



Article

Impact of Foliar-Applied Plant Extracts on Growth, Physiological and Yield Attributes of the Potato (*Solanum tuberosum* L.)[†]

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[†] This article is a revised and expanded version of a paper entitled "Foliar Application with Plant-Derived Extracts Enhances Growth, Physiological Parameters, and Yield of Potatoes (*Solanum tuberosum* L.)", which was presented online at 3rd International Electronic Conference on Agronomy, 15–30 October 2023.

Abstract: The current reliance on pesticides and synthetic fertilizers has been vital to sustain and increase agricultural production. The continuous, excessive use of these traditional practices has negatively affected consumer health and burdened the ecosystem. The use of plant extracts as a tool to minimize agrochemical inputs has been extensively investigated; these extracts have the ability to improve both plant growth and agricultural productivity. Therefore, this study was conducted to determine the effect of foliar plant extract application on potato growth, as well as on certain physiological and yield attributes. From four weeks after planting onwards, five healthy, equal-sized potato plants per treatment received various foliar plant extract applications. These extracts included brown seaweed *Ascophyllum nodosum* extract (ANE), aloe vera leaf extract (AVE), garlic bulb extract (GBE), and moringa leaf extract (MLE). The treatments were repeated weekly until harvesting. Application data regarding growth and physiological parameters were collected weekly. The pre-harvest foliar application of various plant extracts significantly enhanced ($p \leq 0.05$) the plant growth, physiological, and yield attributes of potatoes. The best growth and yield responses were observed following ANE and MLE application. Plant extracts have shown beneficial effects on other crops as well, but further validation of these effects is still necessary in order to popularize and commercialize such applications.

Keywords: biostimulants; food security; plant extracts; potato; sustainable agriculture



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1. Introduction

Potato (*Solanum tuberosum* L.) is a member of the Solanaceae family, native to South America, but it is now grown in most parts of the world [1]. Among all cash crops, potato is one of the world's most important non-grain food crops, exhibiting a global production of about 376 million tonnes, with China as the largest producer, contributing approximately 94 million tonnes annually [2]. Potato is also recognized as a staple food, being the third most-consumed food crop worldwide, following rice and wheat [1]. The worldwide per capita potato consumption reached 33.1 kg in 2020, possibly due to the health and nutritional benefits potato offers [2]. According to Zaheer et al. [3], potato is an excellent source of dietary fiber, carbohydrates, high-quality protein, vitamins, minerals, and other metabolites. Being rich in health-promoting metabolites, potato possesses high antioxidant activity, which helps to reduce the risk of chronic diseases, including heart disease, diabetes, and cancer [4].

Over the last couple of decades, there has been a rapid increase in potato demand. Therefore, it has become of immense importance for all potato growers to increase this crop's profitably, while keeping input costs to a minimum. Additionally, modern agriculture demands sustainable crop production, and the industry is always searching for

alternative methods to sustain plant development with little or no compromise to yield. Both subsistence and commercial farmers are currently facing the major challenge of biotic and abiotic factors aligned with climate change. These include drought, salinity, weed infestation, pests, and diseases, which can all devastatingly affect the growth and yield of the potato [5]. Given these challenges, synthetic pesticides and inorganic fertilizers have become vital for the production of crops and their protection against biotic and abiotic constraints [6]. The reliance on industrially based inputs may, however, pose multiple threats to human health and impart harmful effects on the ecosystem [7]. In addition, Lucas et al. [8] revealed that the continuous, excessive usage of such chemicals might result in the development of new pathogen strains that could become difficult to control, despite the efficacy of the chemical. The aim of modern agriculture is, therefore, to reduce the utilization of these chemicals to a minimum, making crop farming simpler and offering healthier, safer, and sustainably produced goods.

Farmers and researchers are continuously exploring and developing alternative approaches to crop farming, attempting to overcome the challenges of long-term production viability [7]. Among several proposed strategies, the use of plant extracts has been identified as a promising, innovative, eco-friendly, and sustainable approach that could improve crop production and crop protection. Recent studies have tested this method on a broad spectrum of solanaceous crops, such as potato, sweet pepper (*Capsicum annuum* L.), and tomato (*Solanum lycopersicum* L.) [9–13]. The present study aims to evaluate the effect of the foliar application of plant extracts (viz. *Ascophyllum nodosum* extract, garlic bulb extract, aloe vera leaf extract, and moringa leaf extract) on growth, as well as certain physiological and yield attributes of potato.

2. Materials and Methods

2.1. Plant Material and Growing Conditions

A pot experiment was carried out in a glasshouse at the Controlled Environment Facility (CEF) of the University of KwaZulu-Natal, Pietermaritzburg, South Africa (29°37'32.9" S 30°24'18.8" E). Locally obtained baby potatoes, cv. "Sifra", were planted in June (mid-winter) as seed tubers at a depth of 10 cm into 10 L plastic pots filled with a mixture of sandy soil and Gromor[®] (Gromor, Cato Ridge, South Africa) potting mix. The chemical and physical attributes of the soil and growing medium used were analyzed before planting (Cedara College of Agriculture, Department of Agriculture and Rural Development, KwaZulu-Natal). Poultry manure (Nutri-Green Gwano Pellets, Protek, Heidelberg, Gauteng, South Africa), at a rate of 25 g/pot, based on the chemical and physical characteristics of the used medium, was then applied to amend soil nutrition. Thirty days after planting, the same fertilizer was re-applied as a top-dressing at a similar rate (25 g/pot). The environmental conditions inside the glasshouse were maintained at 25 ± 2 °C and 65% relative humidity (RH) during the day, while the temperature and RH were kept constant at 13 ± 2 °C and 72% at night, respectively. The plants were irrigated using automated drip irrigation, dispensing approximately 50 mL per 10 L pot daily.

2.2. Experimental Design and Foliar Application

The study was laid out following a completely randomized design (CRD), with five replications. Five healthy, similar-sized "Sifra" baby potatoes, randomly selected, were used per treatment, with five seed tubers per replicate, yielding 25 experimental units (10 L pots). The experiment consisted of four treatments, namely *Ascophyllum nodosum* extract (ANE), moringa (*Moringa oleifera*) leaf extract (MLE), garlic (*Allium sativum*) bulb extract (GBE), and aloe vera (*Aloe barbadensis* Mill.) leaf extracts (AVE), in addition to the control (no extract application). The above-mentioned treatments were directly applied to potato leaves using a hand-held pressure sprayer, with each plant receiving 50 mL. The first foliar treatment application was performed four weeks after planting (vegetative stage), and treatment applications were repeated weekly until harvest (mid-August, early spring).

2.3. Collection and Extract Preparations

The plant material used for extract preparation was obtained from various suppliers. Brown algae (*Ascophyllum nodosum*) powder (Nature's Choice) was purchased locally (Dis-Chem, Woodburn Mall, Pietermaritzburg, South Africa), whereas healthy aloe vera plants were obtained locally from Woodland Nursery, (Pietermaritzburg, South Africa). Fresh moringa (*Moringa oleifera*) leaf powder (MLP) was supplied by a commercial supplier (runKZN, Pietermaritzburg, South Africa), while fresh Egyptian white garlic was purchased from a local supermarket. The employed extracts, ANE, AVE, MLE, and GBE, were prepared following the procedure described by Noor et al. [14], Ting-Ting et al. [15], and Ngcobo and Bertling [11], with slight modifications. Exactly 10 g of each plant material was weighed out and homogenized in a glass beaker with 450 mL distilled water. The homogenates were then placed onto a hot plate, continuously agitated with a magnetic stirrer, and allowed to boil at 100 °C for 30 min. After 30 min, the solutions were allowed to stand for 2 h to cool down; then, the supernatants were collected and filtered three times through muslin cloth. Next, to yield a final volume of 1 L, serial dilutions were obtained using distilled water. Furthermore, the chemical composition of the extracts was analyzed, as presented in Table 1.

Table 1. Chemical composition of various plant extracts. GAE (gallic acid equivalent); QE (quercetin equivalent); AAE (ascorbic acid equivalent); ANE (*Ascophyllum nodosum* extract); AVE (aloe vera leaf extract); GBE (garlic bulb extract); MLE (moringa leaf extract).

Composition *	ANE	AVE	GBE	MLE	Units
Nitrogen (N)	0.08	0.11	-	0.17	%
Phosphorus (P)	4.2	2.2	16.0	10.4	%
Potassium (K)	102.6	79.4	96.8	106.4	%
Calcium (Ca)	10.4	58.6	-	77.4	%
Magnesium (Mg)	16.6	24.0	2.6	23.2	%
Sodium (Na)	127.2	3.0	2.2	0.8	mg/kg
Manganese (Mn)	-	4.0	-	-	mg/kg
Copper (Cu)	-	-	-	-	mg/kg
Zinc (Zn)	0.9	-	-	1.2	mg/kg
Iron (Fe)	-	-	-	-	mg/kg
Ascorbic acid (Vitamin C)	86.5	78.1	57.1	79.7	mg/g DM
Total phenolics	11.9	2.1	2.4	3.9	mg GAE/g DM
Total flavonoids	28.6	3.3	11.7	51.7	mg QE/g DM
Total protein	104.7	53.7	61.1	81.5	mg/g DM
Antioxidant activity [DPPH (diphenyl-1-picrylhydrazyl)]	105.3	94.9	90.5	95.4	mg AAE/g DM

* For mineral composition, plant extracts were analyzed using inductively coupled plasma mass spectrometry (ICP-MS), whereas the analyses of phytochemicals were carried out following the methods of Boonkasem et al. [16] (ascorbic acid concentration), Wang et al. [17] (total phenolics), Gu et al. [18] (total flavonoids), Bradford [19] (total protein), and Rocchetti et al. [20] (antioxidant activity-DPPH) using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan).

2.4. Determination of Vegetative Growth, Physiological, Morphological, and Yield Parameters

2.4.1. Plant Height and Number of Leaves

Plant growth parameters, including plant height and the number of fully expanded leaves, were recorded from the first treatment application until stage 4 (tuber bulking) of potato growth and development at 7-day intervals. Plant height (cm) was measured from the base of the stem to the tip of the terminal bud using a tape measure. The total number of leaves per plant was counted manually. These parameters were measured in all 25 plants used in this experiment.

2.4.2. Leaf Area

From the first treatment application until the tuber bulking stage, the leaf area of the entire potato plant was estimated directly from leaf length and width measurements. These measurements were obtained from 20 leaves per treatment (≈ 4 leaves per plant). Leaf area was then calculated using the formula described by Bhatt and Chanda [21].

$$LA = 11.98 + 0.06L \times W,$$

where LA = leaf area (cm^2), L = leaf length (cm), and W = leaf width (cm).

2.4.3. Leaf Chlorophyll Index

The leaf chlorophyll index was determined using a portable, non-destructive, and lightweight instrument (CCM-200plus-Opti-Sciences Inc., Hudson, NH, USA). At the tuber bulking stage (2 week after treatment application), the chlorophyll content index was measured using three fully developed functional leaves on each potato plant. A total of four plants, randomly selected from each treatment, were measured.

2.4.4. Fresh and Dry Above-Ground Biomass

Both fresh and dry above-ground biomass (g/plant) were determined using a fine balance in all 25 plants used in this experiment. Fresh mass was recorded immediately after harvesting (mid-August, early spring), whereas dry mass was measured after four days of oven-drying at 80°C .

2.4.5. Yield and Fresh Tuber Mass

At the mature tuber stage, all tubers were harvested from all replicates. Total tuber yield (tuber number/plant) and tuber mass (g) were recorded immediately after harvesting (mid-August, early spring).

2.5. Statistical Analysis

The obtained results were subjected to a one-way analysis of variance (ANOVA) using GenStat statistical software (GenStat[®], 18th edition, VSN International, Hemel Hempstead, UK) and plotted using Microsoft Excel[®]. Means separation were carried out using Duncan's multiple range test, with a difference of $p \leq 0.05$ considered significant; LSD (least significant difference) values were compared at a 5% significance level.

3. Results

3.1. Plant Height

Variations in morphological data revealed a significant effect of plant extract application on the growth of potatoes in terms of plant height. The results indicate that, unlike other treatments, the pre-harvest application of ANE at the vegetative stage significantly influenced ($p \leq 0.05$) potato plant height four weeks after the treatment application (Figure 1). Hence, on the 4th week after treatment application, this treatment resulted in the tallest plants, followed by the MLE treatment (Figure 1). There were, however, no significant differences between values recorded for the AVE, GBE, and the control groups (Figure 1).

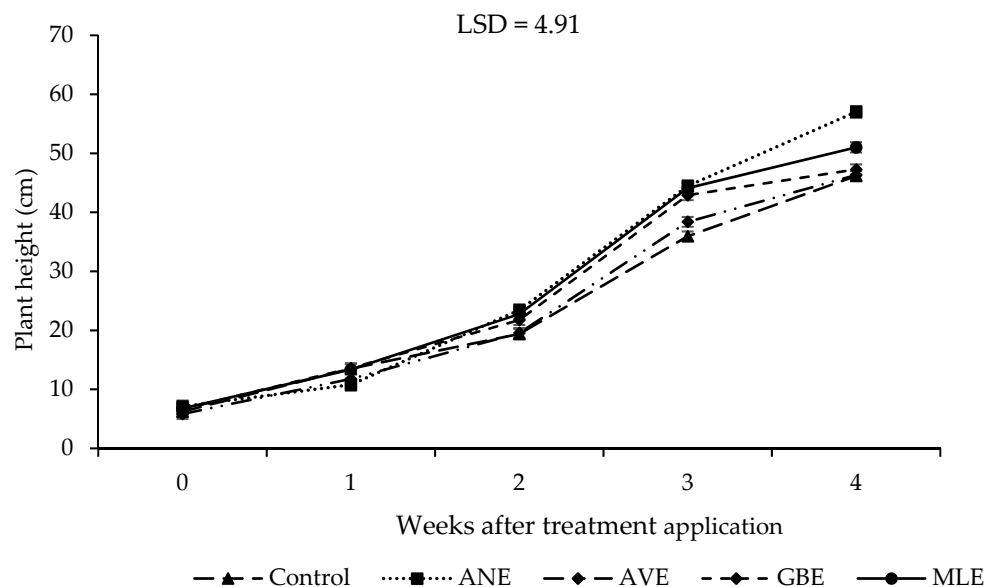


Figure 1. Effect of foliar-applied plant extracts (g/L) on the height of potato plants. Control (no application); ANE (*Ascophyllum nodosum* extract); MLE (moringa leaf extract); GBE (garlic bulb extract); AVE (aloe vera leaf extract). LSD at ($p \leq 0.05$) = 4.91.

3.2. Number of Leaves

Four weeks after treatment application, the number of leaves in all treatments was significantly higher than in the control ($p \leq 0.05$). The MLE treatment recorded the highest number of leaves, followed by the ANE treatment (Figure 2); the number of leaves recorded for the AVE and GBE treatments was less than that for the ANE and MLE treatments.

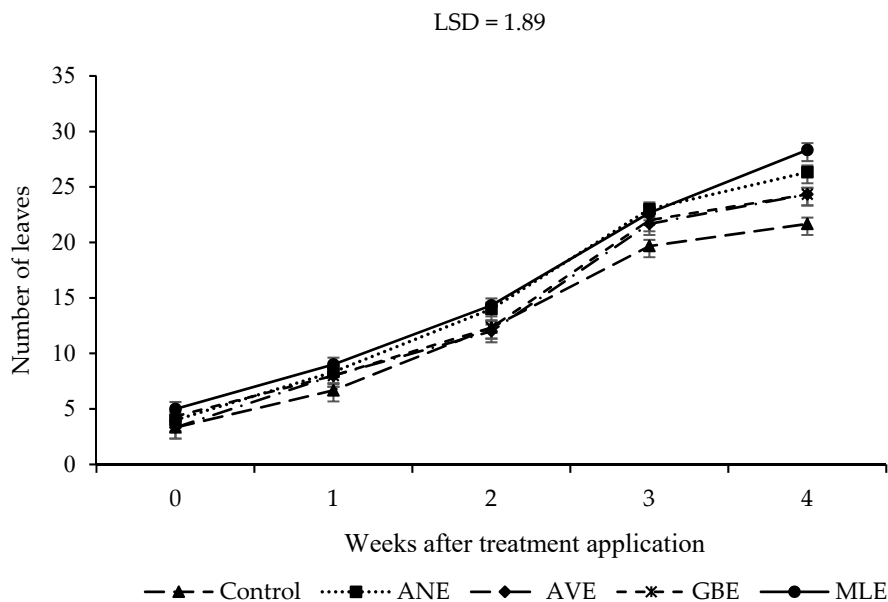


Figure 2. The effect of weekly applied plant extracts (g/L) on the number of potato leaves. Control (no application); ANE (*Ascophyllum nodosum* extract); MLE (moringa leaf extract); GBE (garlic bulb extract); AVE (aloe vera leaf extract). LSD at ($p \leq 0.05$) = 1.89.

3.3. Leaf Area

The treatment of potato plants with various plant extracts positively influenced leaf area development. Four weeks after the treatment application, the largest leaf area was obtained by potato plants treated with ANE, followed by plants treated with MLE, GBE,

and AVE. Moreover, the smallest leaf area was recorded for the control. The obtained results were significantly different ($p \leq 0.05$) from each other (Figure 3).

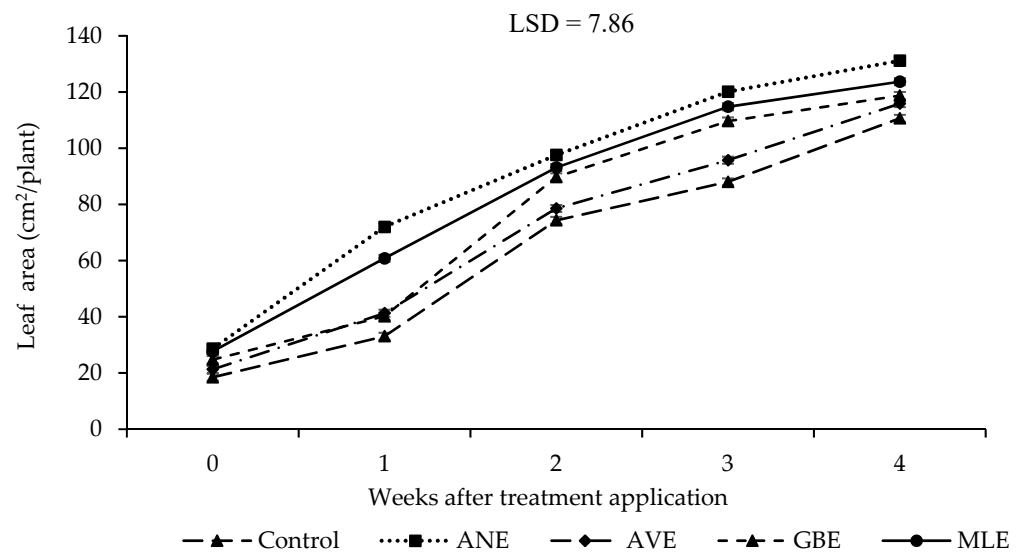


Figure 3. The effect of foliar-applied plant extracts (g/L) on the leaf area of potatoes. Control (no application); ANE (*Ascophyllum nodosum* extract); MLE (moringa leaf extract); GBE (garlic bulb extract); AVE (aloe vera leaf extract). LSD at ($p \leq 0.05$) = 7.86.

3.4. Leaf Chlorophyll Index

The treatment of potato plants with various plant-derived extracts significantly enhanced the leaf chlorophyll index ($p \leq 0.05$). The highest leaf chlorophyll index was obtained in potato plants treated with ANE and MLE; however, the two other treatments did not differ significantly from each other. The control recorded the lowest leaf chlorophyll index, with a value that does not differ significantly from that of either GBE or AVE (Figure 4).

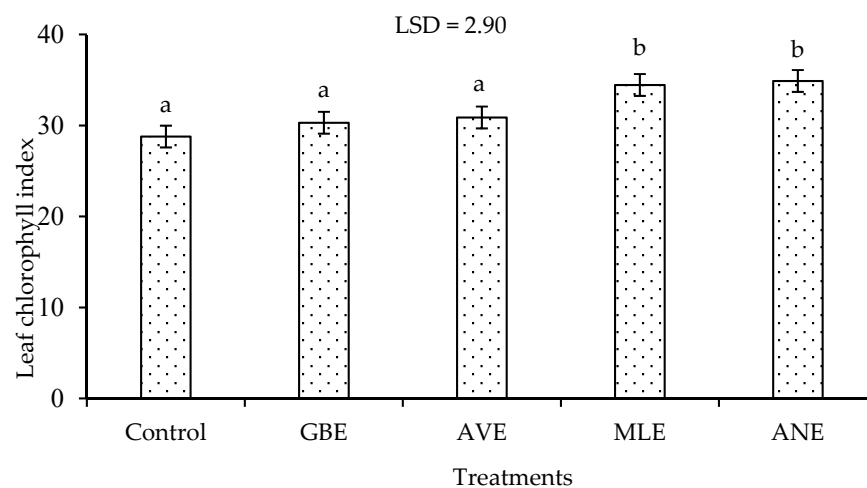


Figure 4. Effect of foliar-applied plant extracts (g/L) on leaf chlorophyll index of potatoes. Control (no application); ANE (*Ascophyllum nodosum* extract); MLE (moringa leaf extract); GBE (garlic bulb extract); AVE (aloe vera leaf extract). Different lowercase letters above each column indicate significant differences between treatments ($p \leq 0.05$), according to Duncan's multiple range test.

3.5. Fresh and Dry Above-Ground Biomass

The analysis of variance of the fresh and dry mass of the above-ground potato plant biomass indicates a significant difference ($p \leq 0.05$) between treatments (Figure 5). The

potato plant treatments showed a pronounced effect on the accumulation of above-ground fresh and dry mass (Figure 5). Specifically, ANE significantly accumulated the highest fresh and dry mass when compared with other treatments, although the results of this treatment do not differ significantly from those of GBE and MLE. The control treatment, however, accumulated the lowest fresh and dry above-ground biomass.

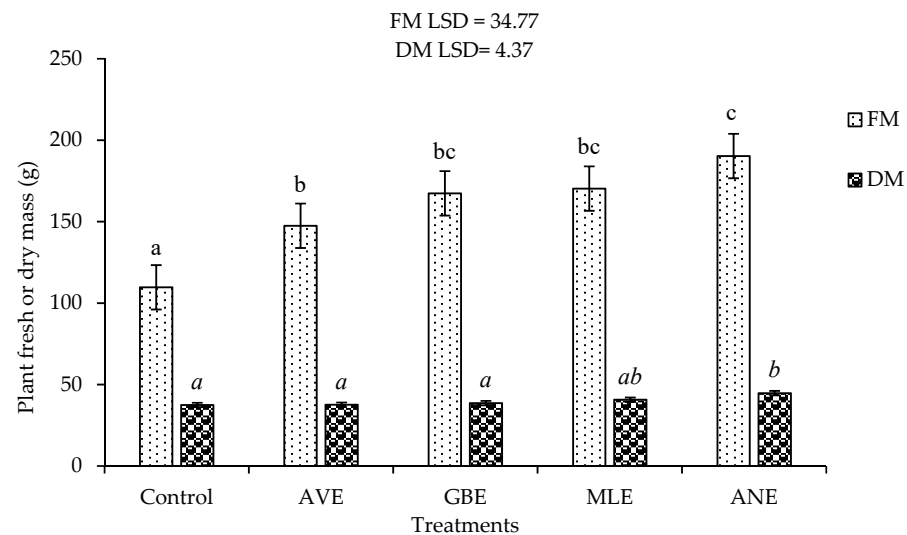


Figure 5. The effect of foliar-applied plant extracts (g/L) on the fresh and dry mass of potato above-ground biomass. Control (no application); ANE (*Ascophyllum nodosum* extract); MLE (moringa leaf extract); GBE (garlic bulb extract); AVE (aloe vera leaf extract); FM (fresh mass); DM (dry mass). Different lowercase letters above each column indicate significant differences among treatments ($p \leq 0.05$), according to Duncan's multiple range test.

3.6. Total Tuber Yield (Number of Tubers/Plant)

The obtained results indicate that the pre-harvest application of all plant extracts significantly influenced ($p \leq 0.05$) the total tuber yield, except for in the case of the GBE treatment (Figure 6), while the ANE treatment resulted in the most significant increase in tuber number when compared with other treatments.

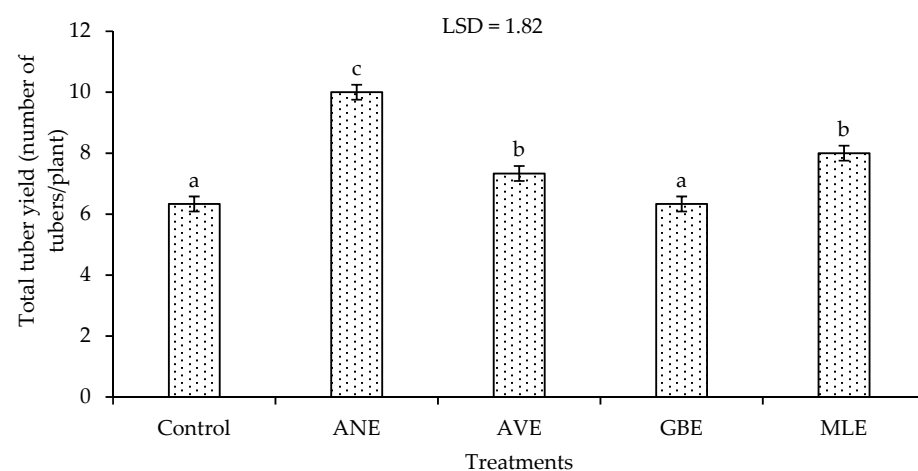


Figure 6. The effect of foliar-applied plant extracts (g/L) on total tuber yield. Control (no application); ANE (*Ascophyllum nodosum* extract); MLE (moringa leaf extract); GBE (garlic bulb extract); AVE (aloe vera leaf extract). Different lowercase letters above each column indicate significant differences among treatments ($p \leq 0.05$), according to Duncan's multiple range test.

3.7. Fresh Tuber Mass

The treatment of potato plants with various plant extracts positively affected fresh tuber mass. The greatest fresh tuber mass was obtained by potato plants treated with ANE, followed by MLE, GBE, and AVE treatments. The control recorded the lowest fresh tuber mass. Overall, the obtained results indicate significant differences ($p \leq 0.05$) in fresh tuber mass between treatments (Figure 7).

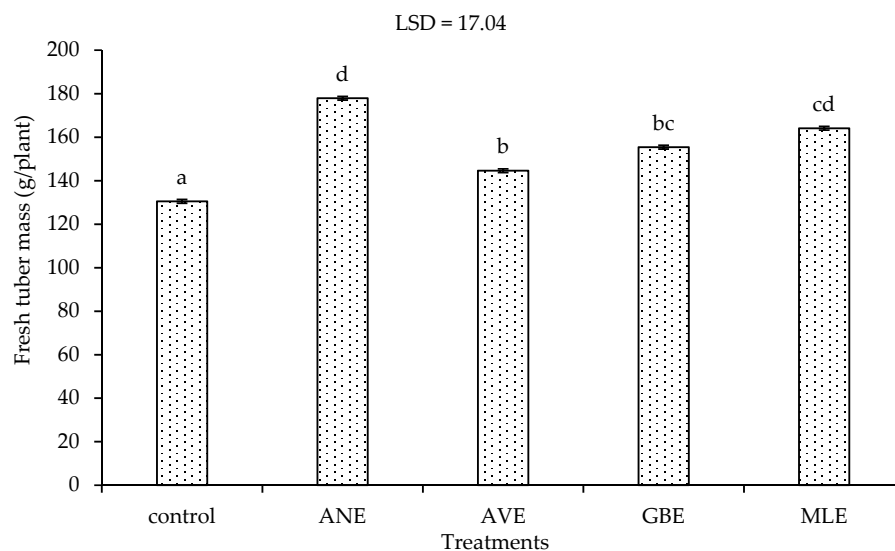


Figure 7. The effect of foliar-applied plant extracts (g/L) on fresh tuber mass. Control (no application); ANE (*Ascophyllum nodosum* extract); MLE (moringa leaf extract); GBE (garlic bulb extract); AVE (aloe vera leaf extracts). Different lowercase letters above each column indicate significant differences among treatments ($p \leq 0.05$), according to Duncan's multiple range test.

4. Discussion

The use of plant extracts as biofertilizers, biostimulants, and soil ameliorants has gained increasing attention owing to the adverse effects of synthetic pesticide and inorganic fertilizer application on human health and the environment [22]. Plant extracts have the ability to increase plant growth and yield attributes by positively interfering with plant physiological processes [7]. Therefore, the present study intended to evaluate the growth and yield promotion effects of various plant extracts. The foliar application of the plant extracts made from *Ascophyllum nodosum* (ANE), aloe vera leaves (AVE), garlic bulbs (GBE), and moringa leaves (MLE) had a positive impact on the growth and yield parameters of the potato.

Increased vegetative growth, leaf chlorophyll concentrations, and yield parameters could be attributed to the biofertilization effect of natural plant extracts, especially ANE and MLE [23]. This phenomenon has been previously reported in a wide variety of crops, such as soybean (*Glycine max* L.), sweet pepper (*Capsicum annuum* L.), wheat (*Triticum aestivum* L.), and tomato [11,24–26]. Both ANE and MLE are excellent sources of minerals, including macro- and micro-nutrients (i.e., N, P, K, Ca, Mg, Zn and Na) [27,28]. Mineral elements, particularly N and P, have been known for their vegetative growth promotion properties in plants; hence, the exogenous application of plant extracts increases the availability of N and P to the plant, thereby boosting vegetative growth and above-ground biomass [29]. In addition, enhanced leaf chlorophyll concentrations could be due the presence of the minerals Mg and N, which are vital components of the chlorophyll molecule [7,27]. Magnesium is the central atom in the porphyrin ring of the chlorophyll molecule, while N is also a structural component of the chlorophyll molecule; this results in a green leaf color, representing a high leaf chlorophyll concentration; both elements are necessary to allow the capture of light energy during photosynthesis [30,31]. Minerals, especially K and Zn, are mainly responsible for yield and quality promotion in crops. Cakmak et al. [30] revealed

that adequate K nutrition is vital in crops to enhance photosynthate translocation (via the phloem from source to sink). Potassium also plays a critical role in the partitioning of carbohydrates by improving photosynthate translocation and the growth rate of sink and/or source organs [30]. Furthermore, Zn is an essential component in the biosynthesis of tryptophan, which is involved in the synthesis of indole acetic acid (IAA) [32]; this plant hormone is responsible for cell growth and cell elongation, thus increasing tuber growth substantially.

In addition to biofertilization, enhanced vegetative growth, leaf chlorophyll concentration, plant above-ground biomass, and yield attributes following plant extract applications could also be ascribed to their bio-stimulatory effects [33]. Plant extracts, especially ANE and MLE, are natural sources of several bioactive compounds, including phytohormones, ascorbic acid, polysaccharides, betaines, and phenolics [27,34]. Exogenous application of such extracts could potentially provide plants with these essential phytochemicals, with many of these compounds working in a synergistic manner [33]. Both ANE and MLE contain phytohormones, such as auxins [indole-3-acetic acid (IAA)], gibberellins, (GAs) and cytokinin (zeatin) [35,36]. The presence of such growth-promoting plant hormones in ANE and MLE could possibly induce cell expansion and cell division, thereby improving vegetative growth, plant biomass, and yield attributes, as observed by Rayorath et al. [27] in *Arabidopsis thaliana* (L.). Rioux et al. [37] reported that, besides containing plant hormones, *Ascophyllum nodosum* extract can exhibit a wide range of growth-stimulatory effects because of the polysaccharides present in the extract. Such compounds include laminarin [β -glucan-(β -D-glucose polysaccharide)] and fucoidans (fucose-rich sulphated polysaccharides, consisting primarily of 1,2-linked α -L-fucose-4-sulfate units with very small amounts of D-xylose, D-galactose, D-mannose, and uronic acid), both exhibiting radical scavenging antioxidant activity [38].

Rayorath et al. [27] also reported significant amounts of betaine (trimethylglycine, a non-protein, methyl-derivative of glycine) present in ANE, which plays a significant role in counteracting metabolic dysfunctions brought on by stress, thereby improving plant growth and survival. In addition to betaines, ANE and MLE contain several antioxidant compounds, including ascorbic acid, tocopherols, flavonoids, and polyphenols; their presence triggers antioxidant biosynthesis, thereby reducing stress caused by reactive oxygen species (ROS) [39]. These ROS can cause cell and membrane degradation, which may speed up chlorophyll degradation and senescence; hence, these antioxidant compounds found in ANE and MLE could promote growth and developmental processes and maintain leaf greenness by reducing ROS levels in potato plants [40]. Increased leaf chlorophyll concentrations due to the foliar application of ANE and MLE could possibly be due to enhanced gene transcripts involved in photosynthesis, cell metabolism, and stress response. The application of ANE and MLE suppresses cysteine protease activity [41], which ultimately results in the inhibition of chlorophyll degradation, thus delaying senescence in plants, as observed by Kałużewicz et al. [42] in broccoli (*Brassica oleracea* var. *italica*).

The results obtained in the present study confirm the findings by Haider et al. [9], who demonstrated a significant improvement in growth attributes of potato plants due to various ANE treatments. Rajendran et al. [22] also demonstrated that growth parameters, such as plant height, number of leaves and branches, and leaf area of sweet pepper plants, were significantly enhanced by foliar ANE and MLE application; these findings correspond well with those from the present study. In addition, ANE and MLE applications to tomato plants grown under water-deficit conditions significantly improved plant height, number of leaves and branches, and leaf area [12]. Ahmad et al. [43] also noted similar effects on leaf chlorophyll in bulbous cut flowers (*Freesia hybrida*), reporting a significant improvement in leaf chlorophyll concentration following MLE application. Similar results were also observed by Elzaawely et al. [44] in snap bean (*Phaseolus vulgaris* L.) and Ali et al. [45] in okra (*Abelmoschus esculentus* L.). Various authors noted a considerable improvement in yield attributes of many crop species, such as lima beans (*Phaseolus lunatus* L.) [46], tomato, and sweet pepper [25], following ANE application, similar to the results observed

in this study. These results also coincide with those of Ali et al. [47], who noted that foliar ANE application improved the total fruit yield and fruit mass of the tomato. Similarly, a significant increase in total tuber yield following foliar ANE application was observed by Haider et al. [9]; our results confirm the findings of these authors. In addition, Taskos et al. [48] demonstrated a profound effect of ANE application on total grape yield; moreover, these results concur with those of Rajendran et al. [22], who noted a positive response in total sweet pepper yield and fruit mass following ANE foliar application. Therefore, the obtained results demonstrate the usefulness of ANE in potato production.

5. Conclusions

The present study encourages the use of various plant extracts in the crop farming community. The pre-harvest foliar application of these plant extracts considerably enhanced vegetative growth, physiological, and yield attributes of the potato. Since modern agriculture necessitates financially feasible and easily accessible organic inputs, the use of plant extracts as biofertilizers, biostimulants, and bio-elicitors could effectively be employed as an ideal multi-active organic input to improve the crop growth and yield potential of agricultural crops. This research has shown that foliar applications of plant extracts, especially from ANE and MLE, have the potential to improve crop productivity and yield. Hence, the results presented in this study are of great significance to commercial, as well as small-scale, potato growers, as the use of organic plant extracts is an environmentally friendly and sustainable approach towards increasing crop productivity. Plant extracts have shown beneficial effects on other crops as well, but further validation of these effects is still necessary in order to popularize and commercialize such applications.

Author Contributions: Conceptualization, S.M. and I.B.; methodology, S.M.; software, S.M.; validation, S.M., I.B. and B.L.N.; formal analysis, S.M.; investigation, S.M.; resources, B.L.N.; data curation, B.L.N.; writing—original draft preparation, S.M.; writing—review and editing, I.B. and B.L.N.; visualization, B.L.N.; supervision, I.B.; project administration, I.B.; funding acquisition, I.B. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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