

EFFECT OF FOLIAR APPLICATION OF HUMIC ACID AND NANOCALCIUM ON SOME GROWTH, PRODUCTION, AND PHOTOSYNTHETIC PIGMENTS OF CAULIFLOWER (BRASSICA OLERACEA VAR. BOTRYTIS) PLANTED IN CALCAREOUS SOIL Ahmed Fahd Rachid, Basem Rahem Bader and Hassan H. Al-Alawy

Department of Soil Science and Water Resource, College of Agriculture, Diyala University, Iraq ahmed.fahdrachid@yahoo.fr

Abstract

A field experiment was carried out at the experimental Station of Agricultural College, University of Diyala, Iraq, during the autumn 2018 agricultural season to investigate the influence of foliar application of humic acid (H) and nanocalcium (Ca) on growth, yield and photosynthesis indicators of cauliflower (Brassica oleracea var. botrytis) planted in calcareous soil. Individual and combined applications of the two factors (H and Ca) each at 0, 2, and 4 ml 1⁻¹ concentrations were sprayed at five weeks after planting. The factorial treatments were distributed in three replicates according to the Complete Randomly Block Design (RCBD). The means of the ANOVA test were compared at 0.05 and 0.01 levels of probability. Results indicate that individual foliar application with low concentration of 2 ml 1^{-1} (H₂ or Ca₂) induced a slight increase ($p \le 05$) of up to 10% in some growth indicators specifically, stem and floral diameters, plant length, and the percentage of dry fruit weight. These same attributes as well as carotenoids increased by 20% when applying individual or combined solutions with overall dose of 4 ml l^{-1} (H₄, Ca₄, or Ca₂ + H₂). Meanwhile, no significant response to the above mention treatments was revealed for leaves number, fresh fruit and above-ground plant weights, and even chlorophyll except in case of H_4 . In contrast, the combined treatments with overall dose of 6 and 8 ml l⁻¹ (Ca₄ + H₂, Ca₂ + H₄, or Ca₄ + H₄) have a large influence. In fact, spraying 4 ml l⁻¹ humic acid in combination with 2 ml l⁻¹ nanocalcium, or inversely, on plants led to an increase close to 30% in all growth and yield parameters. Furthermore, plants receiving 4 ml l ¹ humic acid combined with 4 ml l⁻¹ nanocalcium exhibited the highest increase, representing in average more than 30% as compared with control treatment at $p \le 01$. Improvement in growth and yield led to a remarkably increase in dry fruit weight and photosynthetic pigments content: chlorophyll a, chlorophyll b, and carotenoids. These results suggest that the highest the dosage of application, the greatest the growth, yield, and pigments synthesis. However, no significant effect of factor interaction was found throughout the data, when comparing the treatments of the same overall concentration with each other. Consequently, the significant differences between treatments were related to the levels of concentration, rather than fertilizer type applied.

Keywords : Foliar application, humic acid, nanocalcium, cauliflower quality, calcareous soil.

Introduction

Belonging to the family cruciferae, cauliflower (Brassica oleracea var. botrytis) is the second most important inflorescence vegetables after globe artichoke and before broccoli in many places of the world (Abdel-Razzak et al., 2008). The edible head of the cauliflower is called curd, which is composed of many florets formed of aborted floral meristems. Leaves and stems are also edible as a vegetable broth or discarded. Consumed as fried, soup, and pickles, "curd" has various components with high nutritional and medicinal value, including vitamin-A (51 IU), vitamin-C (56 mg), riboflavin (0.10 mg), thiamin (0.04 mg), nicotinic acid (1.0 mg), calcium (33 mg), phosphorus (57 mg), potassium (138 mg), moisture (90.8 g), carbohydrates (4.0 g), protein (2.6 g), fat (0.4 g), fiber (1.2 g), and iron (1.5 mg) as per 100 g of edible portion of cauliflower curd (Premraj Gocher et al., 2017; Fageria et al., 2012). As an over wintering variety in area with mild winter, cauliflower needs full sun with optimum growing temperatures of 16° to 21° C requires loose, fertile, moist-but-well-drained soil, and ideally clay loam soil with pH as high as 7 to produce the largest and best-quality curds. Nevertheless, in the course of its producing some problems loom up such as low yield, unsuitable curd formation, as well as susceptibility to some pathogenic and physiological disorders, mainly due to unfavorable soil conditions. In fact, like almost most soils cultivated in Iraq, the soil of this research is calcareous with high percentage of $CaCO_3$ (> 31%), low content of organic matter (< 1%), and high level of salinity (EC > 7 dS m⁻¹), which are considered major constraints to plant growth and nutrient availability. Therefore, cauliflower cultivation is still

being so limited in most provinces that the cultivated areas did not exceed 2850 hectares with a low productivity. For example, the amount of production was only 36770 tones for the year 2009 (Alzamili, 2012). Although, neutral and alkali calcium is normally sufficient in soil, it is generally deficient under multiple abiotic stresses, such as high salinity, water shortage, and pH and temperature variations (Nelson and Niedziela, 1998; Durukan et al., 2013). Furthermore, in cases when calcium is available, some plant species can not completely benefit from it because of the competing action with others cations and the plant's inability to translocate Ca²⁺ through xylem into the young, actively growing leaves at a critical point in their development (Kong et al., 2014). Ca-deficiency not only lead to the well-known symptoms called "tipburn" in cauliflower, but also can affect the whole vital role of Ca depended on the molecular and cellular aspects of its action. Several researches have explored the effects of KCl and NaCl salts on Ca deficiency disorders and the associated impacts on plant growth in many horticulture crops. Peck and Macdonald (1986) have proved that increasing the rate of KC1 in hydroponic culture increased the concentrations of K and Zn but decreased Ca and Mg in leaf blades of cauliflower, broccoli, and Brussels sprouts. In another field experiment, increased NaCl salinity has also been found to increase the occurrence of Ca-deficiency related disorders in purslane plant (Kong et al., 2014). In addition to the negative impact of salinity on nutrient uptake in calcareous soil, most of these soils particularly in warmer regions is naturally associated with low organic matter (Celik et al., 2011). Soil organic matter is widely expressed by the term humus, and this latter, as a major component, is

definitely accepted as synonymous for humic substances (Stevenson 1982; Chen and Aviad 1990). Humic substances in the soil have both direct and indirect effects on plants (Nardi et al., 2002; Tan 2003). Indirect effects involve improvements of soil properties, such as aggregation, aeration, permeability, water-holding capacity, solubilization, and availability of microelements especially Fe, Zn and Mn, and some macro-elements namely K, Ca, and P (Chen and Aviad 1990; Tan 2003), and subsequently benefit plant growth. Direct effects are those that require the root uptake and transport of humic substances into the plant tissue (Chen and Aviad 1990; Nardi et al. 2002). Among the other humic substances, fulvic acid and humins, humic acid has alkali soluble property. It is also characterized by the most complex mixture of aromatic organic acids, with diverse functional groups bearing carbon, hydrogen, oxygen, nitrogen, phosphorous, and sulphur, in varying percentages and ions like calcium, potassium, magnesium, copper, zinc, etc. Humic acid induces dominant effects on plants by stimulating enzyme activity, membrane permeability, photosynthesis (Muscolo et al., 2013), respiration (Nardi et al., 2002), maintaining transpiration rate, increasing protein and vitamin contents, and yield of dry matter (Liu et al., 1998). While these positive effects of humic acid on plant growth and productivity have been widely proven in controlled conditions (Rose et al., 2014), less such work is realized in field conditions (Olk et al., 2018), and still much less carried out by foliar-spray practices. Whereas, foliar application is considered more efficient than soil application (Sladky and Tichy, 1959; Zaman and Schumann, 2006) because of its easy availability, equal fertilizer distribution, prompt response, and feasibility in using over large area in less time. The objective of this work is to find out in which extent foliar application could alleviate the adverse effect of soil conditions unfavorable to plant nutrient uptake. So, a series of treatments of humic acid as a bio stimulant (Fernandez, *et al.*, 2013) and nanocalcium as a mineral fertilizer has individually and in combination been applied on cauliflower, followed by some morphological observations and biochemical measurements.

Materials and Methods

Experimental site and soil sampling for analysis

A field experiment was carried out at the experimental Station of Agricultural College, University of Diyala Governorate, Iraq, to study the effect of foliar application of humic acid and nanocalcium element on some growth and yield features of cauliflower (*Brassica oleracea var. botrytis*) planted in calcareous soil with clay loam texture as recorded in table 1. Nanoparticles and nanomaterials are usually defined as particles with dimensions between about 1nm and100 nm, showing properties that are not found in their bulk form (Khan, 2016).

Table 1 : Some physical and chemical characteristics of "LiqHumus" fertilizer and field soil.

Soil					Fertilizer "LiqHumus"	
pH (1:1)					pH 9-	
$EC_{(1:1)} (dS m^{-1})$					CEC (meq/100g)	400-600
Content (%)					Liquid appearance Black	
Organic matter				0.91	Particule size (micron)	<100
CaCO ₃				31.17	Solubility in water 1	
Clay los		Doutiala siza	Clay	30.6	% (w/w)	
soil texture	am	distribution	Silt	40.6	Actively humified organic matter	90
	luie	distribution	Sand	28.8	Humic acids	16
Concentration (ppm)					Fulvic acids	2
e il	Nitrog	itrogen		30.32	Organic Nitrogen (N)	0.2
able	Phosphor		20.11	Potassium (K_2O)	3	
A	Potassium		243.90	Iron (Fe) 0.2		

Composite samples from the upper 30 cm of the experimental field soil were made up before tillage. The samples were air-dried and sieved through 2 mm for laboratory analysis. As mentioned in Page *et al.*, (1982), Organic matter content, available nitrogen, phosphorus, and potassium were analyzed by wet digestion according to Walkley and Black procedures (1934), Kjeldahl distillation unit depending on Bremner and Keeney method (1965), Olsen's bicarbonate following to Olsen and Sommers chart (1982), and ammonium acetate based on Pratt method (1965), respectively; soil texture, CaCO₃, pH, and EC were also measured with hydrometer, calcimeter, digital pH meter, and Conductivity Bridge devices, respectively.

Experimental design, planting, and crop management

The experimental field was prepared by plowing, smoothing and adding organic fertilizer (poultry manure) by 10% on the basis of volume (Alzamili, 2012) at the upper surface of 30 cm, a month before planting seedlings. Meanwhile, seedlings of 'botrytis var.' cauliflower were grown at a plant nursery in propylene trays with space enough for 200 seedlings in "bio plant" organic mineral substratum. The experimental units were distributed in the field according to a randomized complete block design consisting of nine treatment combinations with three replications. Each experimental unit was composed of two five-plant rows, of which only the three central plants were used to obtain the experimental data. Transplantation took place on October 25, 2018 when the seedlings exhibited four leaves. Seedlings were disposed at a distance of 0.6 m between rows and 0.35 m between plants in the row. Irrigation was provided by a drip system during the plant life cycle. As recommended by Alzamili (2012), urea and phosphate di-ammonium as sources of mineral fertilization were applied to soil by feeding method two weeks after transplantation. Foliar treatment combinations include three levels of humic acid (0, 2, and 4 ml l^{-1}) denoted by H₀, H₂, and H_4 and three levels of Nano-calcium (0, 2, and 4 ml l⁻¹) denoted by Ca₀, Ca₂, and Ca₄, applied at once on December 1, 2018: five weeks after seedlings transplantation. Humic acid, commercially labeled "LiqHumus," is manufactured in mines (Stevenson, 1994). Nanocalcium, commercially labeled "G-power Ca," is produced by nanotechnology in the Turkish company Agri-Sciences. This agricultural liquid fertilizer, which composed of 13% of water soluble CaO and 8% of NO₃-N, is recommended to spray with a rate from 1 to 4 ml l^{-1} in the case of vegetable crops. Cauliflower was harvested over two days from 10 to 12, January, 2019.

Morphological and biochemical measurements

Plants were harvested by cutting the stem at 0.5 cm over ground. Morphological measurements were achieved on three plants/replicate. Plant length, stem and floral diameter were recorded using measurement tape and Vernier caliper. Fruit as well as above-ground vegetative fresh weights were measured immediately using a sensitive balance. To determine the percentage of dry fruit weight, "Curd" specimens were weighed before and after oven-drying at 70°C until the stability of weight. Then, percentage of dry fruit weight expressed as $100 \times$ (weight after drying/weight before drying). For the biochemical analysis, photosynthetic pigments were extracted according to the method of Horwitz (1975) by mashing 1 g of fresh plant leaves in a ceramic mortar with 20 ml of 80% acetone for 5 minutes. The filtered extract was placed in a centrifuge for 5 minutes at 1000 rpm. After supplementing the resulting supernatant to 50 ml with the same solvent, the maximum absorbance (Abs) was read with spectrophotometer (ShimadzuMini-1240 UV-Vis, USA) for chlorophyll "a," chlorophyll "b," and carotenoid at 662, 646, and 490 nm respectively. Leaves chlorophyll content (mg l⁻¹) was calculated according to the following formulas (Najla et al., 2012):

Chlorophyll "a" = $12.7 \times Abs_{665} - 2.69 \times Abs_{645}$ Chlorophyll "b" = $22.9 \times Abs_{645} - 4.68 \times Abs_{665}$ Total Chlorophyll = $20.2 \times Abs_{645} - 8.02 \times Abs_{665}$ Carotenoid = $1000 \times Abs_{490} - 2.27$ Chlorophyll "a" - 81.4 Chlorophyll "b" / 227

Depending on sample weigh and solution volume, the chlorophyll content units in the leaves were then converted from mg l^{-1} to mg gm⁻¹.

Statistical analysis

Data in three replicates pertaining to various parameters were subjected to analysis of variance (ANOVA) using Web Agri Stat Package (ICAR Research Complex for Goa, Ela, Old Goa, Goa. 403 402. India). Means were compared by Critical Differences (CD) test at 5 and 1% levels of probability.

Results and Discussion

Growth attributes

Table 2 illustrates the effect of foliar spray of humic acid (H) and nanocalcium (Ca), each at two levels of concentration, 2 and 4 ml 1⁻¹, on some growth parameters of cauliflower (*Brassica oleracea var. botrytis*). Data show that almost all studied parameters were positively affected at $p \le$ 05 and even at a threshold of $p \le 01$ under certain treatments with higher concentration. Compared with control (Ca₀ + H₀), 2 ml 1⁻¹ applications (Ca₂ + H₀) and (Ca₀ + H₂) induced an increase ranged between 0.22 and 0.37 cm in stem diameter, 1.8 and 1.85 cm in floral diameter, and 2.33 and 2.45 cm in plant length, representing an average increase of 8, 13, and 10% respectively. Similarly, this increase went gradually up to 14, 20, and 21% in plants received 4 ml l^{-1} applications individually $(Ca_4 + H_0)$, $(Ca_0 + H_4)$, or in combination ($Ca_2 + H_2$). However, no significant increase in leaves number was observed till the overall concentration reached 6 ml 1^{-1} in Ca₂ + H₄ and beyond in Ca₄ + H₄, where the maximum increase was 25%. For this combined concentration 8 ml l⁻¹, plant morphological features in field were so clearly distinct that stem diameter, floral diameter, and plant length exhibited as highly significant increase as 26, 27, and 39%, respectively as compared to control. The positive effect of nanocalcium found on cauliflower growth and later on yield traits are similar to those obtained by many researchers among them Nelson and Niedziela (1998) on tulip response to Ca applied to soil, Durukan et al. (2013) on cauliflower response to Ca applied to foliage, and Kong et al. (2014) on purslane response to Ca- deficiency. The Ca effect could be explained first by its availability in sufficient quantity to meet plant need at growing time; second, by its well-known functions in plant metabolism processes such as photosynthesis, respiration, cell division, biosynthesis, and ionic absorption. In fact, sufficient concentration of Ca in plant organs ensures cells holding the structure of cell walls and stabilizing cell membranes, increases plant height by increasing mitotic activity in the terminal meristem, and stimulates root growth and early onset of flowering in agronomic and vegetable crops (Mohsin Khadimi, 2013). In parallel, it also has a direct influence on the salt balance within plant cells and activate potassium to regulate the opening and closing of stomata to allow water movement from the plant (Pertuit et al., 2001). Obtained results about the stimulant effect of humic acid on plant growth also confirm findings commonly overviewed and meta-analyzed in the literature (Rose et al., 2014; Barone et al., 2019; Bulgari et al., 2019).

Yield attributes

Yield data shown in table 3 demonstrate the same trend as in table 2, with one exception in terms of effect extent and significance. That is, while dry fruit weight percentage responded noticeably to individual applications at 2 or 4 ml l⁻ ¹, by an average increase of 25 and 80%, respectively, no significant change was observed in fresh fruit and aboveground vegetative weights. In the opposite, increased concentrations through combined applications ($Ca_2 + H_2$, Ca_4) + H₂, or Ca₂ + H₄) have significant impact. Overall 4, 6, and 8 ml l⁻¹ concentrations led to a gradual increase, respectively passing from 14 and 17, to 34% in fresh fruit weight, from 13 and 24, to 33% in above-ground vegetative weight, and from 64 and 84, to 114% in dry fruit weight percentage. These results suggest that the highest the dosage of application, the greatest the yield production and the synthesis of fruit dry matter. The findings of this experiment are largely corroborated by many previous studies on the topic, especially those carried out on vegetables under calcareous soil (Turkmen et al., 2004; Çelık et al., 2011) and saline soil conditions (Aydin et al., 2012; Turhan, 2019). A variety of mechanisms was hypothesized to explain humic substances effect on plant productivity. It can be through stimulation of cell membrane permeability (Nardi et al. 2002; Chen et al., 2004). Foliar spraying of humic acid on asparagus plants has been found to increase uptake of macro and micro elements in shoot and rhizome due to membrane permeability stimulation (Turkmen et al., 2004). Besides, the positive effects on plants could be essentially ascribed to hormonelike activity, as a number of hormones enclosed in the humus structure has already been identified (Chen and Aviad, 1990; Canellas and Olivares, 2014; Nardi et al., 2016). According to previous investigations (Nardi et al., 2002; Muscolo et al., 2013), humic substances are supramolecular aggregates and their stability and reactivity depend on the solution's ionic strength and pH of the surrounding environment. Subsequently, these macro aggregate structures would be broken into subunits of biological active molecules. The likehormone activity of these molecules would be able to regulate the availability of plant growth hormones, such as auxin and indole acetic acid, and induced lateral root and shoot development in plant. These mechanisms are related to the surface activity of humic substances resulting from the presence of both hydrophilic and hydrophobic sites. Hydrophilic interactions occur via anionic groups, especially carboxylic function, and hydrophobic interactions are governed by Van der Waals forces (π - π , ion-dipole) and hydrogen bonds (Nardi, 2016).

Photosynthetic pigments

Spray 2 ml I^{-1} of nano-calcium and humic acid individually $(Ca_2 + H_0)$ and $(Ca_0 + H_2)$ or in combination $(Ca_2 + H_2)$ revealed no significant influence on photosynthetic pigments, as indicated in Table 4. In addition, nano-calcium application at 4 ml I^{-1} $(Ca_4 + H_0)$ seems to be selective in its action, since it affected only the carotenoid content with an increase of 20% at $p \le 5$ compared to control. In the contrary, spraying humic acid at the same concentration $(Ca_0 + H_4)$ resulted in a significant ($p \le 1$) increase of 31% for both the total chlorophyll and carotenoid contents in the leaves. This improvement was consistent in plants receiving a total concentration of 6 ml l^{-1} in the combined application (Ca₄ + H_2) or (Ca₂ + H₄), but, when the concentration passed to 8 ml 1^{-1} in the case of Ca₄ + H₄ treatment, the increase in contents of carotenoids, chlorophyll "a," chlorophyll "b," and total chlorophyll peaked to 38, 45, 57, and 42%, respectively. Halpern et al. (2015), Tahiri et al. (2016), and Turhan (2019) have proved that application of humic substances increased chlorophyll content and accumulation of K, B, Mg, Ca and Fe in leaves. On the other side, Ca foliar application on vegetables was reported to regulate and stimulate nutrient uptake (Durukan et al., 2013; Kong et al., 2014). It could be deduced that the elevated chlorophyll and carotenoids concentration in leaves is likely related to a higher nutrient uptake due to humic acid and / or nano-calcium applications, leading to enhance many biochemical processes, among them photosynthesis in leaves with a synthesis of sugars that are rapidly transported and released into the rhizosphere. These energetic substances consumed by the microorganisms of the rhizosphere, which in turn released micro and macronutrients and synthesize substances needed by the plant at growing and fruiting stages.

Table 2 : Values of some Cauliflower growth attributes as a response to nanocalcium and humic acid foliar applications. Each value is the mean of three replications.

No.	Treatments	Stem Diameter (cm)	Floral Diameter (cm)	Plant Length (cm)	Leaves Number (per Plant)
1	$Ca_0 + H_0$	3.577 ^e	14.043 ^c	24.333 ^e	12.000 ^{cd}
2	$Ca_2 + H_0$	3.800 ^{de}	15.867 ^b	26.667^d	12.000 ^{cd}
3	$Ca_4 + H_0$	4.053^{c}	16.700 ^{<i>ab</i>}	29.667 ^{bc}	13.66 ^{<i>abc</i>}
4	$Ca_0 + H_2$	3.950 ^{cd}	15.890 ^b	26.783 ^{cd}	11.333 ^d
5	$Ca_2 + H_2$	4.127^{bc}	16.857 ^{<i>ab</i>}	28.900 ^{bc}	13.000 ^{bcd}
6	$Ca_4 + H_2$	4.340 ^{<i>ab</i>}	17.423 ^{<i>a</i>}	33.124 ^a	13.000 ^{bcd}
7	$Ca_0 + H_4$	4.087^{c}	17.050 ^{ab}	30.000 ^{bc}	13.333 ^{<i>abc</i>}
8	$Ca_2 + H_4$	4.323 ^{ab}	17.500 ^a	33.433 ^a	14.333 ^{<i>ab</i>}
9	$Ca_4 + H_4$	4.523 ^a	17.890 ^a	33.750 ^a	15.000 ^{<i>a</i>}
ANOVA table	CD (0.05)	0.233	1.270	2.238	1.925
ANOVA lable	CD (0.01)	0.319	1.739	3.774	NS

According to ANOVA table, some treatments found significant at 1% and 5% levels of significance, with Critical Differences: CD (0.01); CD (0.05). When compared with CD (0.05), treatment means were given italicized characters a, b, c, d, e, or their combinations, and those carrying the same character are not significantly different.

Table 3 : Values of some Cauliflower yield attributes as a response to nanocalcium and humic acid foliar applications. Each value is the mean of three replications.

No	Treatmonts	Fresh fruit weight	Above-ground veg.	Dry fruit
190.	Treatments	per plant (kg)	weight per Plant (kg)	weight (%)
1	$Ca_0 + H_0$	1.893 ^d	3.383 ^e	3.300 ^d
2	$Ca_2 + H_0$	2.050 ^{bcd}	3.476 ^{de}	4.133 ^c
3	$Ca_4 + H_0$	2.057^{bcd}	3.383 ^{de}	6.000 ^b
4	$Ca_0 + H_2$	2.000 ^{cd}	3.476 ^{de}	4.100 ^c
5	$Ca_2 + H_2$	2.150^{bc}	3.817 ^{cd}	5.433 ^b
6	$Ca_4 + H_2$	2.200^{bc}	4.117 ^{bc}	6.067 ^b
7	$Ca_0 + H_4$	2.100 ^{bcd}	3.753 ^{cde}	5.883 ^b
8	$Ca_2 + H_4$	2.250 ^b	4.233 ^{<i>ab</i>}	5.967 ^b
9	$Ca_4 + H_4$	2.533 ^a	4.503 ^{<i>a</i>}	7.023 ^a
ANOVA table	CD (0.05)	0.238	0.372	0.799
ANOVA luble	CD (0.01)	0.326	0.509	1.095

According to ANOVA table, some treatments found significant at 1% and 5% levels of significance, with Critical Differences: CD (0.01); CD (0.05). When compared with CD (0.05), treatment means were given italicized characters a, b, c, d, e, or their combinations, and those carrying the same character are not significantly different.

No	Treatments	Leaf Carotenoids	Leaf Carotenoids Leaf Chlorophyll (mg. gm ⁻¹ fresh wei		
INU.		(µg.100gm ⁻¹ fresh weight)	(a)	(b)	(Total)
1	$Ca_0 + H_0$	22.814 ^e	0.534 ^b	0.310 ^e	0.850^{b}
2	$Ca_2 + H_0$	24.326 ^{de}	0.553 ^b	0.308 ^{de}	0.905 ^b
3	$Ca_4 + H_0$	27.357 ^{bcd}	0.591 ^b	0.343 ^{de}	0.946 ^b
4	$Ca_0 + H_2$	25.083 ^{de}	0.573 ^b	0.310 ^{de}	0.937 ^b
5	$Ca_2 + H_2$	25.845 ^{cde}	0.591 ^b	0.362 ^{cd}	0.943 ^b
6	$Ca_4 + H_2$	28.777 ^{<i>abc</i>}	0.727 ^a	0.432 ^{<i>ab</i>}	1.136 ^{<i>a</i>}
7	$Ca_0 + H_4$	30.005 ^{bcd}	0.740 ^{<i>a</i>}	0.416^{bc}	1.113 ^a
8	$Ca_2 + H_4$	30.372^{ab}	0.716 ^{<i>a</i>}	0.466^{ab}	1.202 ^{<i>a</i>}
9	$Ca_4 + H_4$	31.608 ^{<i>a</i>}	0.776 ^a	0.487 ^a	1.205 ^{<i>a</i>}
ANOVA table	$CD(\overline{0.05})$	3.277	0.079	0.056	0.110
ANO VA luble	CD(0.01)	4.488	0.109	0.077	0.151

Table 4 : Photosynthetic pigments content in Cauliflower leaves as a response to nanocalcium and humic acid foliar applications. Each value is the mean of three replications.

According to ANOVA table, some treatments found significant at 1% and 5% levels of significance, with Critical Differences: CD (0.01); CD (0.05). When compared with CD (0.05), treatment means were given italicized characters a, b, c, d, e, or their combinations, and those carrying the same character are not significantly different.

Effect of interaction between humic acid and nanocalcium on studied attributes

In order to clarify the interferential effect between humic acid and nano-calcium on studied attributes, treatments forming the same concentration were tested by a comparative approach. For example, the differences in plant stem diameter between all combined and individual treatments at 4 ml l⁻¹ (Ca₂ + H₂; Ca₄ + H₀; Ca₀ + H₄) were not significant at all. This is almost always true throughout the study data for all the observed parameters, meaning plant improvement seems to depend on treatment dose rather than factor type.

Conclusion

Study results suggest that spraying nanocalcium and humic acid, individually or in combination, improve cauliflower growth, yield, and biochemical attributes. In general, the extent of this improvement is positively correlated to concentration level. The additional increment appears to be 10% on a concentration scale of 0, 2, 4, 6 ml 1^{-1} , and the higher dose (8 ml l⁻¹) represents an increase of more than 30%. On the other hand, no interaction effect between humic acid and nano-calcium are found, allowing to deduce that improvement is attributive to concentration level, not factor type. Finally, these findings support the hypothesis formulated in this study. That is to say, whereas the low values of plant characteristics in the control reflect the already harmful soil conditions of high salinity, low organic matter, and relatively unavailable nutrients in complex forms, these poor conditions can be at least partially avoided by foliar spraying of nutrients and bio stimulants, in the occurrence nanocalcium and humic acid, to improve plant quality.

References

- Abdel-Razzak H.S.; Gamel, T.H. and El-Nasharty, A.B. (2008). Efficiency of Inorganic and Organic Nitrogen Fertilization on Cauliflower (*Brassica oleraceae* var. botrytis, L.) Curds Quality. Alexandria Science Exchange Journal 29(4): 283-297.
- Akinremi, O.O.; Janzen, H.H.; Lemke, R.L. and Larney, F.J. (2000). Response of canola, wheat and green beans to leonardite additions. Can. J. Soil Sci. 80: 437–443.

- Alzamili, N.F.Y. (2012). The role of organic and chemical nutrients on growth and yield of cauliflower Plant. In A Thesis Submitted to the College of Agriculture University of Baghdad. 96.
- Barone, V.; Bertoldo, G.; Magro, F.; Broccanello, C.; Puglisi,
 I.; Baglieri, A.; Cagnin, M.; Concheri, G.; Squartini, A.;
 Pizzeghello, D.; Nardi, S. and Stevanato, P. (2019).
 Molecular and Morphological Changes Induced by
 Leonardite-based Biostimulant in *Beta vulgaris* L.
 Plants 8(181): 1-18.
- Canellas, L.P. and Olivares, F.L. (2014). Physiological responses to humic substances as plant growth promoter. Chemical and Biological Technologies in Agriculture, 1(3):1-11.
- Chen, Y. and Aviad, T. (1990). Effect of humic substances on plant growth. In Humic substances in soil and crop sciences, ed. P. McCarthy, 161-186. Madison, Wisc. ASA and SSSA.
- Çelık, H.; Katkat, A.V.; Aşık, B.S. and Turan, M.A. (2011). Effect of Foliar-Applied Humic Acid to Dry Weight and Mineral Nutrient Uptake of Maize under Calcareous Soil Conditions. Communications in Soil Science and Plant Analysis 42:29-38.
- Durukan, A.; Şahin, S.; Geboloğlu, N.; Aydın, M.; Karaman, M.R.; Sağlam, N. and Turan, M.A. (2013). Effect of Ca and B-Humate Applications on Plant Growth and Quality Characteristics of Cauliflower Leaves. Soil-Water Journal 2(1): 703-712.
- Fageria, M.S.; Choudhary, B.R. and Dhaka, R.S. (2012). Vegetable Crops Production Technology, Volume-II. Kalyani Publication, Noida (UP).
- Fernandez, V.; Sotiropoulos, T. and Brown, P. (2013). Foliar fertilization: scientific principles and field practices. International Fertilizer Industry Association (IFA), Paris, France. 144.
- Horwitz, W. (1975). Official methods of analysis. Association of analytical chemists, (A.O.A.C) Washington, D.C. USA.
- Khan, M.N. (2016). Nano-titanium Dioxide (Nano-TiO₂) Mitigates NaCl Stress by Enhancing Anti oxidative Enzymes and Accumulation of Compatible Solutes in Tomato (*Lycopersicon esculentum* Mill.). J. Plant Sci. 11: 1-11.
- Kong, Y.; Rozema, E. and Zheng, Y. (2014). The effects of NaCl on calcium-deficiency disorder vary with

symptom development stage and cultivar in hydroponic *Portulaca oleracea* L. Canadian Journal of Plant Science 94(7): 1195-1201.

- Liu, C.; Copper, R.J. and Bowman, D.C. (1998). Humic acid application effects photosynthesis, root development, and nutrient content of creeping bent grass. Hort. Sci., 33:1023-1025.
- Mohsen, K. (2013). Foliar Application of Salicylic Acid and Calcium on Yield, Yield Component and Chemical properties of Strawberry. Bull. Env. Pharmacol. Life Sci. 2 (11): 19-23.
- Muscolo, A.; Sidari, M. and Nardi, S. (2013). Humic substance: Relationship between structure and activity. Deeper information suggests univocal findings. J. Geochem. Explor. 129: 57-63.
- Najla, S.; Sanoubar, R. and Murshed, R. (2012). Morphological and biochemical changes in two parsley varieties upon water stress. Physiol. Mol. Biol. Plants 18(2):133-139.
- Nardi, S.; Pizzeghello, D.; Muscolo, A. and Vianello, A. (2002). Physiological effects of humic substances on higher plants. Soil Biology and Biochemistry 34: 1527-1536.
- Nardi, S.; Pizzeghello, D.; Schiavon, M. and Ertani, A. (2016). Plant biostimulants: physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism. Sci. Agric., 73(1): 18-23.
- Nelson P.V. and Niedziela, C.E. (1998). Effect of calcium sources and temperature regimes on calcium deficiency during hydroponic forcing of tulip. Sci. Hortic. 73: 137-150.
- O'Donnell, R.W. (1973). The auxin-like effects of humic preparations from leonardite. Soil Sci. 116: 106–112.
- Olk, D.C.; Dinnes, D.L.; Scoresby, R. and Darlington, J. (2017). Improved soil physical properties with longterm application of humic product in corn-soybean rotations. Proceedings, Annual meeting of the ASA-CSSA-SSSA, Tampa, FL, Oct 22-25.
- Page, A.L.; Miller, R.H. and Kenney, D.R. (1982). Methods of Soil analysis part (2). 2nd Ed. Agronomy 9. Am. Soc. Agron. Madison, Wisconsin.
- Peck, N.H. and Macdonald, G.E. (1986). Cauliflower *Brassica oleracea* var botrytis cultivar imperial 10 6 broccoli *Brassica oleracea* var italica cultivar El Centro and Brussels sprouts *Brassica oleracea* var gemmifera cultivar jade cross responses to concentrated superphosphate and potassium chloride fertilization. Journal of the American Society for Horticultural Science 111(2): 195-201.
- Pertuit, A.J.; Dudley, J.B. and Toler, J.E. (2001). Leonardite and fertilizer levels influence tomato seedling growth. Hort. Sci. 36: 913–915.

- Premraj Gocher, A.K.; Soni, A.K.; Mahawar, S.P. Singh and Koodi, S. (2017). Response of NPK and Sulphur on Nutrient Analysis and quality attributes of Cauliflower (*Brassica oleracea* var. botrytis L.). Int. J. Curr. Microbiol. App. Sci. 6(7): 4364-4371.
- Rose, M.T.; Patti, A.F.; Little, K.R.; Brown, A.L.; Jackson, W.R. and Cavagnaro, T.R. (2014). A meta-analysis and review of plant-growth response to humic substances: practical implications for agriculture. Adv. Agron, 124: 37-89.
- Stevenson, F.J. (1994). Humus Chemistry: Genesis, Composition, Reactions. 2ed. Wiley, New York, NY, USA.
- Tahiri, A.; Delporte, F.; Muhovski, Y.; Ongena, M.; Thonart, P. and Druart, P. (2016). Change in ATP-binding cassette B1/19, glutamine synthetase and alcohol dehydrogenase gene expression during root elongation in Betula pendula Roth and *Alnus glutinosa* L. Gaertn in response to leachate and leonardite humic substances. Plant Physiol. Biochem. 98: 25-38.
- Tan, K.H. (2003). Humic matter in soil and environment: Principles and controversies. New York: Marcel Dekker.
- Turkmen, O.; Dursun, A.; Turan, M. and Erdinc, C. (2004). Calcium and humic acid affect seed germination, growth, and nutrient content of tomato (*Lycopersicon esculentum* L.) seedlings under saline soil conditions. Acta Agriculturae Scandinavica, Section B - Soil and Plant Science 54:168-174.
- Sladky, Z. and Tichy, V. (1959). Applications of humus substances to overground organs of plants. Biologia Plantarum 1: 9-15.
- Halpern, M.; Bar-Tal, A.; Ofek, M.; Minz, D.; Muller, T. and Yermiyahu, U. (2015). The Use of Biostimulants for Enhancing Nutrient Uptake. In Advances in Agronomy; Academic Press: Cambridge, MA, USA 130: 141–174.
- Aydin, A.; Kant, C. and Turan, M. (2012). Humic acid application alleviate salinity stress of bean (*Phaseolus vulgaris* L.) plants decreasing membrane leakage. Afr. J. Agric. Res., 7: 1073-1086.
- Bulgari, R.; Franzoni, G. and Ferrante, A. (2019). Biostimulants Application in Horticultural Crops under Abiotic Stress Conditions. *Agronomy* 9: 306.
- Turhan, A. (2019). The Role of Humic Acid Application in Reducing Detrimental Effects of Salt in Cauliflower (*Brassica oleraceae* L. Var. Botrytis). KSU J. Agric Nat. 22(6): 837-842.
- Zaman, Q.U. and Schumann, A.W. (2006). Nutrient management zones for citrus based on variation in soil properties and tree performance. Precision Agriculture 7: 45-63.