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Impact of Foliar Spray with Some Nutrients as Nano-Particles on Growth, Yield, Enzymatical and Anatomical Changes of Cauliflower

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ABSTRACT

An experiment was carried out at the Experimental Station of Faculty Cairo University during two winter seasons of 2020/2021 and 2021/2022 to study the effect of foliar spray of Ca, Mg and Zn fertilizers in nanoparticles applied alone or in mixed composition on growth , yield, total soluble solids, changes in leaves anatomy structure biochemical components, enzymatic activity. The concentration of used fertilizers was 50 ppm which foliar sprayed cauliflowers compared to control plants that sprayed by water. Results indicated that the calcium fertilizer induced highest values of growth, yield, chlorophyll content, total soluble solids, leaf anatomical structure of relative to control. In addition to calcium nano-fertilizer had a significant effect on chlorophyll concentration and Chl. a/b. All nano-fertilizers had significant reduction on total soluble phenol compared to control. zinc nanoparticle recorded the highest significant total free amino acids as well as free proline accumulation. calcium nanoparticles showed the highest significant specific activity for both catalase as well as peroxidase in cauliflower plant.

KEYWORDS: Cauliflower, Calcium, Magnesium, Zinc, Nano-Fertilizer, Growth, Yield, Anatomy.

1. INTRODUCTION

Cauliflower (*Brassica oleracea var*. Botrytis) is one of the Brassicaceae family, which includes cauliflower. In areas of cool temperate climates, it is one of the developing plants. The plant is very rich in nutrients. In addition to 92 grams of water, 2.4 grams of protein, 25 calories, 4.9 grams of carbohydrates, 2.2 mg of calcium, 1.1 mg of iron, 72 mg of phosphorous, and many vitamins are present in every 100 grames of the edible portion. Abd AL-Hseen and Manea

(2020).Both micronutrients and macronutrients are necessary for healthy crop growth and development, cauliflower responds well to both types of nutrition (Rahman et al,2007).

One such technology that could transform modern science is nanotechnology, which can create many elemental nanoparticles and offer advantages over conventional molecules. (Saleh, 2015). The great solubility of nano-fertilizers distinguishes them apart from other fertilizers, as does their exceptional penetration rate into plant tissues. In addition, they are highly efficient when used in modest amounts, which means they are helpful in solving many of the problems that face agriculture, including those caused by the persistent use of inorganic chemical fertilizer. The goal of incorporating nanomaterials into agricultural practices is to increase the systems' long-term sustainability and productivity (Jyothi and Hebsur, 2017) as well as nanofertilizers have a large surface area, which increases the enzymes and biochemical reactions, they outperform chelated fertilizer in all evaluated features. As they easily dissolve and proliferate, they lead to more reactions, enzymatic activities, and cellular divisions. Additionally, the nanoparticles reduce or inhibit the formation of reactive oxygen species (ROS), which lessens oxidative damage, delays senescence, and promotes vegetative growth in plants (Sorooshzadeh et al., 2012; Morteza et al., 2013). Therefore, the most effective way to solve nutritional shortages and raise crop output and quality is by foliar spray. (Luque, 2017). research on the foliar application of nanofertilizers is currently less comprehensive than that on soil application, making nanofertilizers and foliar fertilization the focus of interest at the moment According to Ding et al. (2023).

Zinc plays a crucial role in the metabolism of nitrogen and is a necessary component of numerous enzymes, including RNA polymerase, alcohol dehydrogenase, carbonic anhydrase, and superoxide dismutase. It is necessary for triggering specific metabolic processes and contributes to the creation of plant growth compounds and enzyme systems. Singh et al, 2017)

Furthermore, according to Ceppi et al. (2012), magnesium is necessary for Grana accumulation in chloroplasts. Plant damage results from a Mg^2+ deficit, according to Senbayram et al. (2015). It is therefore assumed that having enough Mg available is useful to ensure optimal performance under drought stress. Within plant cells, oxidative stress may result from a magnesium deficit. In conditions where magnesium is deficient, CO_2 stabilization damages the RUBP carboxylase by decreasing biosynthesis, which may result in the generation of reactive oxygen species (ROS), damage to photosystems I and II, and a decrease in the plant's growth and yield.

Calcium is essential for the formation of protein, which functions as a valve in various cell contents and the density of cytoplasm vacuoles, cell division, cell elongation, and its effect on meristematic cells and morphological characteristics (Reddy 2001). Nano calcium fertilizer is one of the important things in the growth stages of cauliflower, which needs nutrients to complete the plant life cycle (Monareal et al., 2015)

The aim of this research to study the effect of some Nanofertilizers on plant growth ,yield and its quality on cauliflower plant.

2. MATRIAL AND METHODS

2.1. Experiment layout

The Experimental Station of Cairo University's Faculty of Agricultural, Giza, Egypt (30°01'32.5"N & 31°11'33.0"E) served as the study's location for two successive 2020/2021 and 2021/2022 winter seasons. Cauliflower cv. AD6033 purchased from Vilmorin Company was chosen for this study. Cauliflower seeds were sown in nursery on the first September in both seasons. The mixture of peatmoss: vermiculite (1:1v). Drip irrigation was used, the recommended N, P and K were added according to their commendation of Egyptian Ministry of Agriculture, and the other agricultural practices were done similarly as practiced. The experimental plot area was $4m^2$ (5m length and 0.8 m width, each plot contains one rows. Seedlings were planted at side of drip irrigation line. The number of treatments were 5 with three replicates (15plots). The experimental design were arranged in randomized complete block design with three replicate.

2.2. Preparation of the nano fertilizer

According to [Abdel Wahab M. Mahmoud et al 2020, Mona Awad et al, 2022, and Abdel Wahab M. Mahmoud et al, 2023], nanoparticles were produced using the top to bottom molecular chemical technique.

2.2.1. Nano Zn

Zinc chloride was dissolved in water to create nano zinc for eight hours, the sodium hydroxide solution was added gradually at a molar ratio of 1:2 while being vigorously stirred. Using a high-speed stirrer, the precipitate was filtered and extensively cleaned with deionized water in a mixed water/toluene system. Next, 1.0% polyethylene glycol was added to a volumetric flask, and the mixture was again fully cleaned with ionised water for three hours. The precipitate was dried at 10, 20, 25, 30, 40, and 50 degrees Celsius. It was then placed in an oven at 100 degrees Celsius and exposed to 1.5 pressure points for three days straight, seven hours a day.

2.2.2. Nano Ca

In order to manufacture Nano Ca, $CaCO_3$ was dissolved in calcium hydroxide at a molar ratio of 1:1 and vigorously stirred for six hours, followed by a further twelve hours of stirring. Using a high-speed stirrer, the precipitate was filtered and thoroughly cleaned with ionised water in a mixed water/hexane system. It was then rinsed twice with ionised water alone for a duration of two hours. then centrifuged at 1200 rpm for 20 mintues. After being dried in an oven at 100°C for two days, the precipitate was exposed to 1.5 psi of pressure for five hours each day. It was then moved to a buffer solution and allowed to sit for three hours at room temperature. Finally, the suspension was sonicated for thirty minutes at 0.5 cycles at 80°C.

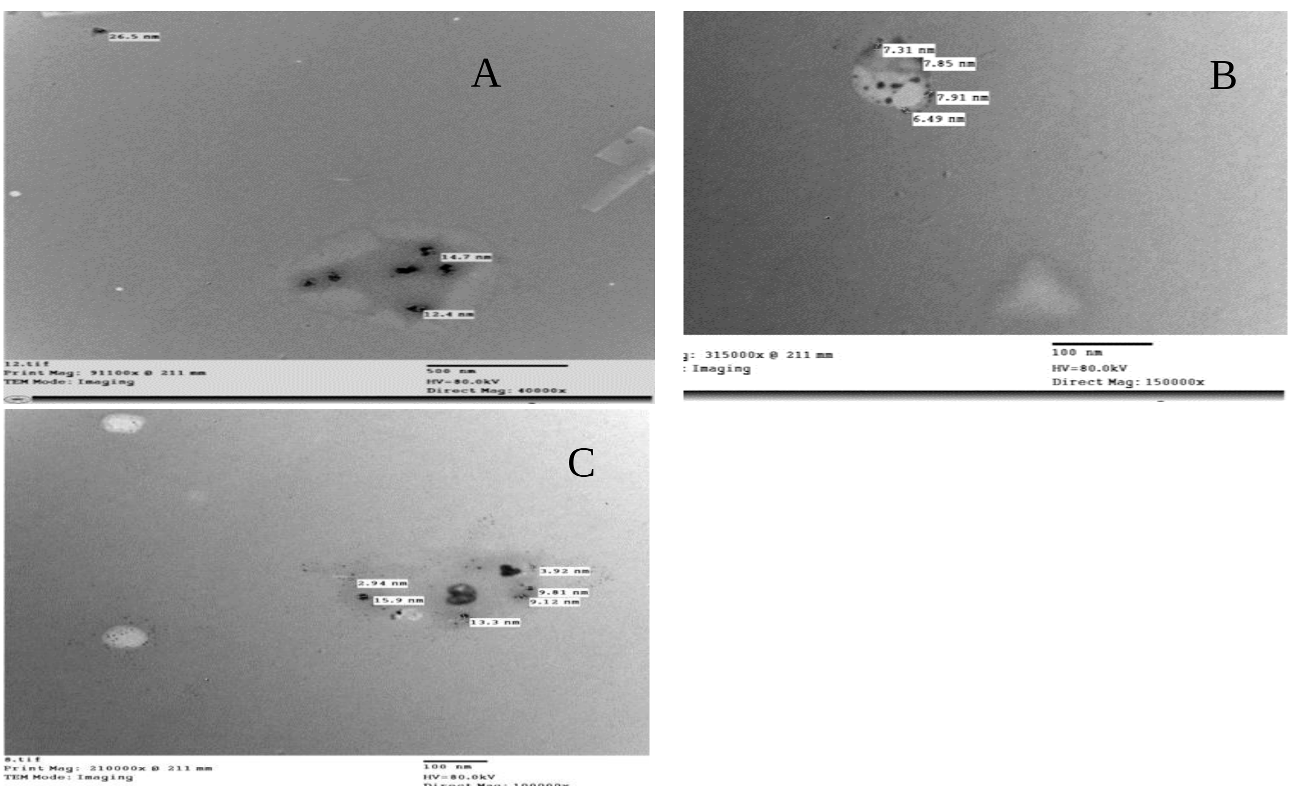

Fig. 1 Image of Nano-calcium (A), Nano magnesium (B) and Nano-Zinc (C).

2.2.3. Nano Mg

Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) and sodium hydroxide (NaOH) were combined to create nano magnesium. After adding the NaOH solutions (2 mol/L) to a 100 mL volumetric flask, 1.0% polyethylene glycol (which represents the mass ratio of $MgCl_2 \cdot 6H_2O$) was added and the mixture was shaken for 45 minutes. Following the rotor's addition to the volumetric flask, 50 mL of $MgCl_2 \cdot 6H_2O$ solutions were carefully added to the flask in two drops at a time. The flask was then extensively cleaned with ionised water using a high-speed stirrer in a mixed water/hexane system, and it was centrifuged for 25 minutes at 1300 rpm.

With an electron acceleration voltage of 60 kV, Transmission Electron Microscopy (TEM) was used to directly observe the size and shape of nanoparticles. The experiment was conducted in the Transmission Electron Microscopy lab (TEM) at the Faculty of Agriculture, Cairo University Research Park (FA-CURP), where a few drops of the solution were applied to a carbon-coated copper grid (Okenshoji Co., Ltd., microgrid B).

2.3. Treatments

1-Untreated
2- Magnesium nanoparticles
3-Zinc nanoparticles
4-Calcium nano particles
5-Combination with Mg, Zn and Calcium nanoparticles with the same concentration 50 ppm .

The control treatment was sprayed with water. The cauliflower plants in the open field were sprayed three times for each season. The first spraying was on the 7^{th} day post transplanting. Two weeks between each with 50 ppm concentration rate

2.4. Plant growth parameters

After harvesting

Plant height, leaf number, head weight, and c hlorophyll contents in leaves using a SPAD (SP AD 502 Minolta Co., Osaka, Japan) Metre. The heads of each plot were weighed to determin e the total yield

2.5. Head quality

At harvest time, six mature heads per experimental plot were selected at random to measure the following: Total soluble solids percentage (TSS %) The TSS was measured in heads by using digital refractometer (PAL-BX/ACID2, Atago Co. Ltd.,Tokyo, Japan).and the results diven as Brix

2.6. Physiological Parameters

Analysis was done on the fifth leaves of twenty plants selected from each treatment with three replications using an infrared gas analyzer, the LICOR 6400 Portable Photosynthesis System (IRGA, Licor Inc., Lincoln, NE, USA), to assess photosynthesis and leaf stomatal conductance. At an approximate light intensity of 1300 mol m^{-2} s^{-1} and 80% relative humidity, measurements were conducted between 9 a.m. and 2 p.m. The volume gas flow rate was 400 mL/min, and the temperature range of the leaf chamber was 25.2 to 27.9 °C. The air had 398 μmol mol^{-1} of CO_2 in it.

2.7. Anatomical of Cauliflower Leaves

Tested samples contain the middle leaf developed on the main stem of caulifower plants were selected during the second growing season of 2022, 10 days after the third foliar application. According to Mohammed and Guma (2015). Transverse sections were done with a Leica Microtome RM 2125, and then micrographed and measured using a Leica Light Image Analysis System DM 750 at the Research Park (CURP), Faculty of Agriculture, Cairo University. The following parameters were recorded: thickness of the midvien (μm), lamina (μm), palisade tissue (μm), spongy tissue (μm), xylem, phloem, upper and lower epidermis (μm), bundles dimension (μm), number of xylem rows in midvein bundle and mean vessels diameter (μm).

2.8. Chemical analysis

Photosynthetic plant pigments (chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids) were determined according to (Moran, 1982). In ethanol extract, total sugars, total free amino acids, total soluble phenols floret of cauliflower are determined are determined in ethanol extract, on fresh basis and expressed as mg/g F.w. Total sugars determination was carried out according to (Dubois et al., 1956). Total soluble phenols were estimated using the folin-Ciocalteau colorimetric method ((Swain and Hillis, 1959). The total free amino acids were determined using Ninhydrin reagent according to (Moore and Stein, 1954). Catalase activity, CAT (EC 1.11.1.6) was estimated using the method described by (Sinha, 1972). Peroxidase activity, POX (EC 1.11.1.7) was determined according to the method of (Herzog and Fahimi, 1973). They expressed as U mg^{-1}P min^{-1}. Total soluble proteins were estimated according to Lowry-Folin as described by Dawson et al. (1986), expressed mg g^{-1}F.w.

2.9. Statistical Analysis

Statistical analysis of the experimental data was performed with CoStat software and ANOVA procedures. The least significant difference (L.S.D.) test, as supplied by Snedecor and Cochran (1976), was used to compare the treatment means using the MSTAT-C v. 2.1 (Michigan State University, Michigan, USA).

3. RESULT AND DISCUSSION

3.1. Vegetative growth

3.1.1. Plant height

As shown in Table 1 that there are significant differences between all treatments .foliar spray with nano-calcium fertilizer had the highest values of plant height by ratio 39.6% and 19.8% respectively in both seasons compared to control, followed by the combination between treatments in the both seasons.

3.1.2. Leaf number

The result appear in Table1 which show the effect of nano-fertilizer foliar spray on leaf number as noticed the superiority of nano-calcium foliar spray in giving the highest leaf number by ratio 48.5% and 48.66% respectively in both seasons compared to control, in addition to foliar spray with nano-magnesium by ratio 39.39% and 37.0 % respectively , in both seasons compared to untreated .According to Ahmed et al. (2011), applying magnesium topically increased plant height as well as the fresh and dry weight of the leaves. Additionally, magnesium is crucial for photosynthesis as well as protein synthesis in leaves (Xiao et al., 2018).

3.1.3. Chlorophyll/SPAD

As shown in Table 1 the chlorophyll /SPAD values were greater in cauliflower plants sprayed with nano-calcium that increased by ratio 7.70% and 13.29% respectively, in both seasons compared to untreated

3.1.4. Total soluble solid

Data in Table 2 it was observed that cauliflower plant that sprayed with nano-calcium showed maximum values of total soluble solid that increased by 25% and 19% respectively, in both seasons compared to untreated. In addition there were not significant differences between all treatments except calcium had the highest value of total soluble in the second season

3.1.5. Head diameter

Similar trend were observed in yield components and their quality .The result presented in Table 2 indicate that there is significant effect of nano-calcium foliar spray on head diameter that gave the highest values of head diameter by ratio 55.26% and 60.50% respectively in both seasons.

Table 1. Vegetative growth parameters of cauliflower as response to foliar spray with some nutrients as Nano-particles in 2020/2021 and2021/2022 successive seasons.

Treatments	Plant height (cm)		Leave number/plant		Chlorophyll/SPAD	
	1st	2nd	1st	2nd	1st	2nd
Control	55.33 c	51.33	11.67 b	12.33 b	57.63 b	55.87 bc
Magnesium	57.67 bc	62.00 b	16.33 a	17.0 a	63.60 a	57.97 b
Zinc	60.0 abc	59.33 b	14.00 ab	13.67 b	55.87 b	53.83 bc
calcium	66.3 a	71.67 a	17.33 a	18.33 a	62.07 a	63.30 a
Ca+Mg+Zn	64.00 ab	66.33 ab	14.33 ab	11.33 b	53.43 b	51.83 c

Table 2. Yield and its component as response to foliar spray with some nutrients as Nano-particles in 2020/2021 and 2021/2022 successive seasons.

Treatments	TSS (Brix)		Head diameter (cm)		Head weight (kg)		yield/fed (ton)	
	1st	2nd	1st	2 nd	1st	2nd	1st	2nd
Control	5.1d	5.2b	15.67 c	14.33c	0.8667 c	0.96 d	8.487c	9.660 d
Magnesium	5.7b	5.53b	18.33 c	19.0b	1.43 b	1.43 bc	14.16 b	14.26 bc
Zinc	5.6 bc	5.4 b	21.33 b	20.67 ab	1.533 b	1.633 b	15.20 b	16.46 b
Calcium	6.5 a	6.3 a	24.33 a	23.00a	2.867 a	2.767 a	28.79 a	27.60 a
Ca+Mg+Zn	5.5 c	5.6 b	18.33 c	18.33 b	1.133 bc	1.167 cd	11.58 bc	11.65 cd

3.2. Yield

Date in Table 2 illustrate that cauliflower plant that sprayed by nano fertilizer had a significant excess on total yield compared to untreated. On the other hand plants that treated by nano-calcium showed a significant improvement resulted in the highest values of head weight 2.86 kg and 2.76 kg .In addition the maximum values of total yield /fed. Observed with plants that sprayed with nano

Calcium 28.79 ton and 27.60 ton respectively, in both seasons compared to control. The results attributed to that the Ca effect may be explained in two ways: first, by its availability in adequate amounts to meet the needs of the plant during growth; and second, by its well-known roles in the metabolism of plants, including respiration, cell division, photosynthesis, biosynthesis, and ionic absorption. Actually, a sufficient concentration of calcium in plant organs ensures that cells maintain the integrity of their cell walls and stabilize their membranes, promotes root growth and the early onset of flowering in agronomic and vegetable crops, and increases plant height by increasing mitotic activity in the terminal meristem (Mohsin Khadimi, 2013).

As well as Fertilizers with nanocalcium base promote the growth of foliage, and leaf nutrient content and chlorophyll (Sabir et al., 2014).

In addition to the function of nanoparticles in modifying gene expression that results in biological processes impacting plant growth and development; also, the special characteristics of nanoparticles modify the physicochemical characteristics of plants in ways that influence plant growth in distinct ways (Aslani et al., 2014)

In addition to that nanocalcium is essential to plant development, its significance in enhancing plant growth .Along with improving cell elongation, it takes involvement in metabolic activities. Furthermore, the enzymatic and hormonal functions of calcium shield plants from heat stress. It increases plant resilience to disease and controls the opening and closing of stomata. Moreover, supplementing with nanocalcium produces better quality (Arvin et al.,2005)

The combination between all treatment gave lowest compared to nano calcium alone because of competing actions with other cations and the plant's inability to translocate Ca2+ through xylem into the young, actively growing leaves at a critical juncture in their development, some plant species may not be able to fully benefit from calcium when it is available (Kong et al., 2014).

Moreover, calcium increases photosynthesis in plants, increasing dry matter content and averting biotic and abiotic stress (Meena et al., 2017).It also contributes to the special qualities of nanofertilizers, which are derived from smaller, more efficient plant cell particles. expanded the surface area of absorption and allowed direct access into plant cells to carry out the necessary tasks (Sabir et al., 2014),

Foliar spray with Ca as Nano-particles increased total yield of cauliflower may be due to that Ca increased plant growth and chlorophyll content in leaf tissues (Table 1) , head diameter and head weight (Table 2) as well as increased thickness of the midvien, lamina, palisade tissue, spongy tissue, xylem, phloem, upper and lower epidermis, bundles dimension, number of xylem rows in midvein bundle and mean vessels diameter (Table 3).

3.3. Physiological Parameters

Moreover all nano-fertilizer that sprayed on cauliflower plants showed significant effect on transpiration rate which concluded the effect of each fertilizer alone had additive effect and when mixed on the transpiration rate of leaves. On the other hand, control showed the lowest significant transpiration rate in cauliflower leaves in both seasons. Furthermore, the effect of the studied ingredient on CO_2 diffusion had been studied. It showed that control recorded the lowest significant value in CO_2 diffusion in cauliflower leaves. However, magnesium nanoparticles and calcium nanoparticles showed the highest significant CO_2 diffusion when compared to the other studied ingredients. Followed by zinc nanoparticles and the mixture ingredients showed significant reduction in CO_2 diffusion in the first seasons in addition that zinc nano fertilizer had a significant effect on difusion of Co_2 in the second season. Concerning the effect of studied ingredients on the quantum in cauliflower leaves, results showed that all plants fed by fertilizers formed as nanoparticles showed a significant increase in quantum when compared to the control. Furthermore, the highest significant quantum values showed by calcium nanoparticles and then followed by mixture of these ingredients in both seasons, in addition to magnesium and zinc nano-fertilizer in the second season. Thus, calcium nanoparticle was recorded the highest significant treatment enhanced quantum values, and ranked highest value in both transpiration rate as well as CO_2 diffusion in cauliflower leaves.

Table 3. Transpiration rate, CO_2 diffusion and quantum in cauliflower as response to foliar spray with some nutrients as Nano-particles in 2020/2021 and2021/2022 successive seasons.

Treatments	Transpiration Rate (mg'nr^s"1)		Diffusion (mm^2/s)		Quantum yield (Io/I)	
	1st	2nd	1st	2nd	1st	2nd
Control	2.810 b	3.46 b	2.04c	1.74 c	216.7 c	186.8 b
Mg	3.770 a	4.36 a	4.00 a	3.08 ab	530.0 b	510.0 ab
Zn	3.990 a	3.98 ab	3.14 b	3.48 a	503.3 b	710.0 a
Ca	4.250 a	4.7 a	3.19 a	3.73 a	716.6 a	572.7 a
Mg+Ca+ Zn	4.330 a	3.89 ab	3.12b	3.31 bc	590.0 ab	503.3 ab

These result may be due to that the surface of a leaf has trichomes, stomata, and phloem pores. There are two pathways via which NPs are absorbed by foliage: the stomatal pathway and the cuticle pathway. According to Wang et al. (2013), nanoparticles (NPs) with a diameter of less than 4.8 nm can enter a leaf directly through the cuticular channel, while bigger particle sizes can enter through stomata. The stomatal route is thought to be a more effective means of NP absorption because of the high density of the stomata themselves (su et al 2019).NFs are transported throughout the plant by the phloem after being absorbed by the stomata. Wu and Li (2022). By facilitating quick nutrient availability at plant development areas, nanofertilizers can raise chlorophyll levels. By facilitating quick access to nutrients at plant growth sites, nanofertilizers can raise the rate of photosynthetic activity, the generation of chlorophyll, and ultimately the growth and development of plants. influence the stratum corneum's ability to absorb NPs efficiently. For instance, NP entrance is influenced by stomatal size, and NPs can influence stomatal size; NP adherence to leaves influences NP delivery to plants effectively (Kah et al 2019).

3.4. Cauliflower Leaf Anatomy

Concerning the anatomical features, Cauliflower leaves have upper and lower epidermis with stomata on both sides. The mesophyll consists of 2-3 layers of palisade parenchyma cells and 3-4 layers of spongy parenchyma cells with small intercellular spaces. In the midrib, the vascular bundle is collateral, with the external phloem and internal xylem (Figures 2,3, and 4). As shown in Table (4) and Figures (2, 3, and 4), all the treatments improved all the anatomical characteristics under study compared to the control plants.The best results were achieved when the plants treated with the Ca NPs followed by the mixture of Zn NPs+ Mg NPs+Ca NPs then Zn- NPs, while the Mg- NPs treatment had the lowest value. The thickness of upper epidermis, the lower epidermis and midvein of the Cauliflower plants treated with the Ca NPs increased by 24.54%, 65.22%, and 48.56%, respectively, when compared with the control (Figures 2, 4 T1 and T4) .

The thickness of leaf lamina was increased by 56.93% due to the increase in the thickness of palisade and spongy tissues by 58.10% and 55.72%, respectively, when compared with the control (Figure 4 T1 and T4). Likewise, the vascular bundle of the midvein also increased on account of the increase in the dimension of the main midvein bundle, thickness of xylem, thickness of phloem, Number of xylem rows in main midvein bundle and the mean diameter of vessels by 41.09%, 45.25%, 60.88%, 66.60% and 52.20%, respectively, when compared with the control (Figures 2, 3 T1 and T4).

A promotive impact on number of vascular bundles was observed in the plants treated with the Ca- NPs by 62.50%, when compared with the control (Figure 2 T1 and T4). The least improvement was achieved in plants that were treated with Mg- NPs for the thickness of midvein,upper epidermis and lower epidermis, number of vascular bundle by 3.00%, 3.94%, 4.17% and 12.50% more than the control; respectively. Likewise, lamina ,palisade and spongy tissues thickness increased by 10.62%, 7.71% and 12.74%, respectively, compared to the control. Additionally, dimension of main midvein bundle increased by 2.84% , this is due to the increase in thickness of xylem, thickness of phloem, number of xylem rows in main midvein bundle and mean diameter of vessels by 5.14%, 2.46%, 10.00% and 21.69%. (Figures 2, 3, 4 T1 and T2).

Table 4. Counts and measurements in micro-meters (µm) of certain histological characters in the transverse sections of the Cauliflower leaf, treated with nanoparticles, 10 days after the third foliar application (Means of three sections from three specimens)

Histological aspects	Treatments				
	control	Mg	Zn	Ca	Zn + Mg +Ca
Thickness of midvein	4198.052	4324.114	5056.670	6237.011	5822.595
Thickness of upper epidermis	11.155	11.595	12.333	13.893	13.063
Thickness of lower epidermis	6.367	6.633	7.196	9.947	8.471
Thickness of lamina	127.810	141.385	191.056	200.573	197.423
Thickness of palisade tissue	56.712	61.086	80.112	89.662	89.126
Thickness of spongy tissue	71.224	80.299	110.944	110.911	108.297
No of vascular bundle	8.000	9.000	10.000	13.000	13.000
Dimension of main midvein bundle	460.54	473.661	499.795	649.821	647.143
Thickness of xylem	93.056	97.845	106.821	135.168	115.692
Thickness of phloem	80.919	82.916	92.671	130.189	96.410
No of xylem rows in main midvein bundle	10. 00	11.00	11.55	16.66	15.66
Mean diameter of vessels	13.941	16.965	18.946	21.219	20.991

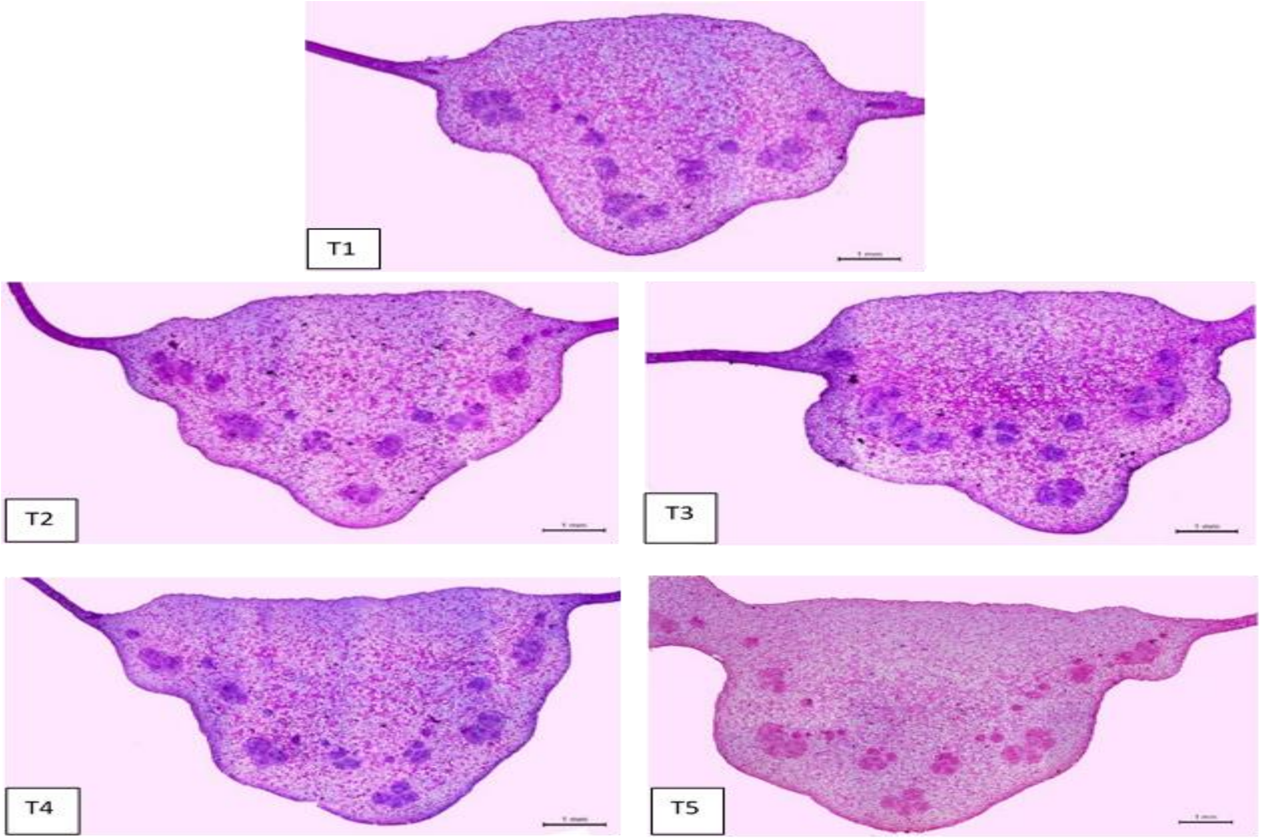

Figure 2. Microphotographs of cross sections through the Cauliflower leaf, treated with nanoparticles , 10 days after the third foliar application Scale bars = 1 mm.

(T1) control (untreated plants); (T2) plants treated with Mg- NPs ; (T3) plants treated with Zn- NPs ; (T4) plants treated with Ca NPs and (T5) plants treated with Zn - NPs+ Mg- NPs+Ca- NPs

This finding is consistent with that of Chmielewska and Michałoj (2009), who discovered that pepper plants treated with calcium had bigger cells as well as more developed layers of subepidermally located collenchyma of the leaf midrib. Abou-shlell et al., 2020 found that the effect of foliar spray with nano-particle treatments under salinity stress led to the stimulative the histological feature of moringa leaflet, the best results were achieved when the plants treated with the zinc for the thickness of palisade tissue and upper and lower epidermis . Mahmoud et al., 2021 found that nano-si spray on barely crop increased the thickness of midvein and mesophyll, mid vascular bundle diameter, and upper sclerenchyma tissue thickness. Hebraism and Hussein 2023 reported that the nano calcium fertilizer at the concentration of 2.5 g/L on Potato tubers increased the thickness of the cortex layer. Similar results were obtained by Sayed et al., 2024 they observed that treated pepper plants with Zn-NPs, Se-NPs individual or combination between them under cold stress increased all anatomical parameters under the study (the thickness of the midvein, lamina, palisade tissue, spongy tissue, upper and lower epidermis, bundles dimension, and mean vessels diameter) when compared with the control plants.

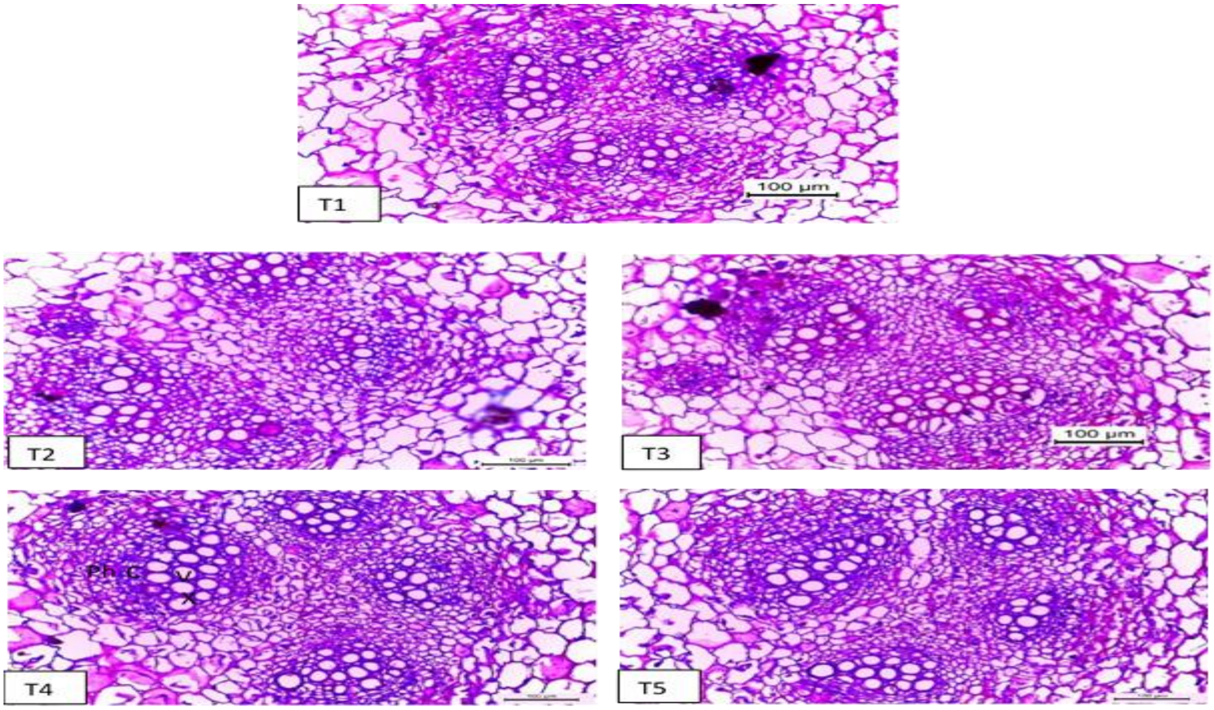

Figure 3. Magnified portions of the leaf midvein bundle through the Cauliflower leaf, treated with nanoparticles , 10 days after the third foliar application Scale bars = 100 µm.

(T1) control (untreated plants); (T2) plants treated with Mg- NPs ; (T3) plants treated with Zn - NPs ; (T4) plants treated with Ca NPs and (T5) plants treated with Zn - NPs+ Mg- NPs+Ca- NPs

Details: mid b, midvein bundle; ph, phloem; v, vessel; x, xylem and c, cambium .

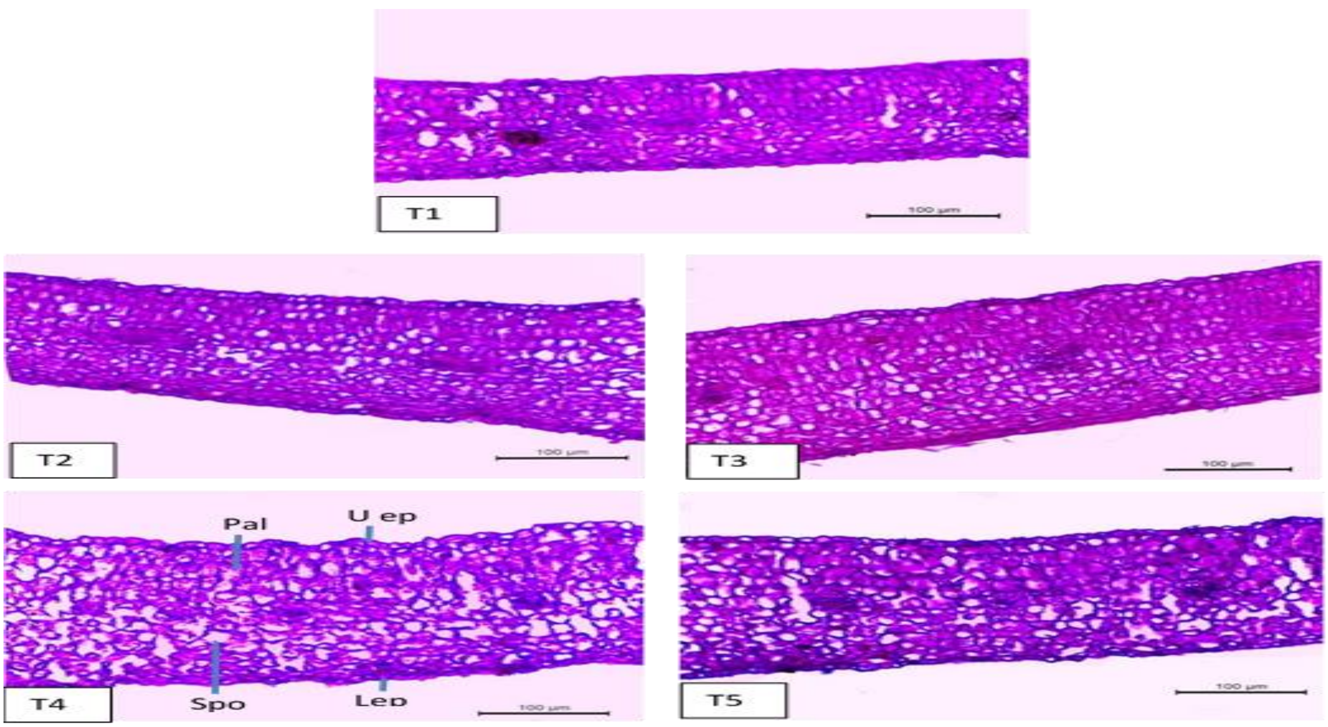

Figure 4. Magnified portions of the leaf blade lamina of through the Cauliflower leaf, treated with nanoparticles , 10 days after the third foliar application Scale bars = 100 µm.

(T1) control (untreated plants); (T2) plants treated with Mg- NPs ; (T3) plants treated with Zn NPs ; (T4) plants treated with Ca NPs and (T5) plants treated with Zn NPs+ Mg- NPs+Ca NPs

Details: l ep, lower epidermis; u ep, upper epidermis; spo, spongy tissue and pal, palisade tissue.

3.5. Chemical content

The physiological and biochemical components in cauliflower plant which supplemented by different nano-fertilizers either mono-fertilizers; magnesium, calcium and zinc or mixed fertilizer of calcium & magnesium and zinc together, were studied and demonstrated in Figures (5-9).

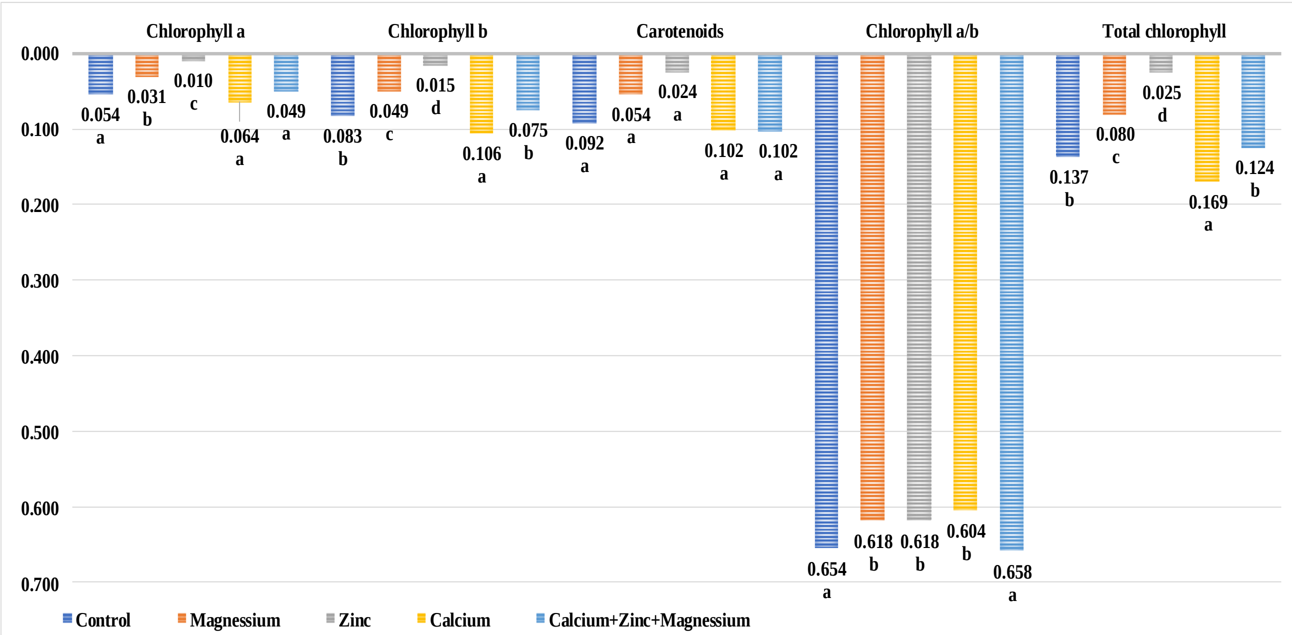

Figure 5. Chlorophyll a, chlorophyll b, carotenoids, chlorophyll a/b and total chlorophyll concentration (mg/g fw) in cauliflower which treated by foliar spray of nano-fertilizers; magnesium, zinc, calcium and mixture of them

3.6. Chlorophyll parameters

Results indicated that cauliflower had various responses to foliar spraying of nano-fertilizers ingredient supplied. Concerning the effect of applied fertilizers on the chlorophyll parameters; chlorophyll a, chlorophyll b, carotenoids concentrations, and relations between chlorophyll a/b and total chlorophyll: carotenoids, results in Figure 5 indicated that total chlorophyll has significantly affected by these fertilizers ingredients, in which calcium nanoparticle fertilizer had recorded the highest significant total chlorophyll concentration when compared to studied fertilizer ingredient. On the other hand, zinc nanoparticle was ranked the lowest significant total chlorophyll concentration. Furthermore, magnesium nanoparticle reduced total chlorophyll concentration significantly. Whereas, mixing these fertilizers together had similarity effect on total chlorophyll concentration to control where there was no significant difference between them. It was concluded that the boost effect of calcium nanoparticle fertilizer in total chlorophyll concentration was resulted from increasing concentration in both chlorophyll a and b together in plants treated with this treatment. Furthermore, calcium nanoparticle was stated the superior fertilizer component among the other studied ingredients depending on its effect on the studied chlorophyll parameters in which the ratio between chlorophyll a and b was recorded the lowest one when compared to other fertilizers and control plants as well. Moreover, carotenoids concentration was enhanced and improved by calcium nanoparticle fertilization as well as mixing Calcium nanoparticle + zinc nanoparticle + magnesium nanoparticle, which recorded the highest values as well as higher than control. In addition, it was observed that chlorophyll b had recorded an increase with compared to chlorophyll a in all studied plants supplemented by various studied fertilizers ingredients. This result may be due that chlorophyll is a green pigment that located in the chloroplasts of green plants. the crucial organelle for photosynthesis process, that fuels plant survival, growth, and development. Chlorophyll a is the primary pigments where chlorophyll b and carotenoids are the accessory pigments. These pigments were serving as antenna collecting light and transferring to the reaction center (Taiz and Zeiger, 2003)

The widespread photosynthetic pigment chlorophyll a is blue or bright green in colour. This pigment, which is the most prevalent type of photosynthetic pigment, is sometimes referred to as primary pigment. In contrast, the yellow-green pigment known as chlorophyll b. In higher plants exposed to light, the amount of chlorophyll b is half that of chlorophyll a, although chlorophyll b is more soluble in polar solvents due to its carbonyl group. However, our study observed an increase in chlorophyll b concentration when compared to chlorophyll a in floret under all studied fertilization ingredients as well as carotenoids concentration had increased. These results were supported by the type of cultivar where the head was colored in warm white or curd-cheese, thus chlorophyll b and carotenoids were abundant in versing to chlorophyll a concentration. This evidence was supported by a study performed which investigated carotenoid and chlorophyll concentrations in differently pigmented cauliflower cultivars. Changes in the pigments of carotenoid metabolic genes were identified in the florets and leaves of cultivars that are orange (Jaffa and Sunset), purple (Di Sicilia Violetto and Graffiti), green (Trevi), and white (Clapton). All carotenoid metabolic genes exhibited distinct transcript levels in their leaves and florets based on cultivar. In contrast to the other cultivars, orange cultivars had the highest levels of lutein in their leaves and β-carotene in their florets, which changed the lutein/β-carotene ratios. In contrast, no increased carotenoid concentrations were found in the green cultivar, which may have been caused by a higher carotenoid turnover in the green cultivar caused by the carotenoid cleavage dioxygenase 4 in the green cultivar. Furthermore, the amount of carotenoid and the transcript levels of phytoene desaturase were low in the purple (Graffiti) and white (Clapton) cultivars. This data confirmed our findings, which showed that cauliflower florets have more chlorophyll b than chlorophyll a (Izadpanah et al., 2023).

Some proteins are identified as light-harvesting complex II (LHCII) proteins because they are mostly linked to photosystem II, whereas other proteins are identified as LHCI proteins because they are linked to photosystem I. Taiz and Zeiger (2003) refer to these antenna complexes as chlorophyll a/b antenna proteins other than that. Apart from their function as supplementary pigments, chlorophyll b. Furthermore, carotenoids are crucial for photoprotection. A protective mechanism is necessary because the huge quantities of energy collected by the pigments might rapidly harm the photosynthetic membrane if this energy cannot be stored by photochemistry. Consider the photoprotection process as a safety valve that lets out excess energy before it harms the organism. According to Taiz and Zeiger (2003), the excited state of chlorophylls is considered to be quenched when the energy it contains is quickly released by excitation transfer or photochemistry When compared to other fertilizer ingredients under investigation, the application of calcium nanoparticles on cauliflower resulted in a reduction in total chlorophyll and an increase in chlorophyll b and carotenoids in the floret, which are essential for fruit quality. Elaborately, this treatment had enhanced the pigments components for the functional purpose by enhancing the primary pigment concentration, the chlorophyll a, besides conserving fruit quality and color.Our findings agreed with a study that looked at the impact of L-aspartic acid nano calcium [Ca (L-asp) - NPs] (nano-Ca) on carotenoid accumulation. It was determined that both Cl–Ca and nano–Ca could raise the carotenoid content of nectarine fruit flesh, with the impact of nano–Ca showing a substantial correlation with the enhancement. It was shown that nano-Ca upregulates the expression of genes associated to carotenoid production and increases calmodulin activity in leaves, peel, and flesh. Additionally, nano-Ca significantly up-regulates genes linked to sucrose transport in stem phloem, encouraging the movement of additional products from photosynthetic processes to fruits and supplying the building blocks needed for the synthesis of carotenoids. These findings were consistent with our research, which explained how nectarine fruit quality is enhanced by nano-fertilizer (Zhu et al., 2023).

3.7. Bio-chemical components

Total sugars, total soluble phenols and protein concentrations in the cauliflower plants fertilized by either calcium, magnesium and zinc in form of nanoparticles as well as the mixture of them in nanoparticles, were demonstrated in Figure 6 and the total free amino acids as well as the free proline concentrations were showed in Figure 7.

Results indicated that the response of biochemical components of cauliflower had different response to various studied fertilizer ingredients. In details, it was found that the effect of mixture of fertilizers ingredients on each biochemical studied components conveyed the effect showed by each fertilizer applied alone on each biochemical component.

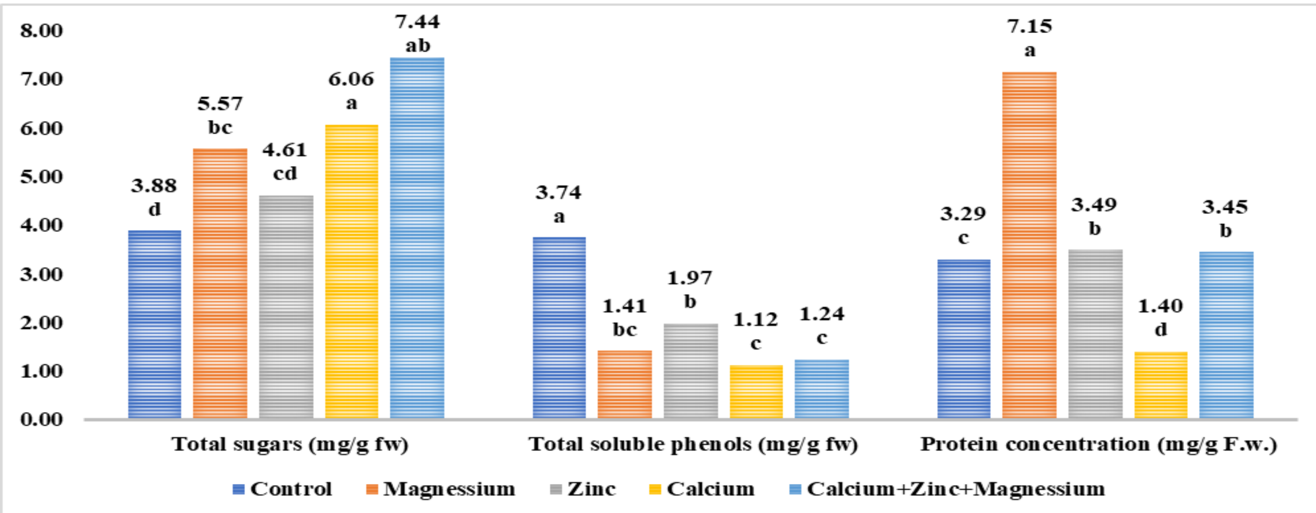

Figure 6. Total sugars, total soluble phenols and protein concentration in cauliflower which treated by foliar spray of nano-fertilizers; magnesium, zinc, calcium and mixture of them.

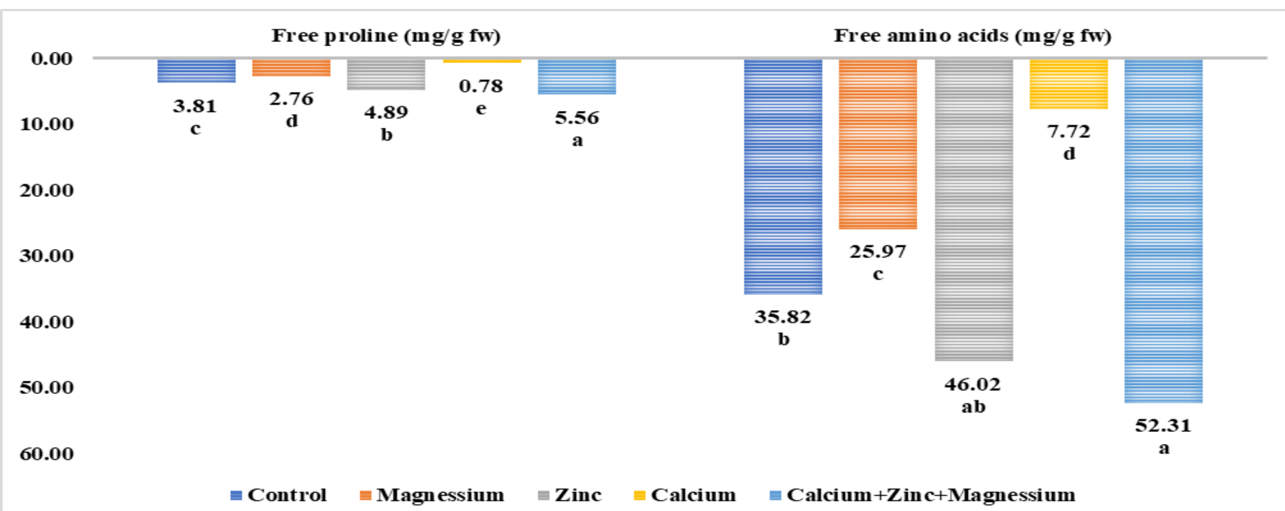

Figure 7. Free proline and total free amino acids in cauliflower which treated by foliar spray of nano-fertilizers; magnesium, zinc, calcium and mixture of them

This result showed in accumulating total sugar in floret where the mixture of fertilizers was significantly increased sugar concentration and recorded the highest value when compared with the other studied fertilizers ingredient. Moreover, the response of total sugar concentration in the floret showed significant increase and accumulation to the various fertilizers' ingredients, when compared to control. However, contradictory results showed the effect of these ingredients on total soluble phenols concentration, free proline, and total free amino acids concentration accumulation in floret. Whereas the increase in the total sugars was observed in all fertilizers supplied when compared to control plants, which indicated accumulating sugars in floret under effect of various studied fertilizers ingredient in respect of fruit quality. However, the highest significant sugars accumulation in floret was showed in mixture of fertilizers ingredient added nanoparticle form. Whereas this could be concluded because of the effect of calcium nanoparticle in the mixture ingredient. That's because it was observed that the mixture ingredient effect showed the similar effect of each fertilizer applied alone in different biochemical components, and mainly likewise to calcium nanoparticle effect.

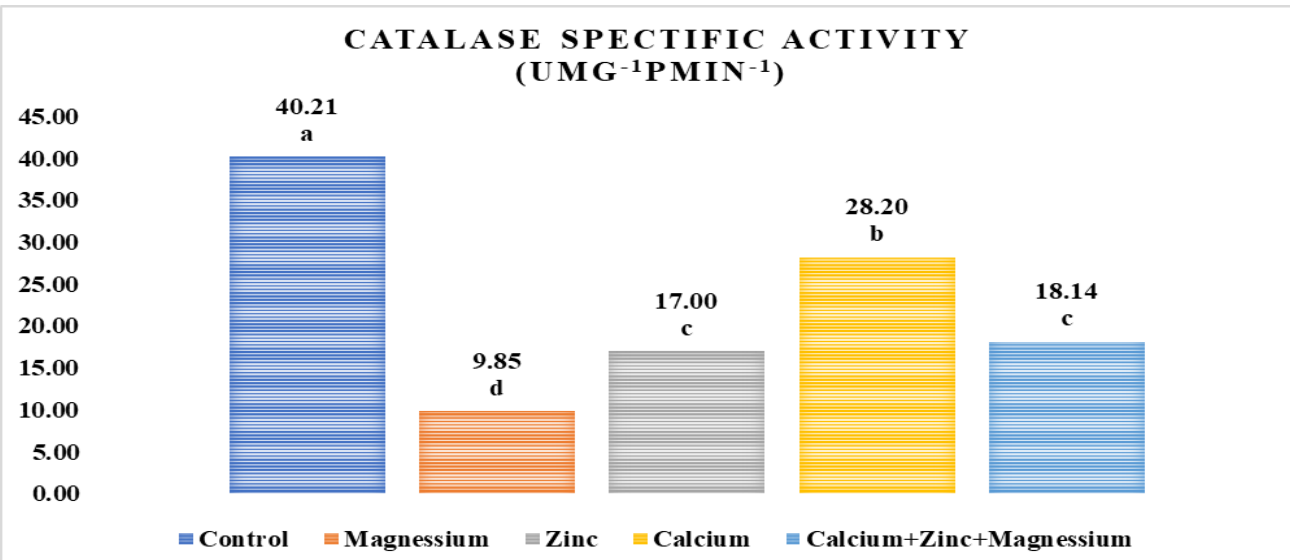

Figure 8. Specific activity of catalase in cauliflower which treated by foliar spray of nano-fertilizers; magnesium, zinc, calcium and mixture of them.

Accordingly, a similar trend was found in total soluble phenols accumulation where results indicated that the effect of each fertilizer applied alone had a significant reduction effect when compared to control. Furthermore, the mixture ingredient was in similarity to this trend. Furthermore, as showed in Figure 6, the mixture ingredient was likewise to calcium nanoparticle effect on total soluble phenols concentration, there is no significant difference between both treatments. By this way, calcium nanoparticle showed beneficial effect on fruit quality parameters as sugars accumulated significantly as well as a reduction showed in total soluble phenols, total free amino acids as well as free proline. Concerning the effect of studied treatments on the total soluble protein, all fertilizers increased their accumulation significantly, when compared to control except calcium nanoparticle. This treatment showed the lowest significant soluble protein accumulation. On the other hand, as shown in Figure 7 the effect of these fertilizers on the total free amino acids as well as free proline, results indicated a significant increase observed by all fertilizer ingredient except calcium nanoparticle. The latter showed the lowest significant effect on total free amino acids accumulation when compared to all other ingredients. Similarly, the free proline accumulation showed a similar trend to the free amino acids' accumulation among all studied treatments. In depth, calcium nanoparticles showed the lowest significant value of free proline accumulation when compared to the other studied fertilizers. Results indicated that magnesium nanoparticle and zinc nanoparticles showed a significant increase in free amino acids and free proline accumulation in the cauliflower . Besides, these treatments increased the soluble protein accumulation and significantly reduced the total sugar concentration. Mainly zinc nanoparticle recorded the highest significant total free amino acids as well as free proline accumulation, in addition to record the second significant treatments in total soluble phenols and total soluble protein accumulation after control and magnesium nanoparticle, respectively.

Concerning the specific activity of catalase and peroxidase in the cauliflower fed by various fertilizers ingredients, calcium, zinc and magnesium in nanoparticle form and the mixture of these fertilizers were demonstrated in Figure 8 and Figure 9. Results showed that cauliflower florets were dependent on peroxidase activity than the activity of catalase, which was showed by the values of both enzymatic activities under effect of various fertilizers ingredients. However, both enzymes activity showed similar trend among the effect of studied fertilizers, in which control showed the highest significant activity recorded in both catalase and peroxidase activities when compared to other fertilizers ingredient.

On the other hand, all studied nano-fertilizers components significantly reduced the catalase specific activity and the peroxidase specific activity as well when compared to control. In depth, the nano-fertilizers effect on specific activities of both catalase and peroxidase showed a significant reduction in cauliflowers treated magnesium nanoparticles which recorded the lowest significant activity.

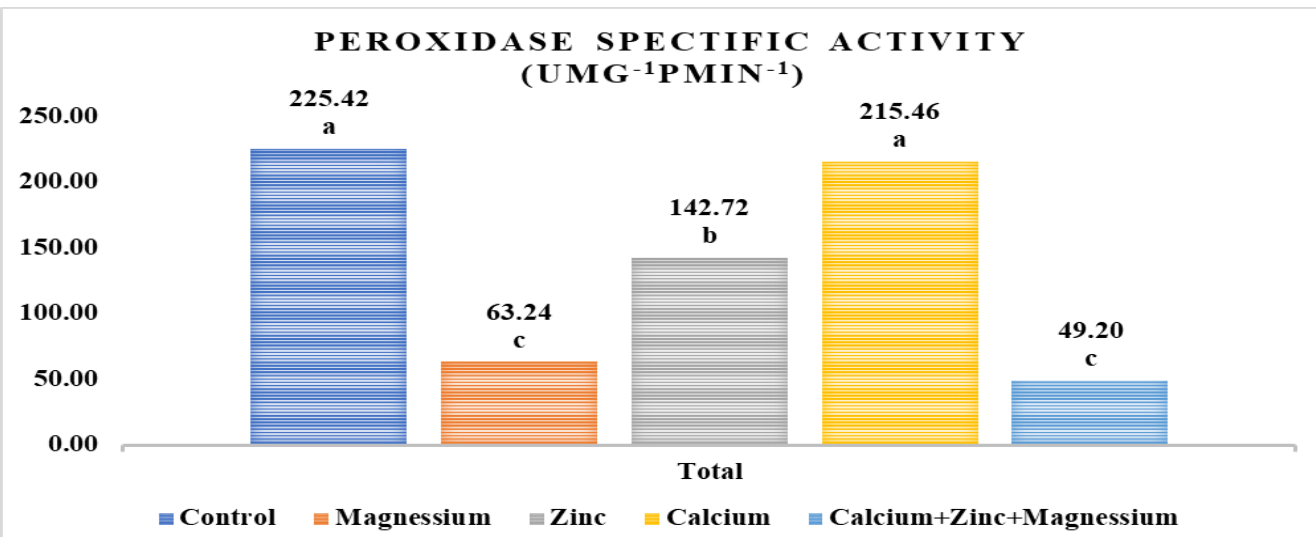

Figure 9. Specific activity of peroxidase in cauliflower which treated by foliar spray of nano-fertilizers; magnesium, zinc, calcium and mixture of them

Furthermore, zinc nanoparticles showed a reduction in catalase and peroxidase activities, in which no significant difference observed between this treatment and mixture ingredients. On the contradictory, calcium nanoparticles showed the highest significant specific activity for both catalase as well as peroxidase in cauliflower plant, when compared to other applied nanoparticle fertilizers ingredients. Zinc, calcium, and magnesium are essential nutrients for plants. Because zinc (Zn) is hazardous to biological systems at certain quantities, it plays an important function in plant biochemistry and metabolism. Zinc supports plant growth, development, and yield in addition to being involved in a number of cellular and physiological processes in plants. It is a crucial part of many proteins and enzymes' structural, enzymatic, and regulatory functions. Given this, the best course of action is to increase the efficiency with which zinc is utilized. This involves developing the root system's architecture, organic acids' ability to absorb zinc complexes, and plants' mechanisms for uptaking and translocation of zinc (Hamzah Saleem et al., 2022).One nutrient that is vital to plants is calcium. The cytosolic $Ca^{2}+$ concentration ([$Ca^{2}+$]) is an obligatory intracellular messenger that coordinates responses to various developmental cues and environmental challenges. It is also necessary for various structural roles in the cell wall and membranes, and it serves as a counter-cation for inorganic and organic anions in the vacuole (White and Broadley, 2003).However, Zn toxicity impairs plant development, leading to physiological changes and even cellular death (Paradisone et al., 2021). Photosynthesis and nitrogen (N) metabolism are two of the main mechanisms in plants that control growth. Per Paradisone et al. (2021) the barley plants were cultivated hydroponically and given two different Zn doses: 0.01 µM ZnSO4 and 100 µM ZnSO4. They were also given CaSiO3. Barley plants with zinc stress had lower biomass and altered zinc content in their leaves. Inhibiting N metabolism and boosting photorespiration, zinc toxicity and deficiency exacerbated stress symptoms. Zn stress effects were lessened by CaSiO3, which also likely improved N metabolism and photosynthesis and controlled Zn levels in plant cells.,(Paradisone et al 2021).Furthermore, a study revealed that when snap bean plants were treated with Nano calcium phosphate (NCaP) instead of traditional P, the dry weights of the shoots and roots, as well as the nutrient content in the shoots and roots and the yield components, the nutrient concentration, and the crude protein percentage in the pods, all increased significantly. The highest increase was achieved by applying 20% NCaP to the soil together with 5% NCaP topically. (Abd El-Ghany et al, 2021).

4. CONCLUSION

This study concluded that the application nano-calcium fertilizer in form of foliar spray on cauliflower affected and enhanced cauliflower growth parameters, productivity, enzymatic activities as well as resulted in changes in anatomical structure of cauliflower plant, which resulted in effective productivity and fruit quality. However, Zinc nanoparticle showed a reduction trend in plant productivity and quality as well as the studied physiological parameters. These results were suggested to be due to the applied dose of zinc, which could need to be increased. Thus, it was suggested that increasing zinc applying dose. Furthermore, mixing these ingredients; calcium, magnesium and zinc nano particle fertilizer resulted in non-significant difference on cauliflower plant growth and fruit quality, compared to calcium nanoparticle. Whereas the effect of mixture was observed to convey the effect of most effected mono-fertilizer of mixture used ingredient.

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الملخص العربي

تاثير الرش ببعض العناصر الغذائية فى صورة نانو على النمو والمحصول والتغيرات الانزيمية والتشريحية للقنبيط

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تم اجراء تجربة بالمحطة التجريبية التابعة لكلية الزراعة جامعة القاهرة خلال الموسيمين الشتويين ٢٠٢٠/٢٠٢١ و٢٠٢١/٢٠٢٢ لدراسة تاثير الرش الورقى باسمدة الكالسيوم , المغنسيوم و الزنك فى صورة جزئيات النانو التى عوملت بصورة منفردة او مختلطة على النمو , المحصول ,المواد الصلبة الذائبة , التغيرات في التركيب التشريحي للأوراق، المكونات البيوكيميائية، النشاط الأنزيمي. و تم رش القنبيط بالأسمدة المستخدمة بتركيز ٥٠ جزء بالمليون مقارنة بالكنترول التي رشت بالماء. أظهرت النتائج إلى أن سماد الكالسيوم أحدث أعلى قيم للنمو والمحصول ومحتوى الكلوروفيل والمواد الصلبة الذائبة الكلية والتركيب التشريحي للأوراق مقارنة بالكنترول بالإضافة إلى ان نانو كالسوم كسماد له تاثير معنوى على تركيز الكلوروفيل، وكلوروفيل ا/ ب. أظهرت جميع الأسمدة النانوية انخفاضاً معنوياً في إجمالي الفينولات الذائبة مقارنة بالكنترول . سجلت جزئيات الزنك النانوية الاعلى معنويا بالنسبة الأحماض الأمينية الحرة بالاضافة الى تراكم البرولين الحر. أظهرت جزيئات الكالسيوم النانوية أعلى تاثير معنوي لكل من الكتاليز والبيروكسيديز لنبات القنبيط.

الكلمات المفتاحية: القنبيط ,كالسيوم ,مغنسيوم ,زنك, الاسمدة النانو , النمو , المحصول , التركيب التشريحى