Response of secondary metabolites and some traits of vegetative growth and yield of *Brassica oleraceae* var. Italica L for spraying with nano-fertilizer and nano-bio-stimulator

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Abstract

A field experiment was conducted during the autumn agricultural season 2020-2021 in Al-Azzawiya area of Al-Mahaweel district, which is located 30 km north of the center of Babylon province, using the RCBD design and arranging Split plot system to study the effect of two factors: the first factor: nano-fertilizer for microelements (zinc 1%, iron 9% and manganese 1%).It was distributed in the main plot with four concentrations (0, 1.5, 1.5, 2) $g.L^{-1}$ nano fertilizer and the second factor: spraying with nano biostimulator nano-treated seaweed and bacteria extract distributed in the sub plot and at three concentrations (0, 1.2 $ml.L^{-1}$ nano biostimulant) and the results showed: The effect of nano fertilizer positively and significantly at a concentration of 1 $g.L^{-1}$ on some characteristics of vegetative growth and yield of one plant and gave the highest averages for the studied indicators, the content of leaves of chlorophyll was 77.52 spad, and the leaves area was 7882 cm^2 $plant^{-1}$, plant yield of one was 2,120 kg $plant^{-1}$,The treatment of nano-fertilizer significantly affected a concentration of 1.5 $g.L^{-1}$ and a concentration of 2 $g.L^{-1}$ in the content of curd of vitamin C, and it reached 151.42 mg per 100 g^{-1}. Also, the treatment gave 1.5 g the highest results in the content of curd of phenols, which amounted to 0.906 mg g^{-1} dry weight, and the 2 $g.L^{-1}$ treatment gave the highest results in curd content of flavonoids, which amounted to 37.739 mg gm^{-1} dry weight, and 1.5 $g.L^{-1}$ treatment gave the highest results in curd content of peroxidase enzyme, which amounted to 118.61 $ml.L^{-1}$, the treatment of 2 $g.L^{-1}$ gave the highest results in the curd content of catalase enzyme, which amounted to 97.56 $ml.L^{-1}$, and the treatment of 2 $g.L^{-1}$ gave the highest results in the curd content of antioxidants, which amounted to 70.76 mg gm^{-1} of dry weight, The nano-bio stimulator had no effect on most of the studied vegetative growth traits, except for some traits, including leaves .The biostimulant also gave the best results in the curd content of vitamin C, where the treatment gave 1.5 ml L^{-1} the best results, amounting to 148.27 mg per 100 g^{-1}, and the treatment gave 1 ml L^{-1} the best results in the curd content of phenols, amounting to 0.908 mg gm-1 dry weight, The treatment of 1.5 ml L^{-1} gave the best results in the curd content of flavonoids, which amounted to 37.316 mg g-1 of dry weight, and the treatment of 1 ml L^{-1} gave the best results in the curd content of peroxidase enzyme, which amounted to 120.34 $ml.L^{-1}$. Also, the treatment of 1 ml L^{-1} gave the best results in the curd content of catalase enzyme , which was 97.23 $ml.L^{-1}$ .As for the interaction, it had a positive and significant effect on all the studied traits, including the curd content of chlorophyll, where the interaction treatment gave 1 $g.L^{-1}$ nano fertilizer and 1 ml L^{-1} nano-stimulator the best treatment and it was 79.29 SPAD.The interaction treatment of 1 $g.L^{-1}$ nano-fertilizer and 1.5 ml l-1 nano-stimulator gave the best treatment for leaves area, which was 8693 cm plant. The interaction between 1.5 $g.L^{-1}$ nano-fertilizer and 1.5 ml L^{-1} gave a higher biostimulant and a percentage in the curd content of vitamin C was 152.33 mg per 100 g^{-1}.The interaction between the spraying with water only treatment and the 1ml L^{-1} nano biostimulant treatment gave the highest value in curd content of phenols, amounting to 0.931 mg. g^{-1} dry weight.The interaction between the spraying with water only treatment and the 1.5 ml L^{-1} nano biostimulant treatment gave the highest value of 38.407 mg g^{-1} dry weight in the curd content of flavonoids.The interaction between the treatment of 1.5 $g.L^{-1}$ nano fertilizer and 1 ml L^{-1} biostimulant gave the highest value and it was 126.44 $ml.L^{-1}$ in the curd content of peroxidase enzyme. The interaction that treatment 1 ml L^{-1} nano biostimulant and

spraying with water only gave the highest value and reached 99.29 ml in curd content of catalase enzyme, treatment reached 2 $g.L^{-1}$ and treatment 1.5 ml L^{-1} the highest value was 72.29 mg g^{-1} dry weight of the total capacity of antioxidants in curd of broccoli plant.

Keyword: broccoli plant. secondary metabolites, biostimulant nano-fertilizer

1- Introduction:

Broccoli is *Brassica oleraceae* var. Italica L. is one of the crops of the Brassicaceae family, which is grown for its edible inflorescences in the process of flowering buds with their thick, smooth stalks. Broccoli has a high nutritional value, It contains many nutrients, vitamins, protein, and carbohydrates, in addition to its high content of glucosides with anti-cancer properties. Broccoli is a vegetable rich in vitamin C, A, carotenoids, fulvic acid, niacin, and riboflavin. It also contains some mineral elements calcium, iron, sodium, phosphorous, and potassium, in addition to that, broccoli has a nutritional and therapeutic value that is not available together in other plants. It builds bones, increases physical strength, helps protect against heart disease, and helps prevent retinal diseases (Kirsh et al., 2007). The availability of the necessary nutrients is an important factor in the growth of plants, where the deficiency of any of them leads to a significant imbalance in the growth and yield, and some of these elements are found in the soil in good quantities, but the ready ones are hardly compatible with the need for the natural growth of the plant.As some mineral elements in most of the lands of Iraq are exposed to many factors that determine their movement and availability for absorption. Therefore, the researchers turned to find modern technical methods and methods, including nano-fertilizers, for the purpose of adopting them in preparing plants with the necessary nutrients by spraying them on the vegetative system for their continued growth and achieving a qualitative and quantitative improvement in growth and the outcome by reducing or reducing the obstacles faced by the nutrients in the soil that reduce Its availability to plant (Martin, 2002). Plant extracts are also considered growth stimulants, which are an important source of macro and micronutrients and amino acids, auxins, gibberellins and cytokines that stimulate plant growth and improve yield characteristics of many plants and increase their resistance to stress and insects (Potter, López, 2005). Based on the foregoing, the study was conducted to find out the effect of spraying the micro-nano elements and spraying the nano-biostimulant alone or interacting with each other on the total plant yield, and some chemical characteristics and secondary metabolism compounds of the broccoli plant.

2. Materials and methods:

A field experiment was conducted during the agricultural season 2020-2021 in Al-Azzawiya area of Al-Mahaweel District, which is located 30 km north of the center of Babylon province. This is for the purpose of studying the effect of small nano-elements zinc, iron, manganese, and nano-bio-catalyst on growth and yield of broccoli .Samples of field soil were taken randomly and from different areas before the start of the experiment, at a depth of 30 cm from the surface area of the soil, and then dried aerobically. It was crushed and passed through a sieve with a pit 2 mm in diameter and analyzed to know some physical and chemical properties of the field soil. and that is after removing the growing plants and weeds, carrying out the process of plowing, smoothing, and leveling, and making the terraces well and homogeneous, then extending the field with a drip irrigation system and the land, which is 45 m long and 5 m wide, was divided into 3 replicates so that each replicate included 12 experimental units, which resulted in 36 experimental units. The distance between one terrace and another was 60 cm, and a distance of 60 cm was left between one plant and another, and a distance of 50 cm was left between the experimental unit and another .Broccoli seeds, Tokita cultivar, produced in Japan, were obtained from one of the private nurseries. The seeds were sown on 25/8/2021 in cork dishes filled

with peat moss, while providing good and suitable conditions for the growth of seedlings. After the seedlings reached the stage of 4-5 true leaves, they were transferred in the morning to the permanent field on 7/10/2021.The planting was conducted inside terraces, with 10 seedlings per experimental unit, and they were planted on the two lines of the terrace, and the distance between one line and another was 60 cm, and between one plant and another 50 cm. Use fungicides to get rid of insects and fungal diseases.As for the irrigation process, it was twice during the week at the beginning of the seedling and then according to the needs of the plant and by means of the drip irrigation system.Nano-fertilization was carried out with nano-microelements in three batches every 30 days from the date of the start of cultivation and according to the recommended concentrations with the nano-bio stimulator Super Fifty seaweed extract also in three batches for every 30 days from the date of the start of cultivation .DAP NPK fertilizer was added to the control plants at the recommended concentration of 400 kg per hectare. The results of the experiments were statistically analyzed according to the method of analysis of variance according to the split-plot system according to the Randomized Complete Block Design (R.C.B.D). The experiment included 12 treatments with three replications, and the significant differences between treatments were calculated at the level of significance 0.05, according to the least significant difference LSD (Al-Sahoki and Waheeb, 1990). The Genstat program was used in the statistical analysis. It was distributed in the main pot with four concentrations (0, 1.5, 1.5, 2 control) $g.L^{-1}$ nano fertilizer, and the second factor: spraying with nano biostimulator nano-treated seaweed and bacteria extract distributed in the sub plot with three concentrations (0 control, 1.2 $ml.L^{-1}$) nano biostimulant . A chlorophyll meter type SPAD-502 estimated the percentage of chlorophyll in broccoli leaves at the flowering time by taking the reading from 5 plants for each experimental unit and then taking the average (Minnotti et al., 1994),It was measured in units SPAD and as mentioned in (Jemison and Williams, 2006). The leaves area was measured according to the method (Watson and Watson, 1953)

The yield of one plant was calculated according to the following law = weight of the main curd + weight of secondary curd.

Vitamin C ascorbic acid in the main curd was determined by oxalic method (Abbas and Abbas, 1992). The activity of POD enzyme was estimated according to the method described by Nezih (1985, 1985). The activity of CAT enzyme was estimated by a spectrophotometer according to the method (1974, Aebi). The total phenols (mg.g-1 dry weight) were estimated according to the method described by Singleton and Rossi, 1965) and modified by (Gregorio et al., 2020). The flavonoids were also estimated according to the method used by (Zhishen et al. 1999). The total antioxidant capacity was estimated by estimating the reducing power according to the method described by (Oyaizu, 1986).

3- Results and discussion:

From Table 1, we notice that the addition of nano-fertilizer significantly affected some indicators of vegetative growth, yield of one plant and secondary metabolism compounds, where the treatment of 1 $g.L^{-1}$ nano-fertilizer gave the best results in the content of chlorophyll in leaves, which amounted to 77.52 SPAD-1.The treatment of 1.5 $g.L^{-1}$ of nano fertilizer gave the best significant positive effect on the leaves area, which amounted to 7882 cm^2 $plant^{-1}$. Also, treatment of 1 $g.L^{-1}$ nano fertilizer gave the best results for the yield of one plant, which amounted to 2.120 kg $plant^{-1}$. The nano-fertilizer treatment had a significant effect at a concentration of 1.5 $g.L^{-1}$ and a concentration of 2 $g.L^{-1}$ on the content of curd of vitamin C, and it reached 151.42 mg per 100 gm-1. The 1.5 g liter treatment also gave the highest results in curd content of phenols, reaching 0.906 mg gm dry weight, and the 2 g L^{-1} treatment gave the highest results in curd flavonoid content, reaching 37.739 mg g^{-1} dry weight. The treatment of 1.5 $g.L^{-1}$ gave the highest results in the content of curd of peroxidase enzyme, which amounted to 118.61 $ml.L^{-1}$, and the

treatment of 2 $g.L^{-1}$ gave the highest results in the content of curd of catalase enzyme, which amounted to 97.56 $ml.L^{-1}$ .The treatment of 2 $g.L^{-1}$ reached the highest results in the antioxidant content of curd and it reached 70.76 mg g^{-1} dry weight. As for adding the nano-biostimulant, the results showed positive significant differences in some of the studied traits. The nano-bio-stimulator had no effect on most of the studied vegetative growth traits except for some traits, including leaves area. The treatment gave 1.5 ml L-1 nano-stimulator the highest value and was 7606 cm 2 plants $^{-1}$, The biostimulant also gave the best results in the content of curd of vitamin C, where the treatment gave 1.5 ml L^{-1} the best results amounted to 148.27 mg per 100 g^{-1}, the treatment gave 1 ml L^{-1} the best results in the content of curd of phenols amounted to 0.908 mg g^{-1} dry weight .The treatment of 1.5 ml L^{-1} gave the best results in the content of curd of flavonoids amounted to 37.316 mg g^{-1} dry weight, and the treatment gave 1 ml L^{-1}.The best results in curd content of peroxidase enzyme amounted to 120.34 $ml.L^{-1}$, also the treatment gave 1 ml L^{-1} the best results in curd content of catalase enzyme amounted to 97.23 $ml.L^{-1}$ .As for the interaction, Table 2 between the studied treatments, the nano-fertilizer and the nano-biostimulant, it improved in many of the studied traits, including chlorophyll, where the interaction treatment (N2S2) gave the highest value of 79.29 SPAD.As well as the effect of the interaction on the leaves area, where the treatment (N2S3)) gave the best results amounted to 8693 cm2 $plant^{-1.}$ We also note the effect of the interaction on the content of curd of vitamin C, where the treatment (N3S3)) gave the best treatment, which amounted to 152.33 mg per 100 g-1. dry weight. As for the curd content of flavonoids, the interaction treatment (N1S3 gave the best results and was 38.407 mg g-1 dry weight for peroxidase enzyme it was the N3S2 interaction treatment). The best results amounted to 126.44 $ml.L^{-1}$, and the catalase enzyme was the interaction treatment (N1S2)) amounted to 99.29 $ml.L^{-1}$, and the interaction treatment gave the total capacity of antioxidants (N4S3)) the best results were 72.29 mg g^{-1} dry weight.

Table . 1 Effect of nano-fertilizer of micro-elements and nano-biostimulant on some vegetative growth traits, plant yield and secondary metabolites of broccoli plant.

Studied factors	Total capacity of antioxidants	curd content of catalase enzyme	curd content of peroxidase enzyme	curd content of flavonoids	curd content of phenols	curd content of Vitamin C	plant yield (kg.plant-1)	leaves area (cm2 plant-1)	Leaves content of chlorophyll SPAD-1
١ Nano Fertilizer N g.L-1									
N1) 0)	69.97	96.43	114.57	37.407	0.901	127.80	1.982	7564	75.74
N2) 1)	69.31	96.54	116.88	37.290	0.890	145.54	2.120	7882	77.52
N3) 1.5)	70.35	96.76	118.61	36.045	0.906	151.42	1.680	6169	76.44
N3) 2)	70.76	97.56	117.73	37.739	0.883	146.99	1.876	6917	75.07
LSD 0.05	1.013	1.044	1.702	0.270	0.010	3.497	0.109	869.6	2.241
nano biostimulant (S) ml.L^{-1}									
S1) 0)	69.87	95.98	115.91	36.743	0.892	136.40	1.932	6285	75.71
(S2) 1	70.28	97.23	120.34	37.302	0.908	145.89	1.913	7508	76.05
S3) 1.5)	70.14	96.88	114.59	37.316	0.885	147.27	1.898	7606	76.82
LSD 0.05	NS	1.423	2.830	0.173	0.011	4.823	0.094	858.9	NS

Table. 2 The effect of the interaction between the nano-fertilizer of micro-elements and the nano-bio-stimulator on some traits of vegetative growth, yield of one plant and secondary metabolites of broccoli plant

Studied factors treatments	Total capacity of antioxidants	curd content of catalase enzyme	curd content of peroxidase enzyme	curd content of flavonoids	curd content of phenols	curd content of Vitamin C	plant yield (kg.plant-1)	leaves area (cm2 plant-1)	Leaves content of chlorophyll SPAD-1
N1S1	68.79	96.21	109.68	35.698	0.926	102.07	1.859	6140	76.33
N1S2	69.56	99.29	122.40	38.116	0.931	140.57	2.311	8538	74.58
N1S3	71.54	93.77	111.61	38.407	0.847	143.77	1.776	8014	76.30
N2S1	71.32	96.59	111.80	36.654	0.859	143.97	2.481	7220	76.23
N2S2	70.81	96.20	121.58	37.378	0.920	148.67	2.168	7732	79.29
N2S3	65.79	96.85	117.26	37.839	0.883	144.00	1.711	8693	77.04
N3S1	70.33	97.54	125.88	36.829	0.888	151.27	1.572	5594	76.55
N3S2	70.26	96.76	126.44	35.450	0.880	150.67	1.470	6684	76.90
N3S3	70.44	95.96	103.50	35.856	0.905	152.33	1.997	6228	75.88
N4S1	69.01	99.63	116.27	37.790	0.879	148.30	1.818	6187	73.71
N4S2	70.48	96.66	110.92	38.263	0.894	143.67	1.702	7079	73.42
N4S3	72.29	94.89	126.00	37.164	0.859	149.00	2.109	7484	78.08
LSD 0.05	1.511	2.453	4.792	0.361	0.019	8.305	0.189	1558.7	3.693

We note from Table. 1 that the treatment of spraying with nano-fertilizer 1 $g.L^{-1}$ has excelled in most of traits of vegetative growth and yield, as we note that the mentioned treatment gave the highest plant yield and the highest content of chlorophyll and leaves area. To the role played by nano-fertilizers in improving traits of vegetative growth and the result that nano-fertilizers have several advantages, including their small size and large surface area, which leads to an increase in the absorption surface and thus leads to a higher photosynthesis process and an increase in the production of active substances in plants (Singh and Prasad, 2016). It may be attributed to its properties as it has a wide surface area due to the small size of its particles and then increases the effective area of the reaction leading to an increase in the efficiency of enzymes and then an increase in biochemical reactions, which in turn leads to an increase in cell divisions (Morteza et al., 2013 and Sorooshzadeh et al., 2012) and the increase in the vegetative indicators represented by the leaves area, cm^2 chlorophyll spad, is attributed to the role of the necessary zinc element in the plant, as it is considered a catalyst for the activity of some enzymes necessary to complete some vital processes. Especially those that form the amino acid tryptophan, which is the basic material for building the plant hormone indole acetic acid (IAA), which is necessary for the expansion and elongation of cells and activating their division (Abu Dhahi and Younes, 1998). Which affects the growth of the plant and thus increases the plant length in addition to being an enzymatic companion of many important enzymes in vital processes, especially the process of photosynthesis, the processes of converting sugars into starch and the synthesis of proteins (Mengel and Kirkby, 2001, Attia and Judua, 1999), Zinc is also necessary for the process of phosphorylation and the formation of glucose sugar, and helps in the construction of chlorophyll through its direct effect in the formation of amino acids, carbohydrates and energy compounds, in addition to its importance in the formation of RNA, which is necessary for the process of protein formation (Abu Dahi and Younis, 1988).Thus, the content of chlorophyll in leaves increased, which was reflected in an increase in the leaves area of the plant, thus increasing the yield of a single plant, and it has an important role in plant productivity, both quantitatively and qualitatively, through its effect on the process of photosynthesis, nitrogen fixation, respiration and other metabolic processes (Naga et al., 2013) As well as the role of iron in improving the characteristics of vegetative growth and yield through its role in many vital processes that occur inside the plant, including photosynthesis and enhancing the production of amino acids (Rui et al., 2016). Also, spraying plants with the biostimulant seaweed extract on the bean plant positively affected some of the studied vegetative traits, including leaves area and chlorophyll (Majwel and Khafaji, 2021).As we note from Table 1, most of the previous studied indicators decreased when spraying at a concentration of 1.5 $g.L^{-1}$ and 2 $g.L^{-1}$. This was accompanied by an increase in secondary metabolic compounds, including vitamin C, phenols, flavonoids, peroxidase enzyme, catalase enzyme, and the total capacity of antioxidants. The addition of nano-fertilizers causes an increase in the concentration of the sprayed elements. This may reflect negatively on the plant in general, which stimulated the plant to produce more antioxidants and the increase in the concentration of nano iron to 1.5 and 2 $g.l^{-1}$.It led to a significant decrease in the above-mentioned characteristics, and the reason for this is due to the increase in the concentration of nanocomposites.It causes a deterioration in the PSII photosynthesis center and an inhibition of electron transfer activity and chlorophyll content, in addition to a decrease in the number of thylakoids and a decrease in the transpiration rate, stomatal conductance and CO2 uptake (Barhoumi et al., 2015 and Khan et al., 2017). This is consistent with what was found (Kazim, 2017) that the increase in iron nanoparticles led to a decrease in the vegetative growth trait of the moringa plant. We also notice from Table . 2 the interaction between the nano-fertilizer and the nano-bio-catalyst, an increase in most of the studied indicators, and this could be due to an increase in the nano-bio-catalyst content of amino acids. It is also a

natural source of plant growth hormones auxins, gibberellins and cytokines as well as carbohydrates and polysaccharides (Ördög et al., 2004 and O'Dell, 2003 and Booth, 1969). This may also be due to the role of seaweed extracts in improving the vegetative growth indicators, which is reflected positively in the increase in the construction of carbohydrates in curd and their transfer to the effective growth areas and the accumulation of manufactured materials, as well as to the extract's containment of nutrients and plant hormones, and to its physiological role in balancing vital processes Therefore, its positive effect was reflected in the vegetative growth traits and the yield of a single plant (Ibrahim, 2015) and (Majoul et al., 2013), during a study on cauliflower, showed that spraying with humic acid at a concentration of 2 ml led to an increase in vegetative growth and yield. The increase in some of the studied indicators can also be explained by the role that nanofertilizers play in improving some of the studied traits, Nano-fertilizers have several advantages, including their small size and large surface area, which leads to an increase in the absorption surface and thus leads to a higher photosynthesis process and an increase in the production of active substances in the plant (Singh and Prasad, 2016). It may be due to its properties as it has a wide surface area due to the small size of its particles and then increases the effective area of the reaction leading to an increase in the efficiency of enzymes and then an increase in biochemical reactions, which in turn leads to an increase in cell divisions (Morteza et al., 2013 and Sorooshzadeh et al., 2012)

4- Conclusions:

The nano-fertilizer treatment of microelements (zinc, iron and manganese) at a concentration of 1 gm gave a positive significant effect on most of the studied indicators.

Increasing the concentration of nano microelements more than 1 g.L^{-1} negatively affected most of the studied indicators.

The use of nano-biostimulant alone had no significant effect on most growth and yield indicators, while its interaction with nanomicro-elements significantly affected most of the studied indicators.

Increasing the concentrations of nano microelements more than 1 g. L^{-1} had a significant effect on secondary metabolites.The interaction had a significant effect on all the studied traits.

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