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# Influence of exogenously applied plant extracts on growth, certain physiological and morphological, as well as yield parameters of Gem squash (*Cucurbita pepo* L.)

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## ABSTRACT

**Purpose:** The study was conducted to evaluate growth, physiological, morphological and yield response of gem squash plants following soil drench application of different plant extracts. **Research method:** A pot experiment conducted in the glasshouse was laid out following complete randomized design (CRD), with five replications. Thirty healthy, similar-sized gem squash plants were grown and treated with different treatments (plant extracts). Treatments included: *Ascophyllum nodosum* extract (ANE), aloe vera leaf extract (ALvE), garlic bulb extract (GBE), ginger rhizome extract (GRE), moringa leaf extract (MLE) and the control (no application). **Findings:** The soil drench application of plant extracts, especially ANE and MLE, had the best growth response of gem squash plants compared with other treatments and the control. Plants treated with ANE and MLE produced a greater number of leaves and branches and simultaneously produced broader leaf area compared to other plant extracts and the control. ANE-treated plants produced the highest leaf chlorophyll concentration, followed by ALvE and MLE. All plant extracts, ANE, MLE, ALvE and GBE, significantly increased the total dry biomass, except GRE was not significantly different from the control. The yield parameters, *viz.* total fruit yield, fruit mass and fruit diameter, were positively affected by all treatments applied, although ANE- and MLE-treated plants yielded the largest number of fruit/plants, heaviest fruit and biggest fruit compared to other treatments. **Research limitations:** There were no limitations identified. **Originality/Value:** Although further studies on plant extracts usage are still required, this study highlight the potential of plant extracts, especially ANE and MLE, as a natural biostimulants to improve growth and yield attributes of gem squash has been demonstrated.

## INTRODUCTION

Plant-produced food, such as fruit and vegetables, has gained increasing popularity, because consuming such food as a component of a well-balanced diet is crucial to meet human dietary requirements, hence, sustaining a healthy body (Zhang et al., 2021). Among fruit vegetable crops, gem squash (*Cucurbita pepo* var. pepo) is a one of the most- produced Cucurbitaceae vegetable crops due to its high nutritive value (Paris, 2001). Judging its actual production is, however, challenging, as the Food and Agriculture Organization statistics (FAOSTAT) and agricultural statistics report the combined production of squash, pumpkins and gourds (Lust et al., 2016). According to FAO (2022), the world's production of pumpkins, squash and gourds was 22 806 million tons in 2022, whereas the South African production was 278 million tons. Gem squash is a seasonal vegetable consumed mashed, roasted or boiled, not only for its low caloric value, but also for its enhanced nutritional status, contributing to its medicinal properties (Blanco-Díaz et al., 2014). Besides carbohydrates, summer squash also contains various other phytonutrients, such as proteins, vitamins, amino acids, polysaccharides, phenolic acids, flavonoids, carotenoids (β-carotene), and minerals (especially K, P, Mg and Fe) (Aliu et al., 2012). All these phytochemicals are vital to human health due to their antioxidant, anti-radical, anti-carcinogenic, anti-inflammatory, antiviral and antimicrobial activities. These compounds, thus, play a pivotal role in reducing the risk of chronic diseases, such as diabetes, cancer, and heart disease (Oloyede et al., 2012).

The world's population is anticipated to rise enormously from the current 7.7 billion to exceed 9.7 billion by 2050 (Van Dijk et al., 2021). As a consequence of this exponentially increasing global population, food demand is also expected to increase by 59-98%, globally (Godfray et al., 2010; Elferink & Schierhorn, 2016). Given such increase in food demand, increasing agricultural production by approximately 60-70% is paramount in order to provide sufficient food for the global population in 2050 (Van Dijk et al., 2021). Agricultural industries are, therefore, facing a major challenge of increasing crop productivity and food production under unpredictable weather conditions due to climate change (Parajuli et al., 2019), contributing to biotic and abiotic constraints, including drought and salinity, as well as weed, pest and disease infestations (Zulfiqar et al., 2020). These constraints could potentially increase in the future and might pose a significant threat to the stability of agricultural crop production (Wheeler & Bruan, 2013). To produce sufficient food, synthetic pesticides and inorganic fertilizers have been traditionally used and became vital for agricultural production and crop protection against biotic and abiotic factors (Baweja et al., 2020). Conversely, complete reliance on chemical inputs to improve crop productivity can also jeopardize human health and compromise the environment (Sharma et al., 2019). In addition, the continuous, excessive utilization of such agrochemicals could result in the development of new resistant strains that could become difficult to control (Alewu & Nosiri, 2011; Ziatabar Ahmadi et al., 2024). In addition, most of these pesticides are expensive; hence, offering an economic incentive to reduce application of such agrochemicals, potentially making farming simpler and safer, partly as this would provide healthier and more sustainable food to consumers (Zulfiqar et al., 2020).

Modern-day agriculture aims to explore and develop alternative strategies of crop production as a stepping stone towards sustainable agriculture (Zulfiqar et al., 2020). Among several strategies proposed, use of plant extracts (like *Moringa oleifera*, *Ascophyllum nodosum*, and *Aloe barbadensis*) as natural biostimulants, has gained increasing interest in the agricultural research field to overcome the above-mentioned challenges (Carvalho et al., 2022; Goordeen & Mohammed, 2021). This emerging, promising, novel and eco-friendly approach has been extensively tested in a wide range of fruit vegetable crops, including eggplant (*Solanum melongena* L.) (Ali et al., 2019), sweet pepper (*Capsicum annuum* L.) (Rajendran et al., 2022) and tomato (*Solanum lycopersicum* L.) (Basra et al., 2016; Ngcobo et al., 2024). Interestingly, this technique has also been tested in some species of the Cucurbitaceae family, such as cucumber (*Cucumis sativus* L.) (Abd-El Gawad & Osman, 2014) and watermelon (*Citrullus lanatus* L.) (Abdel-Mawgoud et al., 2010). Given the importance of safe, healthy and sustainable food, this study aims to evaluate the influence of soil drench-applied plant extracts on the growth, certain physiological, morphological and yield attributes of gem squash.

## MATERIALS AND METHODS

### Site, plant material and growing conditions

The experiment was conducted in a glasshouse at the University of KwaZulu Natal, Pietermaritzburg, South Africa (29°37'32.9"S 30°24'18.8"E). Environmental condition, such as temperature and relative humidity (RH) inside the glasshouse, was maintained at 25±2°C, 60 % RH during the day and 10±2°C, 75 % RH at night. With regards to irrigation, plants were irrigated using the manual irrigation method; water was carefully applied directly to the media surface to avoid water falling on the leave, creating a conducive environment for pathogens to attack. Plants were irrigated at the three-days interval. Summer squash was established directly from seeds bought from Blackwood Nursery, Pietermaritzburg, South Africa. These seeds were planted on the 10 L pots filled with a mixture of clay soil and Gromor® (Gromor, Cato Ridge, South Africa) growing medium. After planting, seeds took two weeks to emerge above the mixture of the soil and growing medium.

### Collection of plant material and extract preparation

Plant materials used for extracts preparation were purchased from different suppliers. Fresh moringa (*Moringa oleifera*) leaf powder (MLP) was supplied by a commercial supplier (runKZN, Pietermaritzburg, South Africa), whereas brown algae (*Ascophyllum nodosum*) powder was purchased from a local supermarket (Dis-Chem pharmacy, Woodburn Mall, Pietermaritzburg, South Africa). Healthy aloe vera (*Aloe barbadensis*) plants were locally bought from Woodland nursery, (Pietermaritzburg, South Africa), while fresh Egyptian white garlic (*Allium sativum*) and ginger (*Zingiber officinale*) rhizome were purchased from a local supermarket. Plant extracts, *i.e.*, *Ascophyllum nodosum* extract (ANE), aloe vera leaf extract (ALvE), garlic bulb extract (GBE), ginger rhizome extract (GRE) and moringa leaf extract (MLE) were prepared according to the protocol described by (Rajendran et al., 2022); Chumark et al. (2008), Ting-Ting et al. (2011); Amuji et al. (2012) and Ngcobo and Bertling (2021), with slight modifications. A mass of 10 g of each plant material was weighed out and homogenized in a volumetric flask (1 L) with 500 mL distilled water. The homogenates were then placed onto a hot plate, continuously agitated with an electromagnetic stirrer and allowed to boil for 15 min at 100°C. After 15 min, the solutions were allowed to stand for 2 hrs to cool down; then the supernatants were collected and filtered three times through muslin cloth. To make a final volume of 1 L, serial dilutions were then made with distilled water.

### Experimental design and extract application

A pot experiment was laid out following a completely randomized design (CRD) with five replications. Five healthy, similar-sized summer squash (*Cucurbita pepo* L.) seedlings were randomly selected and assigned to each of the six treatments, namely control, ANE, ALvE, GBE, GRE and MLE, resulting in 30 experimental units. These treatments (extracts) were directly applied to the root zone via soil drench. The first soil drench application of the treatments was carried out two weeks after seedling emergence and repeated weekly until harvesting. Each plant received 200 mL of the respective extract assigned to that specific treatment. After treatment application, plants were given three days (sufficient time) to absorb the active ingredients of the extracts before the next irrigation cycle to avoid leaching of the minerals and phytochemicals present in the extract.

## Determination of dependent variables

### *Measurement of growth parameters*

Total number of leaves and branches per plant were counted manually, whereas leaf area was measured using LICOR portable leaf area meter (LI-3000CAP). Data on the total number of leaves and leaf area were initially recorded (two weeks after emergence) before treatment application until fruiting, whereas total number of branches was only recorded at fruit set. Then the data was further collected in 14-day (bi-weekly) intervals until fruiting.

### *Determination of leaf chlorophyll concentration*

Immediately after harvesting, the leaf chlorophyll concentration was determined following the destructive procedure described and improved by Lichtenthaler and Buschmann (2001), it was then calculated using the following equations (1, 2, 3, and 4):

$$\mathrm{Ca} = 12.25\ \mathrm{A}663.2 - 2.79\ \mathrm{A}646.8 \quad (1)$$

$$\mathrm{Cb} = 21.50\ \mathrm{A}646.8 - 5.10\ \mathrm{A}663.2 \quad (2)$$

$$\mathrm{Ca} + \mathrm{b} = 7.15\ \mathrm{A}663.2 + 18.71\ \mathrm{A}646.8 \quad (3)$$

$$\mathrm{Cx} + \mathrm{c} = (1000\ \mathrm{A}470 - 1.82\ \mathrm{Ca} - 85.02\ \mathrm{Cb})/198 \quad (4)$$

### *Measurement of total dry biomass*

Immediately after harvesting, fresh plant residues were over-dried for four days at 80°C. After four days of oven drying, the total dry biomass was and then recorded.

### *Measurement of yield parameters*

At full maturity stage, all summer squash fruits were harvested from all replicates. Total fruit yield (number of fruits/plant), fruits size/diameter (cm) and tuber mass (g) were recorded immediately after harvesting.

## Statistical analysis

The data collected was subjected to GenStat statistical software (GenStat®, 21st edition, VSN International, UK), and graphs were plotted with Microsoft Excel. One-way analysis of variance (ANOVA) was used to analyse the obtained data. Mean separation and comparison was performed using Duncan's Multiple Range Tests at a 5 % ($p \leq 0.05$) level of significance.

# RESULTS

## Vegetative growth parameters (number of leaves and branches, and leaf area)

The analysis of variance indicated that the pre-harvest application of treatments (plant extracts) had a significant positive effect on the vegetative growth perspective of summer squash plants (Fig. 1, 2 and 3). Although all treatments ANE, GBE and MLE had the most profound effect on these growth parameters, hence, produced plants with the highest number of leaves (50.50, 45.25 and 54.27, respectively) and greatest number of branches (1.05, 0.95 and 1.30, respectively) at week 8 of treatment application than the GRE and the control (Fig. 1 and 2). Soil drench application of plant extracts, especially ANE and MLE, considerably enhanced leaf area of summer squash plants, thus, recorded the larger leaf area (126.40 and 101.90 $cm^2/cm^2$, respectively) at week 8 of treatment application than other treatments ALvE, GBE, GRE and the control (Fig. 3).

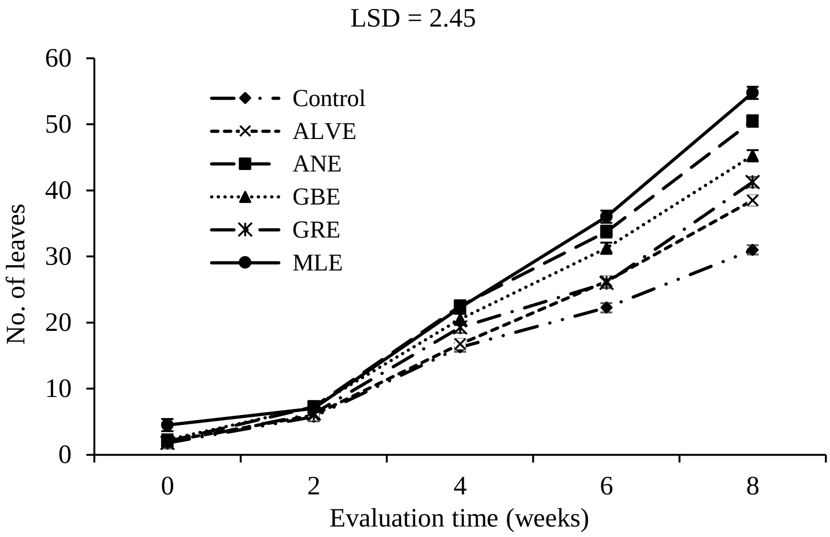


**Fig. 1.** Effect of pre-harvestly applied plant extracts on summer squash number of leaves during growth and development stage. Treatments were control = only distilled water, ANE = *Ascophyllum nodosum* extract, $AL_vE$ = aloe vera leaf extract, GBE = garlic bulb extract, GRE = ginger rhizome extract, MLE = moringa leaf extract were analysed using Duncan's multiple range test at ($p \leq 0.05$) level of significance. LSD = 2.45 and F pr < 0.001.

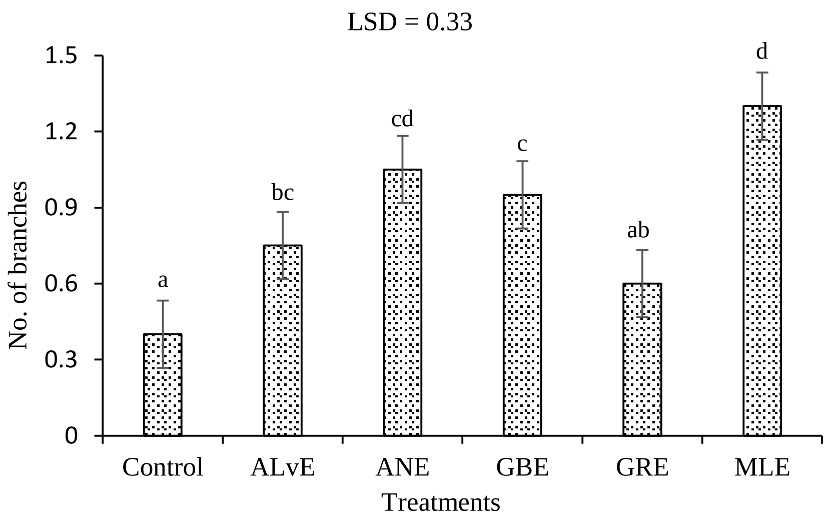


**Fig. 2.** Effect of soil drench application of plant extracts on the number of branches produced by summer squash plants. Treatments were control = only distilled water, ANE = Ascophyllum nodosum extract, $AL_vE$ = aloe vera leaf extract, GBE = garlic bulb extract, GRE = ginger rhizome extract, MLE = moringa leaf extract were analysed using Duncan's multiple range test at ($P < 0.05$) level of significance. Bars of different lower-case letters in each column denote statistically significant differences. LSD = 0.33 and F pr < 0.001.

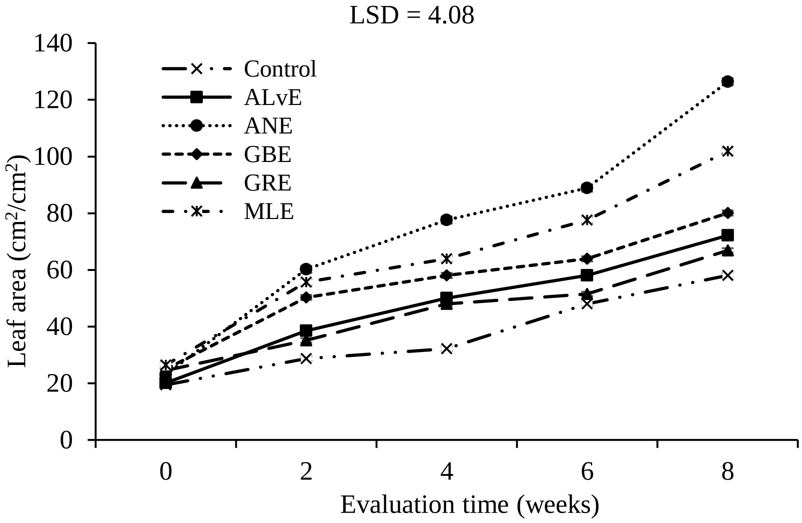


**Fig. 3.** Effect of pre-harvest application of various plant extracts on the leaf area borne by summer squash plants during growth and development stage. Treatments were control = only water, ANE = *Ascophyllum nodosum* extract, $AL_vE$ = aloe vera leaf extract, GBE = garlic bulb extract, GRE = ginger rhizome extract, MLE = moringa leaf extract were analysed using Duncan's multiple range test at ($p \leq 0.05$) level of significance. LSD = 4.08 and F pr < 0.001.

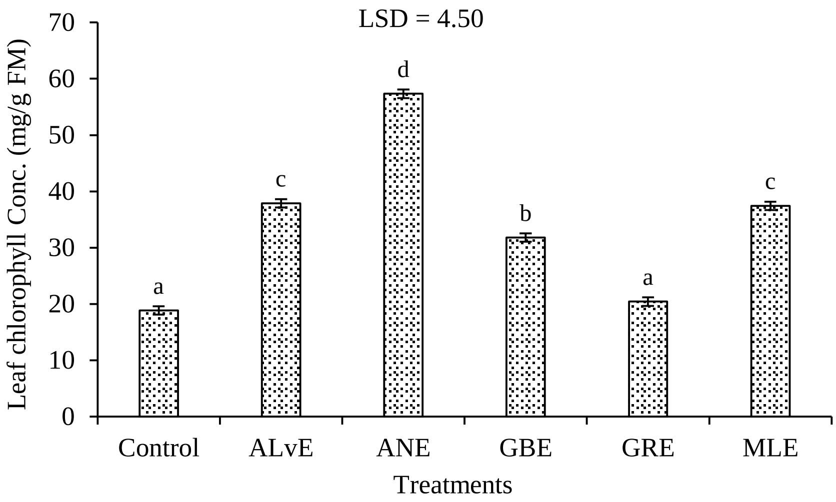


**Fig. 4.** Effect of pre-harvest application of plant extracts on summer squash leaf chlorophyll concentration. Treatments were control = only water, ANE = *Ascophyllum nodosum* extract, $AL_vE$ = aloe vera leaf extract, GBE = garlic bulb extract, GRE = ginger rhizome extract, MLE = moringa leaf extract were analysed using Duncan's multiple range test at ($p \leq 0.05$) level of significance. Bars of different lower-case letters in each column denote statistically significant differences. LSD = 4.50 and F pr < 0.001.

## Physiological parameter (leaf chlorophyll concentration)

The pre-harvest application of plant extracts on summer squash plants, as soil drench application, significantly improved the leaf chlorophyll concentration ($p \leq 0.05$). As a result, the highest leaf chlorophyll concentration was recorded in summer squash plants treated with ANE, ALvE and MLE (57.33, 37.87 and 37.43 mg/g FM) compared with other treatments GBE, GRE and the control (Fig. 4).

### Morphological parameter (total dry biomass)

Variations on the morphological data indicated that soil drench application of plant extracts, as a pre-harvest treatment, significantly influenced ($p \leq 0.05$) the total dry biomass of summer squash plants. Unlike other treatments, ANE, MLE and GBE had a considerable effect on total dry biomass, as they recorded the heavier total dry biomass (57.67, 50.09 and 47.55 g, respectively) than ALvE, GRE and the control (Fig. 5).

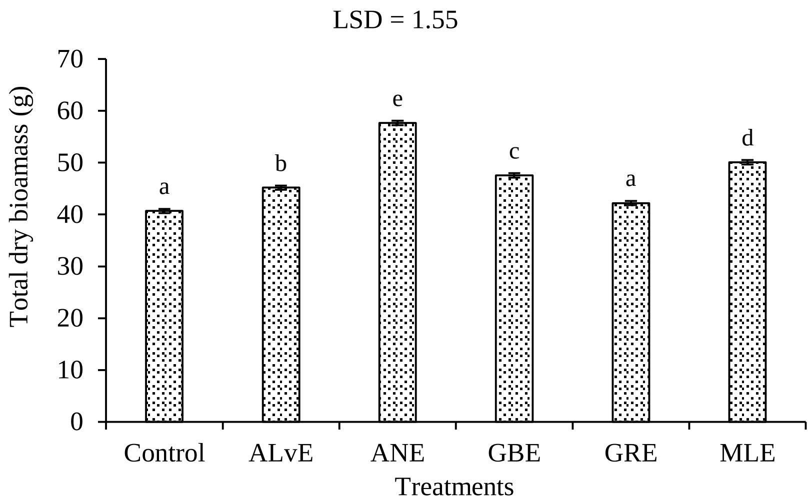


**Fig. 5.** Effect of pre-harvest application of plant extracts on the total dry biomass of summer squash plant residues. Treatments were control = only water, ANE = *Ascophyllum nodosum* extract, $AL_vE$ = Aloe vera leaf extract, GBE = Garlic bulb extract, GRE = Ginger rhizome extract, MLE = Moringa leaf extract were analysed using Duncan's multiple range test at ($p \leq 0.05$) level of significance. Bars of different lower-case letters in each column denote statistically significant differences. LSD = 1.55 and F pr < 0.001.

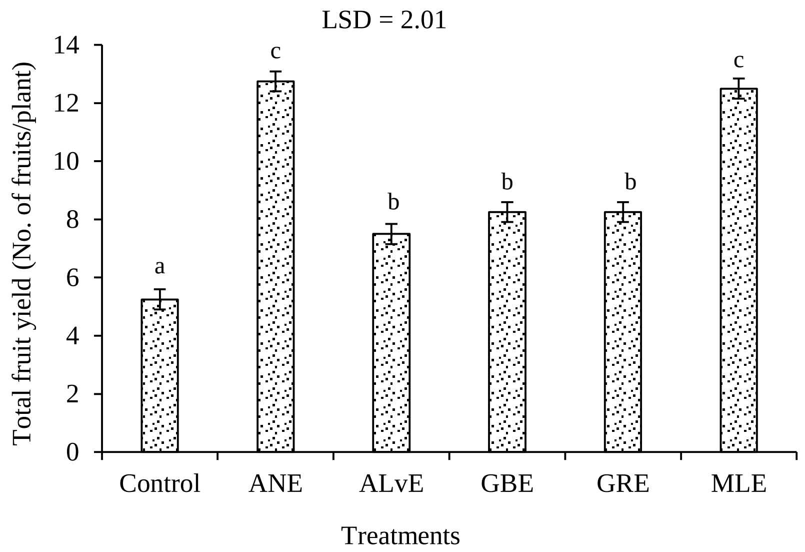


**Fig. 6.** Effect of soil drench-applied of plant extracts on the total fruit yield of summer squash. Treatments were control = only water, ANE = *Ascophyllum nodosum* extract, $AL_vE$ = aloe vera leaf extract, GBE = garlic bulb extract, GRE = ginger rhizome extract, MLE = moringa leaf extract were analysed using Duncan's multiple range test at ($p \leq 0.05$) level of significance. Bars of different lower-case letters in each column denote statistically significant differences. LSD = 4.50 and F pr < 0.001.

### Yield parameters (total fruit yield, fruit mass, and fruit diameter)

The obtained results revealed that the pre-harvest application of plant extracts, as soil drench, had the significant improvement ($p \leq 0.05$) on the yield attributes of summer squash. ANE- and MLE-treated plants notably had the pronounced effect on the measured yield attributes, hence, yielded the highest total fruit yield (12.75 and 12.5 fruits/plant, respectively), heaviest fruit mass (227 and 225.2 g, respectively) and largest fruit diameter (9.5 and 10 cm, respectively) compared to other treatments ALvE, GBE, GRE and the control (Fig. 6, 7 and 8).

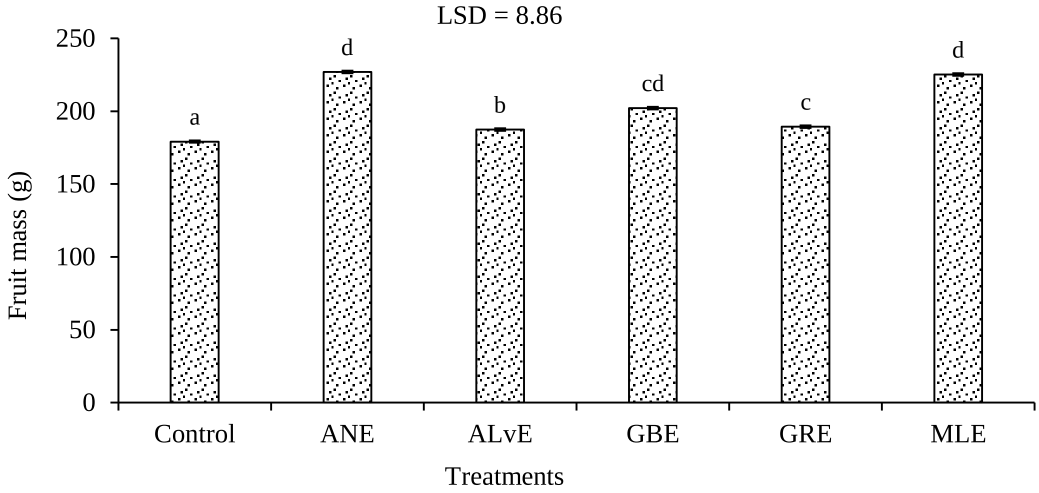


**Fig. 7.** Effect of soil drench-applied plant extracts on summer squash fruit mass. Treatments were control = only water, ANE = Ascophyllum nodosum extract, $AL_vE$ = aloe vera leaf extract, GBE = garlic bulb extract, GRE = ginger rhizome extract, MLE = moringa leaf extract were analysed using Duncan's multiple range test at ($p \leq 0.05$) level of significance. Bars of different lower-case letters in each column denote statistically significant differences. LSD = 8.86 and F pr < 0.001.

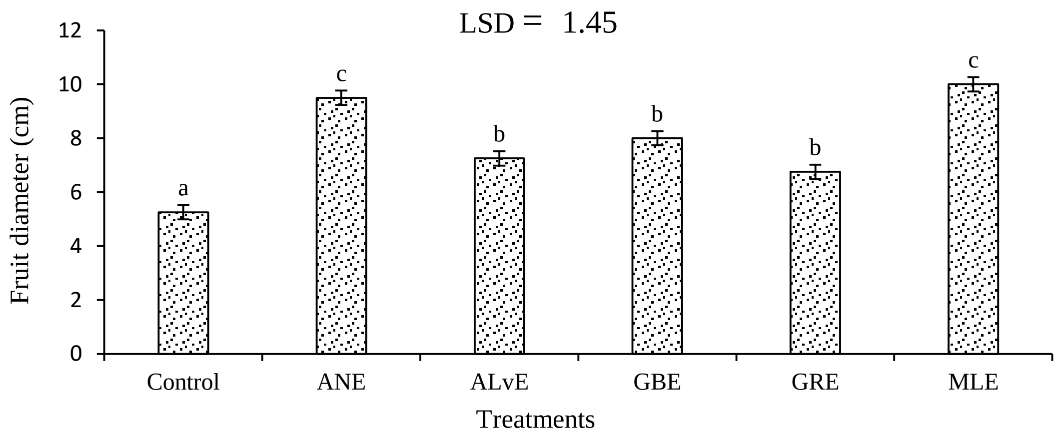


**Fig. 8.** Effect of pre-harvestly applied plant extracts on the fruit size-diameter of summer squash. Treatments were control = only water, ANE = *Ascophyllum nodosum* extract, $AL_vE$ = aloe vera leaf extract, GBE = garlic bulb extract, GRE= ginger rhizome extract, MLE = moringa leaf extract were analysed using Duncan's multiple range test at ($p \leq 0.05$) level of significance. Bars of different lower-case letters in each column denote statistically significant differences. LSD = 1.45 and F pr < 0.001.

## DISCUSSION

Despite the impact of climate change and its aligned factors, there is a need for agricultural industries to increase agricultural production in an eco-friendly and sustainable approach to produce safe and high nutritious food in order to feed the exponentially increasing global population and meet increasing food demand; hence, contributing to global food security and achieving sustainable development goals *viz.* zero hunger, as well as good health and well-being (FSIN, 2020). Pre-harvest application of plant extracts, as natural biostimulants, has received an increasing interest due to its positive effect on plant growth and yield potential of crops (Craigie, 2011; Hayat et al., 2018). Plant extract application has demonstrated its immense potential to increase plant vigour and yield attributes by interfering with plant physiological processes and increasing nutrient acquisition without endangering the environment (Zulfiqar et al., 2020).

The results presented in the present study indicate that soil drench application of plant extracts considerably improved vegetative growth, physiological and yield attributes of gem squash. These results are confirmed by Jang et al. (2021), who noted that application of various plant extracts as soil drench application significantly improved growth and yield attributes of cucumber. According to Kumari et al. (2011), vegetative growth, physiological, morphological and yield attributes of Cucurbitaceae species (*Cucurbita pepo* var. pepo) were enhanced by the application of plant extracts, this could possibly be due to their biofertilization effect. Plant extracts contain significant amounts of mineral nutrients, especially N, P, K, Mg, Ca, Zn, and Fe (Moore, 2004; Moyo et al., 2011). Hala et al. (2017) reported that the presence of such mineral elements in the extracts increases the availability and uptake of these mineral, thus, promoting vegetative growth, physiological, morphological and yield parameters.

The beneficial effect of these plant extracts could also be linked to the biostimulatory effect due to the growth-promoting phytohormones, such as auxins, gibberellins and cytokinins, present in the extracts (Rayorath et al., 2008; Yamaguchi, 2008). The exogenous application of these extracts, therefore, induced the endogenous biosynthesis of plant hormones, thereby promoting cell expansion through cell division and elongation, resulting in growth and yield improvement. Improved leaf chlorophyl concentration following plant extract applications could possibly be attributed to enhanced gene transcripts involved in photosynthesis, cell metabolism and stress response (Wang et al., 2009). Application of these extracts suppressed cysteine protease activity, thus, inhibiting chlorophyll degradation and delayed senescence (Buet et al., 2019). Enhanced leaf chlorophyl concentration due to ANE application could be ascribed to the presence of betaines, which also inhibited the breakdown of leaf chlorophyl (Blunden et al., 2009).

In addition to minerals, phytohormones and betaines, plant extracts contain several antioxidant compounds, including ascorbic acid, phenolics and tocopherols, which their presence induces the antioxidant biosynthesis, hence, triggering plant defence mechanisms against stress caused by reactive oxygen species (ROS) (Wang et al., 2009; Saini et al., 2016; Ngcobo & Mbuyisa, 2024). Plant extracts, especially ANE, are good source of alginic acids and polyuronides, which are responsible for the improvement of soil properties, including soil structure, water -holding capacity aeration, capillary action, hence, boosting soil microbial activity and soil organic matter, which ultimately increase mineral availability and absorption by plants (Moore, 2004). These compounds also increase the mobility and translocation of carbohydrates and other organic compounds, hence, improving fruit development. Rioux et al. (2007) revealed that ANE also contain polysaccharides (*viz*. fucoidan and laminarin), that exhibit radical scavenging antioxidant activity

The results obtained in the present study coincide with Mbuyisa et al. (2023), who reported that application of various plant extracts, especially ANE and MLE, positively influenced growth parameters, such as number of leaves, number of branches, leaf area, total dry biomass and yield parameters of potato (*Solanum tuberosum* cv. Sifra). Ngcobo and Bertling (2021) demonstrated MLE application to cherry tomato (*Solanum lycopersicum* var. cerasiforme) significantly increases vegetative growth parameters, which these results are in line with present study. In addition, these findings coincide with Hidangmayum and Sharma (2017), who reported a significant increase in vegetative growth and yield parameters of onions (*Allium cepa* var. N-53), following ANE application. Furthermore, Manna et al. (2012) also demonstrated that application of ANE to chilli plants increased leaf chlorophyll concentration. Our results are in accordance with Yaseen and Takacs-Hajos (2022), who indicated that applying MLE to different lettuce (*Lactuca sativa*) cultivars (*viz*. May King, Kobak and Great Lakes) improved leaf chlorophyll concentration. In addition, Hala et al. (2017) revealed that MLE application to remarkably improved growth and yield attributes sweet pepper, which their findings correspond with the results of the present study. Similarly, Rayorath et al. (2008) revealed that application of ANE positively affected vegetative growth parameters, such as number of leaves, plant height and root growth, of *Arabidopsis thaliana*. Moreover, the application of ANE enhanced vegetative growth and yield of leafy vegetables, such as spinach (*Spinacea oleracea*) (Fan et al., 2013) and lettuce (Chrysargyris et al., 2018).

## CONCLUSION

The use of plant extracts, especially ANE and MLE, applied as natural biostimulants to increase crop productivity significantly improved growth, physiological and morphological, as well as yield parameters of gem squash. It can, therefore, be concluded that exogenous application of such plant extracts can effectively enhance plant growth and development, as well as yield, of horticultural crops. Hence, the results presented in this research are of high significance, particularly to small-scale cucurbit growers, as hot water extracts present a sustainable, cheap and environmentally friendly approach to increasing crop productivity, while reducing the utilization of synthetic and chemical-based, thereby sustaining the environment and reducing health concerns of consumers. To promote the adoption of such application, further studies on plant extracts, particularly ANE, GBE and MLE, are still required before recommendations for use in large-scale production can be made.

**Conflict of interest**
Authors reported no potential conflict of interest.

**Data availability statement**
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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