	13.7 ± 2.0 a	8.9 ± 1.0 a	982.1 ± 43.1 a	993.2 ± 41.2 a	77.0 ± 0.7 a	104.3 ± 5.1 a
	Zn 100 mg/L	76.8 ± 0.2 ab	75.4 ± 0.2 a	10.7 ± 2.1 a	7.6 ± 1.0 bc	642.0 ± 64.2 b	750.0 ± 65.6 a	53.2 ± 5.8 cd	84.6 ± 7.3 b
	Mo 40 mg/L	79.7 ± 3.9 a	77.0 ± 3.8 a	12.9 ± 1.3 a	8.4 ± 0.6 a	975.8 ± 67.0 a	848.3 ± 60.2 a	68.7 ± 4.2 ab	89.6 ± 7.5 ab
	Control	71.5 ± 2.1 c	56.2 ± 1.5 c	7.0 ± 1.3 d	6.1 ± 1.0 d	365.3 ± 33.2 c	275.1 ± 28.2 d	30.6 ± 2.5 f	39.9 ± 3.2 f
Mean		77.4	72.0	11.1	7.7	741.3	716.7	57.4	79.6
Mycorrhiza 300 spore/g soil	B 100 mg/L	79.7 ± 0.6 a	69.6 ± 0.3 a	12.9 ± 3.5 a	8.3 ± 3.0 a	674.4 ± 74.8 a	659.5 ± 70.2 a	53.5 ± 13.4 b	84.9 ± 16.3 a
	Zn 100 mg/L	72.4 ± 1.3 b	60.7 ± 0.5 c	11.1 ± 1.9 bc	7.9 ± 1.1 bc	572.9 ± 43.5 a	582.0 ± 42.1 a	66.9 ± 16.1 a	86.1 ± 18.7 a
	Mo 40 mg/L	73.3 ± 0.9 ab	63.9 ± 0.4 b	11.7 ± 1.1 ab	8.2 ± 0.5 a	553.6 ± 48.9 a	544.2 ± 45.2 a	67.2 ± 7.2 a	81.3 ± 8.1 a
	Control	67.7 ± 0.5 c	57.3 ± 0.2 c	7.5 ± 1.7 f	5.4 ± 0.8 d	293.8 ± 14.9 f	247.8 ± 12.3 f	30.1 ± 1.9 d	41.8 ± 2.1 d
Mean		73.3	62.9	10.8	7.4	523.7	508.4	54.4	73.5
LSD <sub>p</sub> ≤ 0.05		1.3	2.2	0.6	0.7	14.1	13.1	2.1	1.6

The data represent the means ± standard deviation (SD) of four replications. In the same column, means followed by the same capital letter are not statistically different, according to the Duncan Multiple Range Test (DMRT) at  $p \leq 0.05$ , Interactions take lower case letters, unless otherwise mentioned.

**Table 2.** Effect of arbuscular mycorrhiza (AM) and nano B, Zn, and Mo microelements on growth characteristics of stevia plants during 2019 and 2020 seasons.

Main-Plot Sub-Plot		Fresh Weight of Stem g/plant		Dry Weight of Stem g/plant		Fresh Weight of Herb g/plant		Dry Weight of Herb g/plant	
AM (g)	Nano Fertilizer (mg/L)	2019	2020	2019	2020	2019	2020	2019	2020
Main-plot effect									
	0 (Control)	83.8 ± 2.6 F	34.8 ± 2.7 D	10.4 ± 0.2 D	11.5 ± 0.2 D	161.9 ± 1.2 D	160.0 ± 1.1 F	46.4 ± 0.2 C	46.7 ± 0.2 C
	150 AM spore/g soil	135.2 ± 3.7 D	140.9 ± 3.4 C	13.6 ± 0.6 D	16.5 ± 0.7 C	268.2 ± 1.6 D	265.7 ± 1.5 C	45.5 ± 0.6 F	44.7 ± 0.6 F
	300 AM spore/g soil	75.0 ± 6.7 F	83.2 ± 7.6 D	12.0 ± 1.2 F	13.3 ± 1.3 C	153.2 ± 3.6 D	158.1 ± 3.2 D	31.6 ± 0.7 C	31.0 ± 0.7 C
Sub-plot effect									
	Control	83.8 ± 1.2 F	34.6 ± 1.0 D	10.4 ± 1.1 D	11.5 ± 1.2 D	161.9 ± 1.5 D	160.0 ± 1.2 F	46.4 ± 0.3 C	46.7 ± 0.3 C
	B-100 mg/L	132.2 ± 4.2 A	108.3 ± 3.8 A	19.9 ± 2.5 A	19.0 ± 2.4 A	185.1 ± 3.6 A	192.7 ± 3.2 A	57.6 ± 1.6 A	57.5 ± 1.6 A
	Zn-100 mg/L	143.5 ± 6.4 A	110.0 ± 3.6 A	19.0 ± 3.7 A	16.1 ± 2.3 B	175.3 ± 4.9 B	184.8 ± 5.0 A	52.3 ± 2.6 A	53.8 ± 2.4 A
	Mo-40 mg/L	164.4 ± 4.3 A	88.6 ± 4.9 A	17.4 ± 5.4 A	17.3 ± 5.1 B	184.0 ± 5.7 A	186.3 ± 5.8 A	56.8 ± 3.8 A	55.6 ± 3.6 A
Main-plot x Sub-plot									
0 (Control)	B 100 mg/L	132.2 ± 8.6 a	108.3 ± 7.3 a	19.9 ± 1.1 a	19.0 ± 1.0 a	185.1 ± 2.5 a	192.7 ± 2.8 a	57.6 ± 1.3 a	57.6 ± 1.2 a
	Zn 100 mg/L	143.5 ± 6.4 a	110.0 ± 4.2 a	19.0 ± 0.5 a	16.1 ± 0.4 b	175.3 ± 5.6 b	184.8 ± 6.1 a	52.3 ± 1.5 a	53.8 ± 1.6 a
	Mo 40 mg/L	164.4 ± 8.6 a	88.6 ± 3.1 a	17.4 ± 0.8 a	17.3 ± 0.8 b	184.0 ± 2.7 a	186.3 ± 2.8 a	56.8 ± 0.3 a	55.6 ± 0.4 a
	Control	83.8 ± 9.5 f	34.8 ± 4.6 d	10.4 ± 0.4 d	11.5 ± 0.5 d	161.9 ± 1.6 d	160.0 ± 1.5 f	46.4 ± 1.7 c	46.7 ± 1.6 c
Mean		131.0	85.4	16.7	16.0	176.6	180.9	53.3	53.4
Mycorrhiza at 150 spore/g soil	B 100 mg/L	228.6 ± 18.4 a	189.5 ± 8.2 a	31.2 ± 3.1 a	30.0 ± 3.0 a	295.5 ± 2.1 a	295.1 ± 2.0 a	59.3 ± 0.4 a	61.7 ± 0.5 a
	Zn 100 mg/L	159.3 ± 24.4 a	174.2 ± 21.12 a	28.6 ± 2.1 a	27.0 ± 2.0 b	286.4 ± 0.12 a	289.9 ± 0.12 a	50.5 ± 1.3 b	52.5 ± 1.4 b
	Mo 40 mg/L	191.9 ± 25.4 a	181.3 ± 23.1 a	29.6 ± 0.7 a	28.4 ± 0.6 a	294.7 a ± 7.5	292.1 ± 7.0 a	57.4 ± 0.9 a	57.1 ± 0.9 ab
	Control	135.2 ± 5.3 d	140.9 ± 4.3 c	13.6 ± 0.8 d	16.5 ± 0.9 c	268.2 ± 1.5 d	265.7 ± 1.7 c	45.5 ± 1.0 f	44.7 ± 1.0 f
Mean		178.7	171.4	25.8	25.5	286.0	285.7	53.2	54.0
Mycorrhiza 300 spore/g soil	B 100 mg/L	163.5 ± 23.5 a	145.2 ± 21.6 a	27.5 ± 0.6 a	25.9 ± 0.4 a	189.5 ± 1.3 a	178.7 ± 1.1 a	49.5 ± 0.7 a	49.1 ± 0.6 a
	Zn 100 mg/L	149.9 ± 11.8 a	113.2 ± 9.6 a	25.3 ± 0.9 a	26.5 ± 1.0 a	176.2 ± 7.5 a	172.3 ± 7.2 a	47.3 ± 1.6 b	47.4 ± 1.6 a
	Mo 40 mg/L	157.2 ± 16.2 a	135.9 ± 8.6 a	26.5 ± 0.5 a	25.2 ± 0.4 a	183.3 ± 11.7 a	176.8 ± 10.3 a	48.1 ± 0.8 a	48.1 ± 0.7 a
	Control	75.0 ± 5.5 f	83.2 ± 6.1 d	11.9 ± 1.7 f	13.3 ± 1.8 c	153.2 ± 8.0 d	158.1 ± 8.5 d	31.6 ± 0.7 c	31.0 ± 0.6 c
Mean		136.4	119.3	22.8	22.7	75.5	71.5	44.1	43.9
LSD <sub>p</sub> ≤ 0.05		14.1	8.2	1.1	1.6	4.7	2.7	2.3	1.4

The data represent the means ± standard deviation (SD) of four replications. In the same column, means followed by the same capital letter are not statistically different, according to the Duncan Multiple Range Test (DMRT) at  $p \leq 0.05$ , Interactions take lower case letters, unless otherwise mentioned.



Generally, the combination between AM and B, Zn, and Mo nano micronutrients at different levels, significantly increased biomass production and vegetative growth aspects of stevia at 70 DAT during both seasons.

### 3.2. Chlorophyll Contents

Chlorophyll A content of stevia leaves was increased from 1.05 mg/100 g fresh weight to 1.26 and 1.31 mg/100 g f. w. for control and nano B at 100 mg/L during both seasons. In addition, chlorophyll A significantly increased with other nano microelements (i.e., Zn at 100 mg/L and Mo at 40 mg/L) compared with the control. Furthermore, AM alone had no effect on chlorophyll content. Meanwhile, the interaction between AM at 150 spore/g and nano B at 100 mg/L produced the highest value of chlorophyll A when compared with the control at 70 DAT during the first and second seasons. The interaction between AM at 300 spore/g and nano B at 100 mg/L, Zn at 100 mg/L, and Mo at 40 mg/L increased chlorophyll A but did not reach the highest value during both seasons. Chlorophyll b and carotenoids recorded the same sequence with AM at 150 spore/g and 300 spore or B at 100 mg/L, Zn at 100 mg/L, and Mo at 40 mg/L alone the combination between them at different levels at 70 DAT during 2019 and 2020 seasons. The interaction between AM at 150 spore/g and B at 100 mg/L recorded the highest value for these traits compared with untreated treatments during the first and second seasons. Combination of AM at 150 spore/g with Mo at 40 mg/L reached the second highest value of these traits during first and second seasons. Generally, all other treatments significantly increased chlorophyll B and carotenoid content compared to the control during both seasons. Chlorophyll A and chlorophyll B were increased by all applied treatments (i.e., AM at 150 and 300 spore/g and nano B at 100 mg/L, Zn at 100 mg/L and Mo at 40 mg/L) at 70 DAT during the 2019 and 2020 seasons. During both seasons, the highest response was obtained with applying AM at 150 spore and nano B at 100 mg/L (Table 3).

### 3.3. Stevioside Contents

Data presented in Figures 1 and 2 showed that soil addition of AM at 150 spore/g combined with foliar spraying of nano B at 100 mg/L resulted in a significant increase in stevioside content in stevia plants during the second growing season when compared with other treatments and the control. Inoculation with AM at 150 spore/g + Zn at 100 mg/L show the second highest stevioside content in stevia plants, followed by AM at 150 spore/g + Mo at 40 mg/L.

### 3.4. Nutrient and Sugar Contents

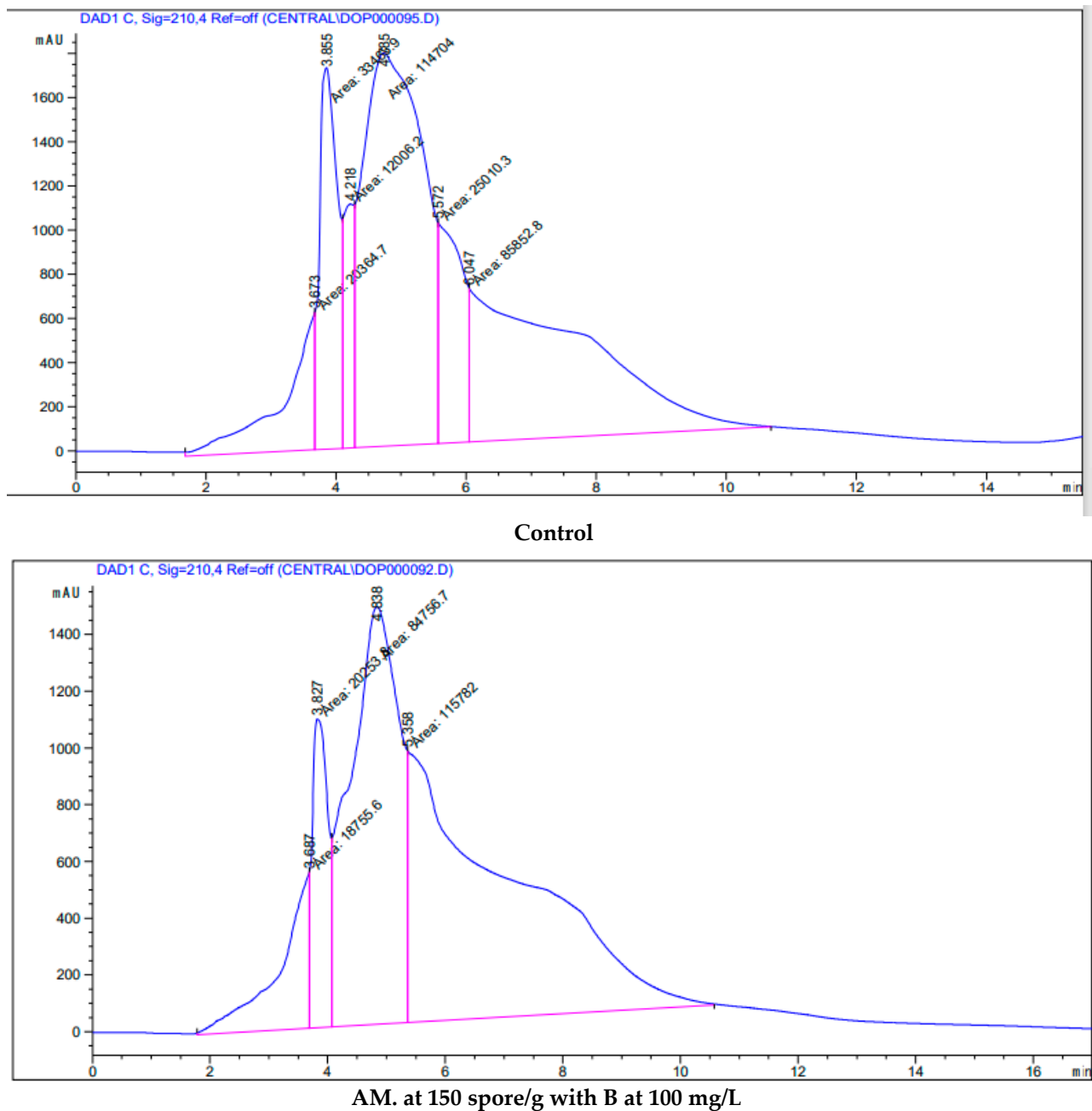
Leaf mineral concentrations i.e., (N, P, K, B, and Zn) were increased by soil additions of AM at 150 and 300 spore/g and foliar application of nano B at 100 mg/L, Zn at 100 mg/L, and Mo at 40 mg/L, alone or in combination during 2019 and 2020 seasons (see Table 4). The combination between AM at 150 spore/g with nano B at 100 mg/L significantly increased these parameters more than control by 34.4%, 39.2%, 157.9%, 161.1%, 38.7%, 68.7%, 198.2%, 198.5%, 84.7%, and 99.4% for N, P, K, B, and Zn during 2019 and 2020 seasons, respectively. The subsequent most effective treatment was AM at 150 spore/g with Mo at 40 mg/L when compared to the control during the first and second seasons. AM at 150 spore/g with nano B at 100 mg/L was the best treatment during both seasons.

**Table 3.** Effect of arbuscular mycorrhiza (AM) and nano B, Zn, and Mo microelements on chlorophyll contents of stevia leaves at 70 days after transplanting during 2019 and 2020 seasons.

Main-Plot Sub-Plot		Chl. a Mg/100 f. w		Chl. b Mg/100 f.w.		Chl. a + b Mg/100 g f.w.		Carotenoids	
AM (g)	Nano Fertilizer (mg/L)	2019	2020	2019	2020	2019	2020	2019	2020
Main-plot effect									
	0 (Control)	1.05 ± 0.0 F	1.05 ± 0.0 F	0.30 ± 0.1 D	0.40 ± 0.01 C	1.35 ± 0.01 F	1.520 ± 0.02 F	0.32 ± 0.05 D	0.30 ± 0.05 D
	150 AM spore/g soil	1.04 ± 0.1 F	1.12 ± 0.1 D	0.44 ± 0.1 F	0.43 ± 0.01 F	1.48 ± 0.13 F	1.55 ± 0.12 F	0.33 ± 0.04 D	0.30 ± 0.05 D
	300 AM spore/g soil	1.17 ± 0.1 D	1.12 ± 1 D	0.42 ± 0.1 D	0.40 ± 0.1 F	1.57 ± 0.11 D	1.53 ± 0.12 D	0.29 ± 0.07 D	0.37 ± 0.07 D
Sub-plot effect									
	Control	1.05 ± 0.0 F	1.05 ± 0.0 F	0.30 ± 0.1 D	0.40 ± 0.1 C	1.35 ± 0.03 F	1.52 ± 0.05 F	0.32 ± 0.05 D	0.30 ± 0.05 D
	B-100 mg/L	1.26 ± 0.1 A	1.31 ± 0.1 A	0.41 ± 0.0 B	0.43 ± 0.02 B	1.67 ± 0.02 A	1.74 ± 0.03 A	0.43 ± 0.02 BA	0.34 ± 0.02 B
	Zn-100 mg/L	1.17 ± 0.1 A	1.10 ± 0.1 B	0.46 ± 0.0 A	0.45 ± 0.03 A	1.63 ± 0.13 A	1.55 ± 0.14 A	0.47 ± 0.03 A	0.39 ± 0.03 A
	Mo-40 mg/L	1.11 ± 0.1 AB	1.17 ± 0.1 A	0.40 ± 0.03 B	0.45 ± 0.04 A	1.51 ± 0.16 A	1.62 ± 0.17 A	0.46 ± 0.05	0.39 ± 0.05 A
Main-plot x Sub-plot									
0 (control)	B 100 mg/L	1.26 ± 0.1 a	1.31 ± 0.1 a	0.41 ± 0.1 b	0.43 ± 0.1 b	1.67 ± 0.17 a	1.74 ± 0.18 a	0.43 ± 0.05 b	0.34 ± 0.04 b
	Zn 100 g/L	1.17 ± 0.1 a	1.10 ± 0.1 b	0.46 ± 0.1 a	0.45 ± 0.1 a	1.63 ± 0.18 a	1.55 ± 0.21 a	0.47 ± 0.06 a	0.39 ± 0.06 a
	Mo 40 mg/L	1.11 ± 0.1 ab	1.17 ± 0.1 a	0.40 ± 0.1 b	0.45 ± 0.1 a	1.51 ± 0.22 a	1.62 ± 0.23 a	0.46 ± 0.05 a	0.39 ± 0.05 a
	Control	1.05 ± 0.1 f	1.05 ± 0.1 f	0.30 ± 0.03 d	0.40 ± 0.3 c	1.35 ± 0.24 f	1.52 ± 0.24 f	0.32 ± 0.03 d	0.30 ± 0.03 d
Mean		1.15	1.16	0.39	0.45	1.54	1.61	0.42	0.36
Mycorrhiza at 150 spore/g soil	B 100 mg/L	1.56 ± 0.1 a	1.62 ± 0.1 a	0.69 ± 0.03 a	0.67 ± 0.03 a	2.25 ± 0.13 a	2.29 ± 0.15 a	0.58 ± 0.05 a	0.59 ± 0.05 a
	Zn 100 mg/L	1.38 ± 0.1 ab	1.42 ± 0.1 ab	0.52 ± 0.04 a	0.55 ± 0.04 ab	1.90 ± 0.08 ab	1.97 ± 0.09 a	0.54 ± 0.05 b	0.51 ± 0.05 b
	Mo 40 mg/L	1.54 ± 0.1 a	1.61 ± 0.1 a	0.59 ± 0.03 a	0.64 ± 0.03 a	2.13 ± 0.13 a	2.25 ± 0.13 a	0.56 ± 0.05 a	0.53 ± 0.05 a
	Control	1.04 ± 0.1 f	1.12 ± 0.0 d	0.44 ± 0.03 f	0.43 ± 0.04 f	1.48 ± 0.11 f	1.55 ± 0.12 f	0.33 ± 0.06 d	0.30 ± 0.06 d
Mean		1.38	1.44	0.56	0.57	1.94	2.01	0.52	0.48
Mycorrhiza 300 spore/g soil	B 100 mg/L	1.32 ± 0.2 a	1.35 ± 0.1 a	0.48 ± 0.1 a	0.48 ± 0.1 a	1.80 ± 0.23 a	1.83 ± 0.25 a	0.49 ± 0.07 a	0.48 ± 0.07 a
	Zn 100 mg/L	1.36 ± 0.1 a	1.37 ± 0.1 a	0.47 a ± 0.01	0.48 ± 0.02 a	1.83 ± 0.04 a	1.85 ± 0.05 a	0.48 ± 0.08 a	0.45 ± 0.08 a
	Mo 40 mg/L	1.33 ± 0.1 a	1.39 ± 0.1 a	0.52 ± 0.0 a	0.53 ± 0.01 a	1.85 ± 0.05 a	1.92 ± 0.06 a	0.46 ± 0.05 a	0.47 ± 0.05 a
	Control	1.17 ± 0.0 d	1.12 ± 0.0 d	0.42 ± 0.03 d	0.40 ± 0.04 f	1.57 ± 0.07 d	1.53 ± 0.08 d	0.29 ± 0.02 d	0.37 ± 0.2 d
Mean		1.30	1.31	0.48	0.49	1.78	1.79	0.43	0.44
LSD <sub>p</sub> ≤ 0.05		00.05	0.03	0.07	0.06	0.02	0.03	0.05	0.02

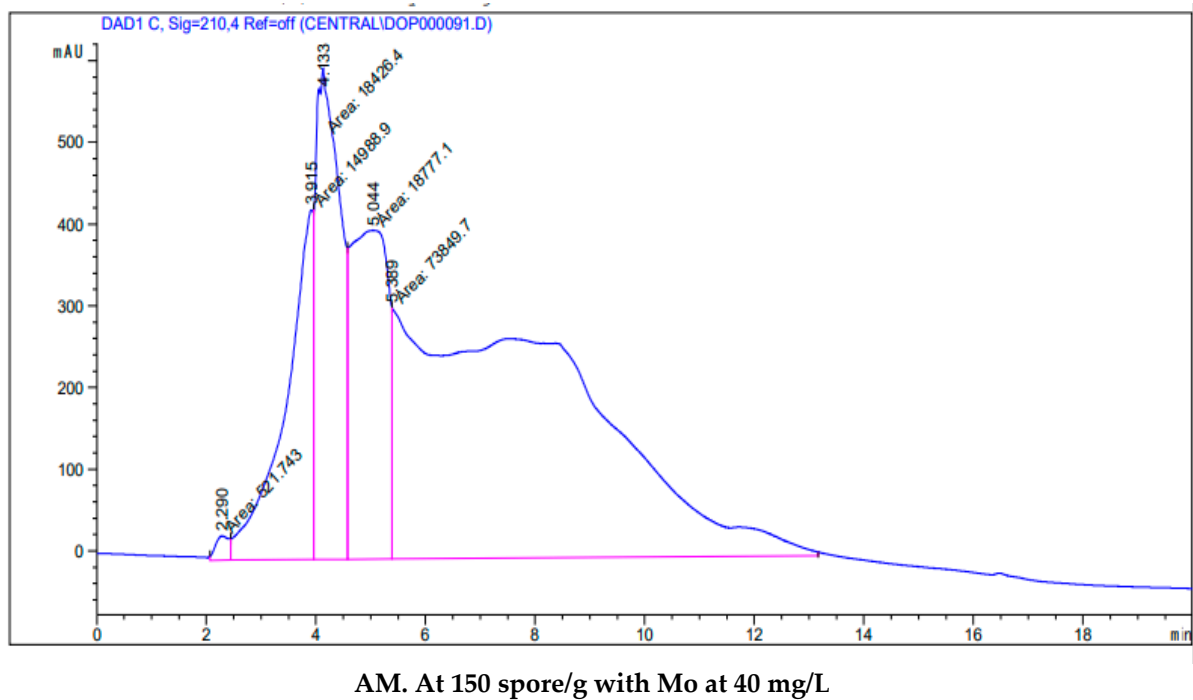
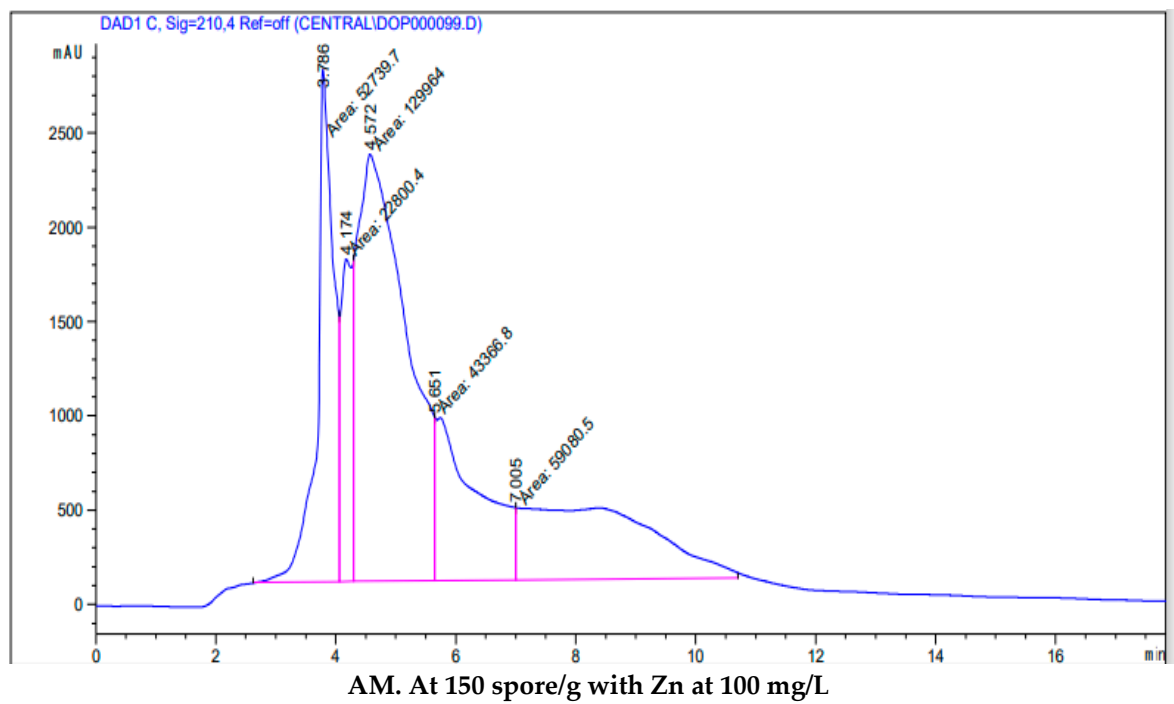
The data represent the means ± standard deviation (SD) of four replications. In the same column, means followed by the same capital letter are not statistically different, according to the Duncan Multiple Range Test (DMRT) at  $p \leq 0.05$ , interactions take lower case letters, unless otherwise mentioned.





**Figure 1.** Effect of Arbuscular Mycorrhiza (AM) and Nano B, Zn, and Mo nano microelements on stevioside contents of stevia leaves during 2020 season.

According to the data in Table 5, the combination between AM at 150 spore/g and B at 100 mg/L resulted in the highest value for total sugars, reducing and non-reducing sugars, and total carbohydrates in leaves during both seasons. Using nano foliar spray of B at 100 mg/L, Zn at 100 mg/L, and Mo at 40 mg/L significantly increased carbohydrates compared with AM at 150 spore/pot and 300 spore/g and the control. In addition, soil application of the combination of AM at 150 and 300 spore/g and foliar application of nano B at 100 mg/L, Zn at 100 mg/L, and Mo at 40 mg/L significantly increased reducing sugars, non-reducing sugars, total sugars, and total carbohydrates in leaves during 2019 and 2020 seasons. The combination between AM at 150 spore/g with foliar spraying of nano Mo at 40 mg/L was the second most effective treatment for increasing the traits mentioned above during the first and second seasons.



**Figure 2.** Effect of Arbuscular Mycorrhiza (AM) and Nano B, Zn, and Mo microelements on stevioside contents of stevia leaves during 2020 season.

**Table 4.** Effect of arbuscular mycorrhiza (AM) and nano B, Zn, and Mo microelements on mineral contents of stevia plants during 2019 and 2020 seasons.

Main-Plot Sub-Plot AM (g) Nano Fertilizer (mg/L)		N %		P %		K%		B ppm		Zn ppm	
		2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Main-plot effect											
0 (Control)		2.07 ± 0.2 F	2.00 ± 0.15 F	0.13 ± 0.01 E	0.14 ± 0.02 E	1.47 ± 0.12 E	1.51 ± 0.11 F	27.72 ± 1.2 G	25.34 ± 1.0 F	159.26 ± 2.0 G	169.41 ± 2.2 F
150 AM spore/g soil		2.05 ± 0.15 E	2.04 ± 0.14 E	0.19 ± 0.05 F	0.18 ± 0.06 F	1.96 ± 0.20 E	1.57 ± 0.15 E	41.11 ± 2.1 H	44.25 ± 2.3 H	154.85 ± 2.1 G	144.84 ± 1.8 G
300 AM spore/g soil		2.07 ± 0.12 F	2.00 ± 0.1 F	0.22 ± 0.06 F	0.21 ± 0.07 F	2.00 ± 0.19 F	2.00 ± 0.19 E	31.70 ± 1.5 H	39.44 ± 1.8 G	172.93 ± 2.5 G	169.37 ± 2.0 G
Sub-plot effect											
Control		2.07 ± 0.12 F	2.00 ± 0.09 F	0.13 ± 0.01 E	0.14 ± 0.02 E	1.47 ± 0.12 E	1.51 ± 0.15 F	27.72 ± 1.1 G	25.34 ± 1.0 F	159.26 ± 2.0 G	169.41 ± 2.2 F
B-100 mg/L		2.35 ± 0.25 A	2.34 ± 0.25 A	0.28 ± 0.05 A	0.29 ± 0.06 A	2.36 ± 0.26 A	2.46 ± 0.27 A	91.82 ± 3.6 A	85.82 ± 3.1 A	247.77 ± 3.5 A	246.42 ± 3.2 A
Zn-100 mg/L		2.19 ± 0.35 AB	2.28 ± 0.39 AB	0.26 ± 0.09 A	0.25 ± 0.09 A	2.39 ± 0.28 A	2.46 ± 0.30 A	83.97 ± 2.8 A	81.64 ± 2.2 A	214.59 ± 4.1 A	208.09 ± 3.8 A
Mo-40 mg/L		2.31 ± 0.30 A	2.30 ± 0.30 A	0.27 ± 0.10 A	0.27 ± 0.10 A	2.30 ± 0.25 A	2.45 ± 0.28 A	73.26 ± 2.1 A	78.51 ± 2.0 A	230.01 ± 2.3 A	247.61 ± 1.7 A
Main-plot x Sub-plot											
0 (control)	B 100 mg/L	2.35 ± 0.33 a	2.34 ± 0.32 a	0.28 ± 0.07 a	0.29 ± 0.07 a	2.36 ± 0.26 a	2.46 ± 0.28 a	91.82 ± 9.7 a	85.82 ± 9.0 a	247.77 ± 5.23 a	246.42 ± 3.0 a
	Zn 100 mg/L	2.19 ± 0.36 ab	2.28 ± 0.40 ab	0.26 ± 0.06 a	0.25 ± 0.06 a	2.39 ± 0.23 a	2.46 ± 0.25 a	83.97 ± 8.8 a	81.64 ± 8.1 a	214.59 ± 3.47 a	208.09 ± 3.2 a
	Mo 40 mg/L	2.31 ± 0.46 a	2.30 ± 0.51 a	0.27 ± 0.03 a	0.27 ± a	2.30 ± 0.26 a	2.45 ± 0.32 a	73.26 ± 16.9 a	78.51 ± 7.6 a	230.01 ± 15.8 a	247.61 ± 11.1 a
	Control	2.07 ± 0.31 f	2.00 ± 0.32 f	0.13 ± 0.01 e	0.14 e	1.47 ± 0.40 e	1.51 ± 0.43 f	27.72 ± 2.7 g	25.34 ± 2.2 f	159.26 ± 0.98 g	169.41 ± 0.99 f
Mean		2.23	2.23	0.24	0.24	2.13	2.22	69.19	67.83	212.91	217.88
Mycorrhiza 150 spore/g soil	B 100 mg/L	2.75 ± 0.25 a	2.84 ± 0.32 a	0.49 ± 0.08 a	0.47 ± 0.09 a	2.72 ± 0.33 a	2.65 ± 0.31 a	122.63 ± 13.5 a	132.12 ± 14.2 a	287.61 ± 284 a	288.93 ± 12.1 a
	Zn 100 mg/L	2.40 ± 0.28 a	2.41 ± 0.32 ab	0.35 ± 0.06 ab	0.37 ± 0.07 ab	2.58 ± 0.27 a	2.59 ± 0.28 a	113.30 ± 1.1 a	101.03 ± 1.0 a	257.27 a ± 13.81	266.99 ± 12.3 a
	Mo 40 mg/L	2.55 ± 0.40 a	2.58 ± 0.42 a	0.43 ± 0.05 a	0.45 ± 0.05 a	2.66 ± 0.20 a	2.61 ± 0.19 a	118.54 ± 18.1 a	113.93 ± 12.2 a	268.38 ± 9.86 a	275.07 a ± 9.9

Table 4. Cont.

Main-Plot Sub-Plot AM (g) Nano Fertilizer (mg/L)		N %		P %		K%		B ppm		Zn ppm	
		2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Mycorrhiza 300 spore/g soil	Control	2.05 ± 0.36 e	2.04 ± 0.32 e	0.19 ± 0.04 f	0.18 ± 0.03 f	1.96 ± 0.42 e	1.57 ± 0.32 e	41.11 ± 2.9 h	44.25 ± 3.1 h	154.85 ± 4.38 g	144.84 ± 3.6 g
	Mean	2.44	2.47	0.37	0.37	2.48	2.36	98.90	97.83	242.03	243.96
	B 100 mg/L	2.37 ± 0.25 a	2.42 ± 0.29 a	0.34 ± 0.05 a	0.40 ± 0.06 a	2.46 ± 0.25 a	2.50 ± 0.22 a	110.56 ± 5.6 a	113.66 ± 5.8 a	251.33 ± 9.49 a	252.30 ± 8.24 a
	Zn 100 mg/L	2.37 ± 0.30 a	2.41 ± 0.29 a	0.26 ± 0.04 a	0.36 ± 0.08 a	2.44 ± 0.13 a	2.61 ± 0.23 a	92.64 ± 2.0 ab	91.30 ± 2.1 a	260.22 ± 3.90 a	254.04 ± 3.6 a
	Mo 40 mg/L	2.52 ± 35 a	2.47 ± 0.32 a	0.32 ± 0.04 a	0.37 ± 0.07 a	2.56 ± 0.19 a	2.58 ± 0.20 a	102.96 ± 2.6 a	101.04 ± 2.5 a	258.10 ± 12.10 a	246.53 ± 10.2 a
	Control	2.07 ± 18 f	2.00 ± 0.15 f	0.22 ± 0.03 f	0.21 ± 0.04 f	2.00 ± 0.16 f	2.00 ± 0.16 e	31.70 ± 1.2 h	39.44 ± 2.2 g	172.93 ± 7.75 g	169.37 ± 2.6 g
	Mean	2.33	2.33	0.29	0.34	2.37	2.43	84.47	86.36	235.65	230.56
LSD <sub>p ≤ 0.05</sub>		0.02	0.05	0.02	0.01	0.05	0.11	7.59	4.38	40.16	23.19

The data represent the means ± standard deviation (SD) of four replications. In the same column, means followed by the same capital letter are not statistically different, according to the Duncan Multiple Range Test (DMRT) at  $p \leq 0.05$ , interactions take lower case letters, unless otherwise mentioned.

**Table 5.** Effect of arbuscular mycorrhiza (AM) and nano B, Zn, and Mo microelements on some bio constituents of stevia plants during 2019 and 2020 seasons.

Main-Plot Sub-Plot AM (g) Nano Fertilizer (mg/L)		Reducing Sugars %/g d.w.		Non Reducing Sugars %/g.d.w.		Total Sugars %. /g.d.w.		Total Carbohydrate mg/L	
		2019	2020	2019	2020	2019	2020	2019	2020
Main-plot effect									
0 (Control)		4.12 ± 1.1 F	4.43 ± 11 F	9.12 ± 0.23 F	9.60 ± 0.25 F	13.24 ± 0.68 F	14.03 ± 0.75 F	152.34 ± 0.96 F	137.48 ± 0.85 G
150 AM spore/g soil		5.53 ± 0.3 F	4.45 ± 0.25 F	9.03 ± 0.21 F	9.61 ± 0.35 F	14.56 ± 0.75 F	14.06 ± 0.88 H	155.67 ± 0.95 F	158.39 ± 0.89 G
300 AM spore/g soil		5.23 ± 0.56 F	4.89 ± 0.72 G	9.81 ± 0.24 F	8.77 ± 0.15 G	16.04 ± 0.89 F	13.66 ± 0.74 H	162.12 ± 1.2 F	137.46 ± 0.98 G
Sub-plot effect									
Control		4.12 ± 0.1 F	4.43 ± 0.12 F	9.12 ± 0.18 F	9.60 ± 0.32 F	13.24 ± 0.69 F	14.03 ± 0.71 F	152.34 ± 1.1 F	137.48 ± 0.78 G
B-100 mg/L		5.39 ± 0.3 A	5.77 ± 0.35 A	10.10 ± 1.24 A	10.27 ± 1.23 A	15.49 ± 1.3 A	16.04 ± 1.6 A	168.37 ± 1.2 A	163.35 ± 1.0 A
Zn-100 mg/L		5.98 ± 0.30 A	5.86 ± 0.42 A	10.37 ± 1.2 A	10.01 ± 1.0 A	16.35 ± 1.6 A	15.87 ± 1.9 A	160.12 ± 1.0 A	166.78 ± 1.6 A
Mo-40 mg/L		5.53 ± 0.14 A	5.42 ± 0.21 A	10.25 ± 1.0 A	10.30 ± 1.1 A	15.78 ± 1.9 A	15.72 ± 1.78 A	166.21 ± 1.3 A	167.28 ± 1.32 A
Main-plot x Sub-plot									
0 (control)	B 100 mg/L	5.39 ± 0.93 a	5.77 ± 0.95 a	10.10 ± 1.24 a	10.27 ± 1.32 a	15.49 ± 2.9 a	16.04 ± 3.0 a	168.37 ± 1.9 a	163.35 ± 1.8 a
	Zn 100 mg/L	5.98 ± 0.89 a	5.86 ± 0.86 a	10.37 ± 1.0 a	10.01 ± 1.3 a	16.35 ± 2.7 a	15.87 ± 2.5 a	160.12 ± 1.4 a	166.78 ± 1.6 a
	Mo 40 mg/L	5.53 ± 0.82 a	5.42 ± 0.85 a	10.25 ± 0.64 a	10.30 ± 0.68 a	15.78 ± 2.4 a	15.72 ± 2.4 a	166.21 ± 3.7 a	167.28 ± 3.1 a
	Control	4.12 ± 1.1 f	4.43 ± 1.3 f	9.12 ± 0.93 f	9.60 ± 0.94 f	13.24 ± 2.9 f	14.03 ± 3.1 f	152.34 ± 3.7 f	137.48 ± 4.3 g
Mean		5.26	5.37	9.96	10.05	15.215	15.41	161.76	158.72
Mycorrhiza at 150 spore/g soil	B 100 mg/L	7.33 ± 0.08 a	7.62 ± 0.09 a	11.59 ± 1.15 a	11.91 ± 1.25 a	18.92 ± 0.68 a	19.53 ± 0.72 a	193.12 ± 2.5 a	184.73 ± 2.2 a
	Zn 100 mg/L	6.54 ± 0.04 a	6.94 ± 0.05 a	10.51 ± 0.63 a	10.76 ± 0.68 a	17.05 ± 1.4 a	17.70 ± 1.9 a	181.16 ± 8.5 a	178.02 ± 7.5 a
	Mo 40 mg/L	6.84 ± 0.02 a	7.37 ± 0.12 a	10.97 ± 0.56 a	11.82 ± 0.69 a	17.81 ± 1.2 a	19.19 ± 2.3 a	183.95 ± 4.6 a	183.50 ± 4.3 a
	Control	5.53 ± 0.2 f	4.45 ± 0.15 f	9.03 ± 0.32 f	9.61 ± 0.35 f	14.56 ± 1.1 f	14.06 ± 0.95 h	155.67 ± 1.9 f	158.39 ± 2.1 g
Mean		6.56	6.60	10.53	11.03	17.08	17.62	178.48	176.16
Mycorrhiza 300 spore/g soil	B 100 mg/L	6.58 ± 0.11 a	6.42 ± 0.10 a	10.47 ± 0.68 a	10.63 ± 0.73 a	17.05 ± 0.60 a	17.05 ± 0.62 a	180.86 ± 2.1 a	181.73 ± 2.0 a
	Zn 100 mg/L	6.95 ± 0.22 a	6.49 ± 0.12 a	10.44 ± 0.83 a	10.58 ± 0.87 a	17.39 ± 0.81 a	17.07 ± 0.75 a	181.78 ± 6.0 a	180.12 ± 5.4 a
	Mo 40 mg/L	6.65 ± 0.16 a	6.73 ± 0.21 a	10.86 ± 0.65 a	10.76 ± 0.89 a	17.51 ± 0.79 a	17.49 ± 0.75 a	182.99 ± 2.7 a	181.58 ± 2.3 a
	Control	5.23 ± 0.12 f	4.89 ± 0.19 g	9.81 ± 0.48 f	8.77 ± 0.25 g	16.04 ± 0.42 f	13.66 ± 0.40 h	162.12 ± 2.7 f	137.46 ± 2.7 g
Mean		6.35	6.13	10.40	10.19	17.00	16.32	176.94	170.22
LSD <sub>p</sub> ≤ 0.05		0.60	0.04	0.39	0.22	0.47	0.41	9.72	5.61

The data represent the means ± standard deviation (SD) of four replications. In the same column, means followed by the same capital letter are not statistically different, according to the Duncan Multiple Range Test (DMRT) at  $p \leq 0.05$ , interactions take lower case letters, unless otherwise mentioned.

#### 4. Discussion

A significant increase in stevia growth in response to 150 and 300 AM spore/g soil alone, or in combination with 100 mg B/L, 100 mg Zn/L, and 40 mg Mo/L was due to the improved uptake of nutrients, especially P, and micronutrients associated with plant metabolism [39]. AM has been shown to have a positive effect on plant development in the Asteraceae family [40]. They indicated that AM associations were shown to increase colonization and shrub development at low concentrations of P and therefore have an impact on the growth of plants.

The collaborative effects of nano B, Zn, and Mo enhanced plant growth and development, Marzouk and colleagues [41]. The significant improvement in vegetative growth could be attributed to the beneficial effects of nano micronutrients on increasing photosynthetic rates and other metabolic actions that are essential for the development of several plant metabolites responsible for cell division and elongation [42]. This could be preceded by an optimistic adjustment in the hormonal profile, especially the promoter ones (i.e., auxins, gibberellins, and cytokinins). The biomass of the stevia plant is also reliant on its ability to increase photosynthesis. An enhanced nutrient status has also been described to contribute to the net assimilation in plant photosynthesis [43]. In this case, AM increases biomass production, and its components improve photosynthesis (mentioned above). Moreover, AM provides stable stevia hosting components which lead to better growth and dry weight production.

During both seasons, chlorophyll contents were increased by applying AM at 150 spore/g and nano B at 100 mg/L. Thus, this could be attributed to the fact that AM inoculation show the highest biomass production, which suggests that the frequency of photosynthesis is increased via increased uptake of mineral nutrients. Consequently, AM provides sensible nutrition to growing plants, improving growth aspects and biomass production. AM application increased photosynthesis efficiency to reward biomass production by increasing chlorophyll pigment content [44]. AMF application increased chlorophyll pigment content in stevia leaves [45].

Increasing stevioside contents in stevia leaves via the application of the combination of AM at 150 spore/g and nano B at 100 mg/L during the second growing season could be attributed to the beneficial effects of AM on mineral uptake and content of total carbohydrates in plants. Carbohydrates assimilation also increases stevioside concentration by attracting the accumulation of methylerythritol phosphate (MEP) [46]. Furthermore, altering the expression of some enzymes in the MEP pathway increases the transcription of the stevioside biosynthesis. Cordoba et al. [47] indicated this action upon stevioside accumulation in the plant tissue during plant growth and development. In addition, sugar concentration stimulates the glycosylation of the entikaurene to form biosynthesis of stevioside production in a higher content of zero-calorie sweeteners, i.e., stevioside [48]. Furthermore, the effects of AM and nano B can increase stevioside contents by a minor increase in the concentration of secondary metabolite biosynthesis combined with improved growth, mediated through increased accumulation of photosynthate partitioning and allocation, as reported by [45]. The application of AM on Dutch fennel plants increased oil contents, as reported by [43].

The above-mentioned findings for AM-applications and nano-micronutrient-foliar spraying applications showed that the greatest value for N, P, K, Zn, and B in stevia leaf was achieved with the application of AM 150 spore/g and B at 100 mg/L. AM and B have an effect on the sugars and nutrients of the photosynthesis parts (leaves) in order to snick the storage section (fruit), as well as the special functions of yield production, through improved pollination and seed quality, could be attributed to this increase when applied to AM and B according to Lakshmi [19]. Zewail et al. [24] stated that foliar spraying of sugar beet plants with B at 50 mg/L increased the sugar content and some other bio constituents. Similarly, Giri et al. [43] reported that AM application increased photosynthesis portioning and allocation through the enhanced uptake of minerals, thus, offering stable elements to host stevia in improving growth and bio constituent contents.

The application of AM and nano microelements (i.e., B, Zn and Mo) increased some bio constituents of stevia, i.e., (reducing, non-reducing, total sugars, and total carbohydrates) during the 2019 and 2020 seasons. This increase in bio constituent contents could be attributed to carbon assimilation and photosynthesis. Wright et al. [44] reported that AM inoculum increased the photosynthesis efficiency to reward the carbon necessity of the AM. Thereby, assimilation of photosynthesis products such as sugars and carbohydrates and increased the accumulations of glycosides content in the active ingredient materials (stevioside). In addition, B plays a significant role in the translocation of photosynthesis portioning and allocation from sink to source [49]. Zewail et al. [24] reported that foliar spraying of sugar beet plants with boron increased mineral element content and root quality. Standard B-containing fertilizer mergers are failing to achieve uniform delivery of nutrients. Given the need for this essential nutrient, B is the world's second most common micronutrient deficiency concern after zinc [24]. Boron is an essential element for division of the cell transport of sugars, cell wall development, fruits, and hormone synthesis. B deficiency affects some processes in plants like elongation of the root, metabolism of carbohydrates, sugar translocation, and activity of IAA oxidase [50]. Boron is essential for reproduction. Hence adjusting reproductive B status is important to plant productivity and sugar content [51]. Sanjeev and Sanjay [52] found that foliar spraying of stevia with B at 100 mg/L increased total carbohydrates and protein concentration in stevia leaves.

AM has an essential role in improving adaptation and mitigating biotic and abiotic plant stress. It is an environmentally safe method of increasing plant growth and production while reducing the use of harmful pesticides and artificial fertilizers. A further research is necessary in order to assess the outcomes in the lab and in greenhouse. This knowledge is needed in accordance to different biogeographical areas to promote and enhance their wide industrial production to ensure that low calories and sweeteners material is enough for every person on the planet today and, in the future, [18].

## 5. Conclusions

The present study concluded that using AM at 150 or 300 spore/g soil, and B, Zn, and Mo at 100, 100, and 40 mg/L, respectively, and their combination, improves vegetative growth characteristics, mineral contents, and some bio constituents (i.e., reducing, non-reducing, and total sugars) in stevia. The stevioside content of stevia is also affected by the application of AM and micronutrients and their combination, because the consistency of the sugars and some other bio constituents are dependent on stevioside biosynthesis. It is recommended to combine AM at 150 spore/g with nano B at 100 mg/L during plant growth and development to obtain stevia with high growth performance, bio constituents, mineral contents, and stevioside concentration in leaves. In this study we obtained increased stevioside contents as a low calorie sweetener by using safe and environmentally friendly materials such as AM and nano B.

**Author Contributions:** Conceptualization, M.A., A.A.G., E.A., I.S.H.E.-G., S.H.A.A.-M., M.E., E.A. and H.S.E.-D.; formal analysis, R.M.Y.Z., A.A.G., E.A., A.A.G., N.E., M.H.M.M., K.R.I., I.S.H.E.-G. and H.S.E.-D.; investigation A.A.G., N.E., E.A. and H.S.E.-D.; supervision A.A.G., N.E. and E.A.; writing—original draft review and editing, M.A., A.A.G., E.A., I.S.H.E.-G., S.H.A.A.-M., M.E., E.A. and H.S.E.-D. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Data Availability Statement:** Available upon request from the corresponding author.

**Acknowledgments:** We thank Taif University Researchers Supporting Project number (TURSP-2020/13), Taif University, Taif, Saudi Arabia.

**Conflicts of Interest:** The authors declare no conflict of interest.



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