

## Response of Broccoli to Irrigation Water Quantities and Foliar Application with Antitranspirants and Humic Acid as Soil Amendment on Yield, Quality and some Antioxidant

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### ABSTRACT

This study was conducted at El-Baramoon Research Station, Dakahlia Governorate, Egypt during the growing seasons of 2015 and 2016 to study the effect of three water quantities (800.34, 1600.73 and 2400.40 m<sup>3</sup>/fed as common used treatment) and some antitranspirants, *i.e.*, kaolin solutions 4%; as aluminum silicate, calcium carbonate (6%) as well as humic acid as potassium-humate (80% humic acid, 11-13% K<sub>2</sub>O) and their interactions on growth, yield, quality and some antioxidants of broccoli. The second level of water irrigation (1600.73 m<sup>3</sup>/fed) with application of kaolin or humic acid or the first level (2400.40 m<sup>3</sup>/fed) with application of kaolin or humic acid had the most significant effect of plant height, number of leaves/plant, dry weight/plant, leaves area/plant, central and lateral head weights/plot, total head yield/plot and total head yield/fed in both seasons of study, without significant differences between them. Increasing irrigation quantity from 800.34 to 2400.40 m<sup>3</sup>/fed increased broccoli growth characters in the both seasons. No significant differences were found in vegetative growth and yield attributes between water quantities of 1600.73 and 2400.40 m<sup>3</sup>/fed in both seasons. Kaolin or humic acid led to significant increase in most vegetative growth and yield traits compared to control. The third level of irrigation water quantity (800.34 m<sup>3</sup>/fed) with kaolin application had significant increases in antioxidants (vitamin C, anthocyanin, phenolic compounds and total flavonoids content). On the other hand, the application of high level of irrigation water quantity (2400.40 m<sup>3</sup>) and application of kaolin had a significant effect on protein content, in both seasons. The low level of irrigation water (800.34 m<sup>3</sup>) significantly increased the antioxidants content and decreased protein content in both seasons. Application of kaolin was significantly increased in all antioxidants and protein content in the both seasons in comparison with other treatments. Generally, it could be concluded that kaolin or humic acid application under water quantity of 1600.73 m<sup>3</sup>/fed was the best combination for broccoli production aimed at maximum water use efficiency in this study. This in turn encourages such amendments to reduce water consumption by 33.3%.

**Keywords:** Water quantities, Kaolin, Calcium carbonate, K-humate, yield, antioxidants.

### INTRODUCTION

Broccoli (*Brassica oleracea* var. *italic* Plenck) is a widespread international vegetable crop belongs to the family of Cruciferae. It is considered as a functional food (Villarreal-García *et al.*, 2016). It is a good source of vitamins, riboflavin, niacin, chlorophyll, and contains essential minerals such as phosphorus, potassium, calcium, copper and magnesium, with a few quantities in microelements like iron and zinc. Moreover, antioxidant compounds, like phenolic compounds, including phenolic acids, flavonoids and anthocyanin, which having chelating effect against free radical that lead to oxidative stress; therefore, these compounds played important roles in preventing of cardiovascular diseases, and a lowered risk of cancer (Baenas *et al.* 2014). Broccoli is important source of health-promoting compounds like glucosinolates, which has long been recognized for their outstanding benefits to human nutrition and plant defense. The glucosinolates that are divided into three major categories: aliphatic, indole and aromatic glucosinolates (Yan and Chen, 2007). Because of its varied uses and high nutritional value; broccoli is considered a vegetable of important economic value. At the same time, consumers have a rising concern with product safety of broccoli (Rangkadilok *et al.*, 2004). In Egypt, broccoli is occupied 48<sup>th</sup> rank worldwide, and quantity of 163 on of product was exported in 2017 (FAO, 2018).

Due to floods and water stresses caused by climate change, pollution of rivers and lakes, urbanization, over-extraction of ground water and expanding populations mean that many nations like Egypt face serious water scarcity. Therefore, the water is considered as the most critical resource for sustainable agricultural development and productivity. Broccoli is growing in Egypt during winter when there is few precipitation and high evapotranspiration. Crop cultivation during this dry period usually requires irrigation. Broccoli being a shallow rooted crop requires

frequent irrigation to keep the plant vigorous. Studies of various worker indicated that frequent irrigation gives the highest yields of curd; plant requirement is approximately 440 mm (Gomes *et al.*, 2000). The current management practices of irrigation water cause not only wastage of scarce water resources and fertilizer uses but also decreases the yield of Cole crop (Rahman *et al.*, 1988). Head, curd development and storability of broccoli will decrease with the water deficits at any growth stage (Selim and Mosa, 2012).

The amount of water lost within the plant via transpiration can be incredibly high. Consequently, water uptake from the land by the distributor of the roots is very high. The uptake of water by plant roots and evaporative losses gradually reduce the moisture content of the soil below the field capacity. If not completed, soil water content may reaches a wilting point for plant growth. Broccoli grows are likely to over -which in result water stresses from the system (Pasakdee *et al.*, 2006).

Incorrect irrigation management not only reduces available water resources, but also response nutrient losses by leaching, runoff, and denitrification of nitrogen in ecosystems. Irrigation scheduling is a critical management input to maximize soil moisture content for proper growth of plant, development, optimum yield, water use efficiency and economic benefits (Himanshu *et al.*, 2013). To estimate the quantity of water required in a plant, it is more important to consider two major parameters (Hanson *et al.*, 1999): the amount of water required by the crop, the rates of precipitation and evapotranspiration during the two seasons. The important use of irrigation can be characterized as the rooting area and avoiding the leaching of nutrients into soil layers (Kruger *et al.*, 1999). Therefore, predicting the water content in the root zone can be use to helping the farmer decide when, and how much to irrigate.

Humic acid and antitranspirants with considerable resistance to soil moisture deficient has been considerable

an economic and efficient means of utilization drought-prone areas when appropriate management practices to reduce water losses are needed (Ezzat *et al.*, 2009). In general, humic acid has a number of potent benefits for plants: increased water and nutrient holding capacity; increased reserve of slow release nutrients; enhanced solubility of phosphorus, zinc, iron, manganese, and copper; increased resistance to soil pH change; improved soil aggregation; enlarged root system and improved stimulation of plant-growth due to hormones (Mikkelsen, 2005). Selim and Mosa (2012) indicated that, humic substances (HS) affected spatial water distribution and increased water retention in the root zone. Furthermore, usage of HS increased total marketable yield and head diameter of broccoli and quality parameters (i.e., total soluble solids, protein, and vitamin C). Highest nutrient concentrations were found in the broccoli heads and concentrations of plant-available nutrients in soil after harvesting were also higher, indicating an improvement in soil fertility. Humic acid and zinc application had no effect on plant height, curd length and dry weight. Leaf number, lateral curd number and marketable total yield of broccoli were increased significantly (Yilmaz *et al.*, 2013).

Gawish and Fattahallah (1997) stated that covering all leaf surface of *Colocasia esculenta* with kaolin (9 g/m<sup>2</sup>) combined with wilt-pruf (4 g/m<sup>2</sup>) reduced the transpiration rate by 50-60%. Anwar (2005) found that the combination between water quantity at the level of 1500 m<sup>3</sup>/fed and spraying with kaolin or calcium carbonate CaCO<sub>3</sub> at 6%

was the superior treatment regarding plant growth and potato tuber weight/plant as well as total yield/feddan. Ezzat *et al.* (2009) indicated that irrigation of potato plants with 1600 m<sup>3</sup>/fed with application of humic acid as K-humate or spray with Kaolin "antitranspirant" was the most efficient treatment for growth, yield, quality and WUE of potato plants grown in clay loam soil.

The objective of this study was to investigate the impact of different levels of irrigation water quantities with reducing water requirement substances and their interactions on quantitative, qualitative yield of broccoli crop.

## MATERIALS AND METHODS

### 1. Materials of plant and conditions of growing

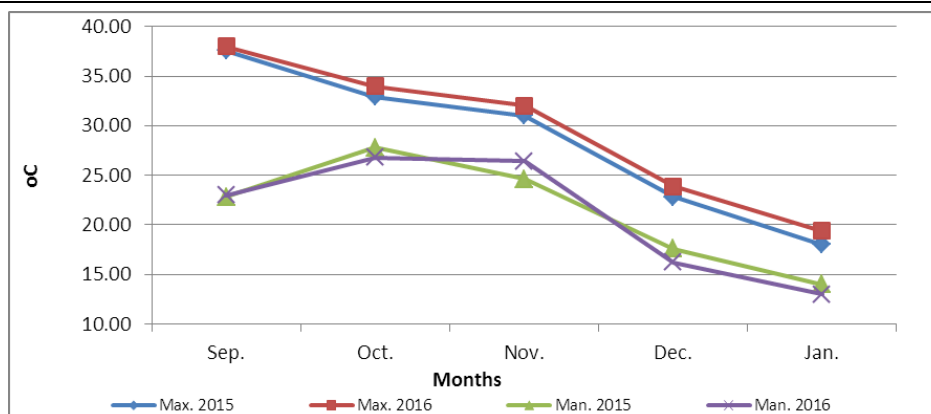
The experiments were conducted with Broccoli (*Brassica oleracea* var. *italica*; cv. F1 hybrid) at El-Baramoon Research Station, Mansoura, Dakahlia Governorate, Egypt during seasons of 2015 and 2016 to study the effect of water quantities, some antitranspirant and humic acid and their interactions on growth, productivity, and water use efficiency of broccoli. Analyses of the soil (physical, chemical and hydro-physical) are presented in Table 1 (a and b) (Page, 1982; El-Hady and El-Sherif, 1988). The temperature and precipitation during the growing seasons are shown in Figures 1 and 2. The collection of data was obtained from Agrometeorological Services, Central Management of Agriculture Guideline, Agriculture Research Center.

**Table 1. Analysis of experimental clay loam soil during the seasons of 2015 and 2016.**

<b>(a) Mechanical and chemical analysis.</b>								
Physical properties	Value		Chemical Properties	Value				
	2015	2016		2015	2016	2015	2016	
Coarse sand (%)	2.56	2.59	Organic matter (%)			2.53		2.48
Fine sand (%)	12.93	12.95	Total N (ppm)			32.51		33.0
Silt (%)	17.67	17.72	Available P (ppm)			6.03		6.08
Clay (%)	66.84	66.74	Available K (ppm)			341		347
Texture class	Clay	Clay	Fe (ppm)			12.05		12.10
CaCO <sub>3</sub>	1.85	1.86	Mn (ppm)			7.04		7.06
pH value	7.78	7.85	Zn (ppm)			3.05		3.02
EC dSm <sup>-1</sup> (1.5)	1.48	1.53	Cu (ppm)			0.97		0.95

<b>(b) Hydrophysical analysis.</b>								
Soil depth (cm)	Saturation percentageS. (%)		Field capacity FC (%)		Wilting point WP (%)		Available water AW(%)	
	2015	2016	2015	2016	2015	2016	2015	2016
0-15	80.3	80.5	40.0	40.1	16.4	16.4	18.6	19.2
15-30	81.7	81.4	40.5	40.7	16.7	16.6	19.2	19.3
30-45	82.8	82.4	41.3	41.2	16.9	16.8	20.1	20.0



**Fig. 1. Monthly averages of temperature at Dakahlia Governorate during the growing seasons of 2015 and 2016.**

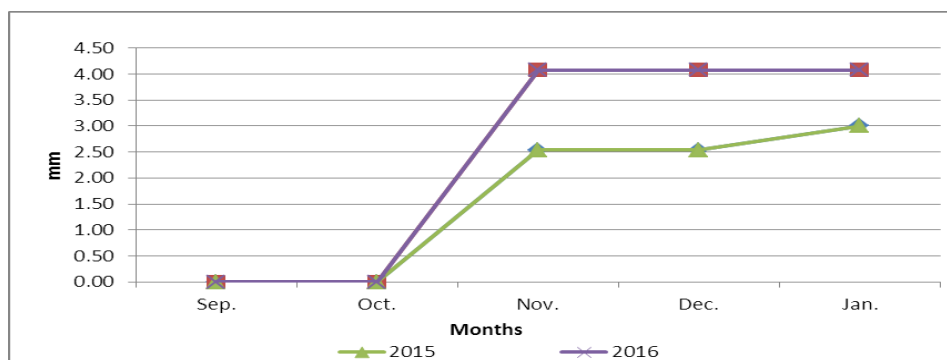


Fig. 2. Monthly averages of precipitation at Dakahlia Governorate during the growing seasons of 2015 and 2016.

## 2. Treatments and experimental design

The split-plot design in a randomized complete blocks design with three replicates was used. Three water irrigation quantities (2400.40 "common used", 1600.73, and 800.34 m<sup>3</sup>/fed, i.e., 54.1, 50.0 and 45.8% from field capacity, respectively) which equal the irrigation intervals every 10, 20 and 30 days' starting after the first irrigation were assigned to main plots. The number of irrigation and seasonal precipitation are in Table 5. Soil moisture prior to irrigation and after the irrigation according to treatment was determined. Scheduling irrigation and applied was done to bring the soil moisture up to field capacity FC considering the effective root zone depth (Table 1b). Irrigation treatments began after the plants establishment and irrigation water was calculated using the following equation (Michael, 1978):

Total irrigation water applied (m<sup>3</sup>/fed) = d x No. irrigation applied and  $d = (FC - MCI) / 100 \times As \times D$ . Where, d= Depth of irrigation (cm), FC= Field capacity of the soil (%), MCI= Soil moisture at the time of irrigation (%), As= Apparent specific gravity of the soil (g/cc) and D= Depth of effective root zone (cm).

Seasonal water requirement was calculated as a following equation according to water balance as follows:

Seasonal water requirement (m<sup>3</sup>/fed) = Total irrigation water applied (m<sup>3</sup>) + Seasonal rainfall (mm) from rainfall data + Soil water contribution (mm) from soil moisture analysis (Table 5). Total irrigation applied (m<sup>3</sup>/fed) = water quantity at the first irrigation (m<sup>3</sup>/fed) + total irrigation applied (m<sup>3</sup>/fed) during the season.

The sub-plots were devoted to three effective procedures to improve irrigation water efficiency including, kaolin, calcium carbonate and humic acid as well as check treatment (water). The antitranspirant (Kaolin solutions as aluminum silicate) at 4% was applied as foliar application at 30 and 45 days after planting. Plants were sprayed with a fine mist of Kaolin till run-off, with care being taken to cover all plant parts. Calcium carbonate at 6% was used and sprayed on plants at the same times of Kaolin treatment. Humic acid in a form of potassium-humate (80% humic acid, 10-12% K<sub>2</sub>O) was used after dilution with water (1: 100) beside broccoli plants. The humic acid were conducted out by dissolving it in a tap water to make a liquid humic acid solution and added at the rate of 200 ml plant<sup>-1</sup> before 1<sup>st</sup> irrigation then two times later at 15 and 30 days after transplanting. Control, sprayed plants with tap water at the same time of antitranspirants application was used.

The total numbers of plots were 36 plots. The plot area was 14.4 m<sup>2</sup> (3 ridges, each 6 m length and 0.8 m width). The transplants were sown on 22<sup>th</sup> September in both

seasons. Plant distances were 60 cm apart. A distance of 2 m was left between each two irrigation treatments to avoid the overlapping infiltration of irrigation or spraying solutions. Irrigation by flooding was used from the beginning to the end of the two seasons. The other agricultural practices were carried out according to the recommendation of Ministry of Agriculture.

## 3. Data and measurements

### Vegetative growth characteristics

Representative samples of three plants were taken randomly from each treatment after 71 days from transplanting and the vegetative growth parameters of plant height, number of leaves per plant, and fresh and dry weights per plant were recorded. Leaves area /plant were calculated according to the formula described by Koller (1972) as follows:

$$\text{Total leaves area} = \frac{\text{Area of the disks (cm}^2\text{)} \times \text{Fresh weight of leaves/plant (g)}}{\text{Fresh weight of leaves (g)}}$$

### Total heads yield:

At harvest time, 72 days after planting, the total heads yield / feddan, total main head yield / plot and lateral heads yield / plot were recorded. Marketable yield / feddan were recorded using good shapes healthy and green florets.

### Chemical analysis of plant and quality:

Chemical analyses were performed after harvest and after storage for one month. Samples were taken from recently expanded leaves and heads of each plot. In order to determine the antioxidant contents of heads, heads samples were oven-dried at 70°C for 48 h and then ground. Total Chlorophylls (a + b) were determined in fresh leaves and pods as described by Goodwine (1965). Vitamin C was determined in juice using 2,6-dichlorophenol indophenols dye according to A.O.A.C. (1990). Protein content was determined as total nitrogen multiplied by 6.25 to calculated total crude protein. Total phenolics content (TPC) was determined as a method described by Chaovanalikit and Wrolstad (2004). Total flavonoids content (TFC) was measured by a spectrophotometric, method was used for TFC measurement according to Sultana *et al.*, 2009. Total anthocyanin (TA) was examined according to Masukasu *et al.* (2003).

### Water use efficiency (WUE)

Water use efficiency WUE is defined as crop yield divided by irrigation water used to produce the total yield (Begg and Turner, 1976). Thus, WUE as a fresh head weight (kg) obtained per unit volume of irrigation water quantity as follows:

$$\text{Water use efficiency} = \frac{\text{Head yield (kgfed}^{-1}\text{)}}{\text{Irrigation water quantity (m}^3\text{fed}^{-1}\text{)}} = \text{kg/m}^3$$

**Statistical analysis**

The data were statistically analyzed as split plot design according to the procedure described by Snedecor and Cochran (1982). Comparisons among means of treatments were tested using Duncan multiple range test.

**RESULTS AND DISCUSSION**

**1. Effect of irrigation water quantities, foliar application with antitranspirants and humic acid as soil amendments and their interactions on vegetative growth characters of broccoli plants.**

The vegetative growth characters of broccoli were significantly influenced by water quantities in both seasons. The highest water quantity (2400.40 m<sup>3</sup>/fed) or medium level (1600.73 m<sup>3</sup>/fed) had significant increases in

all vegetative growth characters without any significant differences between them, compared to the lowest level or water stress (800.34 m<sup>3</sup>/fed) in both seasons (Table 2). Moreover, the application of Kaolin or humic acid had higher effects on vegetative growth parameters compared to control (Table 2). The interaction between irrigation quantity and reducing water requirements treatments had significant effects on growth parameters in both season of study (Table 2). The second level of water irrigation (1600.73 m<sup>3</sup>/fed) or the first level (2400.40 m<sup>3</sup>/fed) with application of kaolin or humic acid had the most significant effect of plant height, number of leaves/plant, dry weight/plant, leaves area/plant, without significant differences between them.

**Table 2. Vegetative growth of broccoli plants as affected by water quantities, antitranspirants, humic acid and their interactions in 2014/15 and 2015/16 seasons.**

Treatments		Plant height (cm)		Number of leaves/plant		Fresh weight /plant (g)		Dry weight /plant (g)		Leaves area /plant (m <sup>2</sup> )	
Irrigation quantities (m <sup>3</sup> )	Antitranspirants and humates	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
		/15	/16	/15	/16	/15	/16	/15	/16	/15	/16
2400.40 m <sup>3</sup>		70.18 A	71.67 A	19.65 A	21.97 A	2373.36 A	2375.72 A	330.45 A	335.24 A	396.32 A	401.16 A
1600.73 m <sup>3</sup>		70.16 A	70.17 A	19.34 A	24.03 A	2393.33 A	2395.58 A	331.18 A	336.92 A	398.81 A	402.39 A
800.34 m <sup>3</sup>		67.46 B	65.33 B	18.33 B	18.57 B	1831.07 B	1834 B	315.33 B	315.51 B	367.28 B	367.75 B
	Control (tap water)	61.00 C	62.11 C	16.90 C	18.25 C	1950.44 C	1953.22 C	283.53 D	285.08 C	321.88 C	323.88 C
	Kaolin	73.88 A	74.89 A	20.26 A	23.42 A	2321.55 A	2325.11 A	347.84 A	353.37 A	417.44 A	402.78 A
	Calcium carbonate	69.55 B	67.78 B	18.47 B	21.28 A	2109.55 B	2111.11 A	329.12 C	332.41 C	394.91 B	396.44 B
	Humic acid	72.33 A	73.44 A	20.65 A	23.15 A	2355.22 A	2357.44 A	340.44 B	343.84 B	412.44 A	416.35 A
2400.40	Control (tap water)	63.67 d	65.00 d	17.83 ef	17.76 ef	2164.00 bc	2166.66 bc	297.33 f	298.35 f	356.15 e	362.82 e
	Kaolin	74.00 a	76.00 a	20.89 ab	24.08 a	2535.66 b	2539 a	351.00 a	360.51 a	421.21 a	424.88 a
	Calcium carbonate	69.67 c	69.33 c	19.05 cd	22.05 bc	2302.00 b	2303.66 b	329.67 de	329.53 de	395.21 c	396.80 cd
	Humic acid	75.00 a	76.33 a	21.50 a	24.00 a	2551.00 a	2552.5 a	350.5 a	361.22 a	420.50 a	429.66 a
1600.73	Control (tap water)	61.00 ef	60.00 f	17.59 f	21.00 c	2163.33 bc	2166 bc	292.66 f	296.29 f	351.15 e	351.15 f
	Kaolin	74.33 a	75.67 a	20.55 b	25.85 a	2553.66 a	2557 a	351.86 a	358.95 a	422.39 a	428.72 a
	Calcium carbonate	72.33 abc	71.00 bc	17.85 ef	23.80 b	2306.66 b	230766 b	335.85 cd	345.85 b	403.03 bc	406.29 bc
	Humic acid	73.00 ab	74.00 ab	21.36 ab	25.47 a	2549.66 a	2551.66 a	344.33 ab	346.59 b	418.66 a	423.38 a
800.34	Control (tap water)	58.33 f	55.33 i	15.33 g	16.00 f	1524.00 e	1527 e	260.60 g	260.60 g	258.33 f	257.66 g
	Kaolin	73.33 ab	73.00 abc	19.33 cd	20.33 c	1875.33 d	1879.33 d	340.66 bc	340.66 bc	408.74 b	408.74 bc
	Calcium carbonate	66.67 d	63.00 ef	18.52 de	18.00 d	1720.00 d	1722 d	321.84 e	321.84 e	386.23 d	306.33 bc
	Humic acid	70.33 bc	70.00 c	19.70 c	19.98 cd	2111.5 c	2114.5 c	332.5 d	333.10 cd	403.68 bc	404.43 bc

Means of each column for every separate factor and interaction followed with the same letters are not significantly different according to Duncan multiple range test at the probability of 0.05 levels

The increasing of water quantity applied to broccoli plants led to keep highest moisture content in the soil and this in turn might favored the enhances nutrient availability which improves nitrogen and other macro- and micro-elements absorption, plant metabolism and photosynthetic pigments (Table 3) that lead to increase the plant growth characters and to produce higher dry matter (Table 2). This finding is in agreement with Hussain *et al.* (2016) who reported that the higher vegetative growth and total yield of broccoli was observed from 15 days interval irrigation. The saved water, by application of deficient irrigation strategy, can be used for other purposes or to irrigate extra lands. Application of a deficient irrigation strategy put crop plants under water stress (drought), adversely affecting plant growth and physiological processes (Table 2 and 3; Loutfy *et al.*, 2012; Hussain *et al.*, 2016). It has been reported that responses of crop plants to drought depend on species and genotype, length and severity of water stresses, age and development stage, and perhaps on exogenous application of antitranspirants or humic acid for

stressed plants (Selim and Mosa, 2012). Moreover, these results may be due to the role of humic acid to keep more water content in plant tissue, and this in turn led to enhance the growth rate photosynthesis and enzymes activities that finally led to increase in dry matter and leaf area (Table 2; Bahawireth, 2011; Selim and Mosa, 2012; Yilmaz, 2013). On the other hand, Marschner (1995) reported that, under sufficient water conditions, there were decrease in abscisic acid (ABA) and increase in cytokinin (CYT), gibberellins (GA) and indole-3-acetic acid (IAA) reflecting good growth and dry matter content.

**2. Effect of irrigation water quantities, foliar application with antitranspirants and humic acid as soil amendments and their interactions on total head yield and yield components of broccoli plants.**

The low level of irrigation water quantity (800.34 m<sup>3</sup>) and medium level (1600.73 m<sup>3</sup>) with Kaolin had a significant effect on chlorophyll content in comparison with other treatments. No significant differences were found in

chlorophyll content, central and lateral head weights/plot and total head yield per plot and feddan between water quantity of 1600.73 and 2400.40 m<sup>3</sup>/fed in both seasons (Table 3). Application of humic acid or Kaolin was significantly

increased in chlorophyll content, central, lateral and total head yield per plot and feddan compared with the control (tap water) in both seasons (Table 3).

**Table 3. Total chlorophyll, total yield and yield components of broccoli plants as affected by water quantities, antitranspirants, humic acid and their interactions in 2014/15 and 2015/16 seasons.**

Irrigation quantities (m <sup>3</sup> )	Antitranspirants and humates	Total Chlorophyll in heads (mg/100 g F.W.)		Central head weight (Kg/plot)		Lateral head weight (Kg/plot)		Total head yield (Kg/plot)		Total head yield (ton/fed.)	
		2014 /15	2015 /16	2014 /15	2015 /16	2014 /15	2015 /16	2014 /15	2015 /16	2014 /15	2015 /16
2400.40	Control (tap water)	1.40 h	1.42 f	19.56 g	18.88 e	25.15 e	35.38 f	44.71 e	54.26 c	13.042 e	15.828 c
	Kaolin	1.90 cd	1.83 bc	22.19 ab	22.38 a	52.47 a	50.00 ab	74.56 a	72.38 a	21.774 a	21.113 a
	Calcium carbonate	1.79 de	1.85bc	20.65 d	20.00 c	46.49 b	47.72 cd	67.14 b	67.72 b	19.583 b	19.754 b
	Humic acid	1.80 de	1.61 de	22.13 b	21.10 b	52.36 a	48.50 b	74.49 a	69.60 ab	21.726 a	20.302 ab
1600.73	Control (tap water)	1.52 g	1.57 de	19.21 h	18.63 f	25.21 e	30.88 g	44.42 e	49.51 de	12.960 e	14.442 cd
	Kaolin	2.30 a	2.06 a	22.41 a	22.52 a	52.62 a	50.40 a	75.03 a	72.92 a	21.899 a	21.271a
	Calcium carbonate	1.99 c	1.92 ab	21.61 c	21.00 b	46.44 b	48.12 bc	68.06 b	69.12 ab	19.855 b	20.162 ab
	Humic acid	2.28 a	2.04 a	22.20 ab	22.41 a	52.25 a	50.62 a	74.45 a	73.03 a	21.711 a	21.303 a
800.34	Control (tap water)	1.60 f	1.61 de	18.36 i	18.00 g	22.14 f	27.55 h	40.50 f	45.55 e	11.812 f	13.287 d
	Kaolin	2.32 a	2.10 a	19.98 f	18.77 ef	30.79 c	47.32 d	50.77 c	66.09 b	14.814 c	19.278 b
	Calcium carbonate	2.14 b	1.84 bc	19.76 fg	19.14 d	27.62 d	40.22 e	47.38 d	59.63 bc	13.822 d	17.394 bc
	Humic acid	2.22 ab	2.22 a	20.27 e	19.00 de	30.07 c	47.34 d	50.34 c	66.34 b	14.683 c	19.351 b
2400.40 m <sup>3</sup>		1.72 B	1.67 C	21.13 A	20.59 A	44.12 A	45.40 A	65.25 A	65.99 A	19.03 B	19.249 A
1600.73 m <sup>3</sup>		2.02 A	1.89 B	21.36 A	21.14 A	44.14 A	45.01 A	65.50 A	66.15 A	19.10 A	19.294 A
800.34 m <sup>3</sup>		2.07 A	1.99 A	19.59 B	18.73 B	27.65 B	40.61 B	47.24 B	59.40 B	13.78 C	17.330 B
	Control (tap water)	1.50 C	1.53 B	19.04 C	18.50 C	24.17 C	31.27 C	43.21 C	49.77 C	12.60 C	14.52 C
	Kaolin	2.17 A	1.99 A	21.53 A	21.22 A	45.30 A	49.24 A	66.83 A	70.46 A	19.49 A	20.55 A
	Calcium carbonate	1.90 B	1.95 A	20.67 B	20.04 B	40.18 B	45.35 C	60.85 B	67.64 B	17.75 B	18.81 B
	Humic acid	2.10 A	1.95 A	21.53 A	20.83	44.89 A	48.82 A	65.42 A	69.66 A	19.37 A	19.93 A

Means of each column for every separate factor and interaction followed with the same letters are not significantly different according to Duncan multiple range test at the probability of 0.05 levels

The interaction between water quantities and antitranspirants and humic acid had significant effects on central and lateral head weights/plot, total head yield/plot and total head yield/fed in both seasons (Table 3). highest results for these parameters were appeared from the application of Kaolin or humic acid under 1600.73 m<sup>3</sup>/fed irrigation water quantity treatments.

It could be suggest that adding sufficient water + reducing water requirements treatments like humic acid markedly favored the total head yield via its favorable effect on growth of plant, as it is well known that water plays great role and has important functions in all physiological processes starting from minerals absorption from the soil up to build different components inside the plant and finally the yield is the sum of plant growth and development (Table 2) and its availability to store foods in their storage organs; i.e., heads (Table 4). These results were in agreement with those Selim and Mosa, (2012), Hussain *et al.* (2016) and Ramadan and Omar (2017).

There is a few information available on the effect of exogenous Kaolin on broccoli growth under drought conditions. In the present study, the exogenous application of Kaolin found to help to minimize the deleterious effects of drought stress in broccoli plants. It was noticed in this study that minimum level of irrigation water quantity (800.34 m<sup>3</sup>/fed) significantly decreased growth characteristics and head yield of broccoli plants (Tables 2 and 3) which was observed by Hussain *et al.* (2016) that may be attributed to a severe damage in plant growth under

drought conditions. In a report, Boutraa (2010) reported that water stress conditions cause a multitude of molecular, biochemical and physiological changes, adversely impacting in plant growth and development. The decline in plant growth in response to drought stress might be attributed to reductions in cell elongation due to inhibition of growth-promoting hormones, leading to decrease in cell turgor, volume and eventually limitations of photosynthesis, growth and yield (Tezara *et al.*, 2005; Hussain *et al.*, 2016). On the other hand, the improve of water quantity applied to plant led to increase in fresh and dry weight of broccoli, and this lead to significant increase on leaves number, dry and fresh weight of plant, and these increments led to favorable role in production of broccoli. The obtained results are in good harmony with those of (Kurunç and Unlukara, 2009) on okra and Ezzat *et al.* (2015) on Jerusalem artichoke plants. The exogenous application of Kaolin led to form a layer on the foliage surface, which in turn decreased transpiration rate, and hence led to keep more water in plant tissues that would reflect favorable effect on plant metabolism, photosynthetic rate and increased outward transportation of photosynthesis from the foliage to the tubers. These results are in harmony with Lipe (1979), Anwar (2005) and Ezzat *et al.*, (2011) they found that the use of antitranspirants of potato plants during the tuber enlargement reduces plant water use and increase the yield of larger potatoes. Similar results were found by Suryanarana and Venkateswarlu (1981) on tomato, and Gawish and Fattahallah (1997) on taro.

**Table 4. Antioxidants content of broccoli heads as affected by water quantities, antitranspirants, humic acid and their interactions in 2014/15 and 2015/16 seasons.**

Treatments		Vitamin C		Total anthocyanin		Total phenolic content (mg GAE/100 g F.W.)		Total flavonoids content		Crude protein	
		(mg/100 g F.W.)	(mg/100 g F.W.)	(mg/100 g F.W.)	(mg/100 g F.W.)	(mg/100 g F.W.)	(mg/100 g F.W.)	(g/ 100 g D.W.)	(g/ 100 g D.W.)		
Irrigation quantities (m <sup>3</sup> )	Antitranspirants and humates	2014 /15	2015 /16	2014 /15	2015 /16	2014 /15	2015 /16	2014 /15	2015 /16	2014 /15	2015 /16
2400.40	Control (tap water)	80.13 k	74.43 h	7.15 i	7.45 h	72.19 k	75.50 k	81.00 l	83.1 l	1.57 f	2.30 f
	Kaolin	89.22 g	91.00 f	9.97 f	10.17 e	85.30 f	84.60 de	95.32 g	91.30 h	2.85 a	2.95 a
	Calcium carbonate	85.81 i	88.13 g	9.40 g	9.70 f	82.33 h	82.83 ef	89.50 i	89.80 i	2.76 b	2.74 bc
	Humic acid	87.86 h	88.16 g	9.50 g	9.80 f	84.25 g	82.56 ef	91.05 h	95.30 g	2.83 a	2.82 b
1600.73	Control (tap water)	83.74 j	84.03 j	8.42 h	8.72 g	75.31 j	76.60 jk	87.23 k	87.30 k	1.10 g	2.22 fg
	Kaolin	95.61 cd	95.60 d	11.31 d	11.60 c	87.33 d	87.33 c	102.40 d	99.30 e	2.75 b	2.89 ab
	Calcium carbonate	91.06 ef	92.36 e	10.14 f	10.46 e	86.24 e	85.56 cd	98.22 f	98.30 f	2.63 c	2.63 c
	Humic acid	92.03 e	92.33 e	10.62 e	10.93 d	86.31 e	85.60 cd	99.12 e	101.30 d	2.67 c	2.93 a
800.34	Control (tap water)	85.32 i	87.76 i	8.45 h	8.75 g	78.68 i	80.80 f	88.10 j	88.30 j	1.08 g	2.00 g
	Kaolin	98.20 a	98.50 a	12.70 a	13.00 a	91.78 a	92.10 a	112.44 a	112.36 a	1.98 d	2.52 d
	Calcium carbonate	96.53 bc	96.83 c	11.74 c	12.06 b	89.20 c	89.50 b	103.45 c	107.33 b	1.82 e	2.41 e
	Humic acid	97.00 b	98.00 b	12.07 b	12.36 b	90.40 b	91.70 a	107.21 b	103.10 c	1.85 e	2.34 ef
2400.40 m <sup>3</sup>		85.75 C	85.43 C	9.00 C	9.28 C	81.02 C	81.7 C	89.12 C	89.87 C	2.50 A	2.70 A
1600.73 m <sup>3</sup>		90.61 B	91.08 B	10.12 B	10.43 B	83.79 B	83.77 B	96.50 B	96.55 B	2.28 B	2.66 B
800.34 m <sup>3</sup>		94.26 A	95.27 A	11.24 A	11.54 A	87.51 A	88.52 A	102.50 A	102.77 A	1.68 C	2.31 C
	Control (tap water)	83.06 D	82.07 D	8.00 D	8.30 D	75.39 D	77.63 D	85.33 D	86.23 C	1.25 D	2.17 D
	Kaolin	94.34 A	95.03 A	11.32 A	11.59 A	88.14 A	88.01 A	103.25 A	100.98 A	2.52 A	2.78 A
	Calcium carbonate	91.13 C	92.44 C	10.42 C	10.74 C	85.93 C	85.96 C	96.83 C	100.31 A	2.40 C	2.59 C
	Humic acid	92.29 B	92.99 B	10.73 B	11.03 B	86.99 B	86.62 B	99.23 B	98.48 B	2.45 B	2.69 B

Means of each column for every separate factor and interaction followed with the same letters are not significantly different according to Duncan multiple range test at the probability of 0.05 levels

The beneficial effect of humic acid on studied morphological and yield characters may be attributed to its role in increase of availability of elements and water holding capacity, consequently increasing their absorption by plant and transfer to the storage part (Table 4). The role of humic acid in increasing broccoli yield and quality parameters could be attributed to direct or indirect effects on plant growth. Concerning the real effects, it has been demonstrated that humic acid can induce an increase in the root surface by affecting root morphology (Schmidt *et al.*, 2007). Canellas *et al.* (2008) founded that humic acid affected organic acid exudation by root media led to changes in root area, primary root length, number of lateral roots, and lateral-root density. Furthermore, it has been shown that humic acid enhanced the respiration rate of plants (Nardi *et al.*, 2002).

**3. Effect of irrigation water quantities, foliar application with antitranspirants and humic acid as soil amendments and their interactions on antioxidants content of broccoli heads.**

Regarding antioxidant content and crude protein, they were significantly affected by irrigation water quantity as shown in Table 4. The low level of irrigation water (800.34 m<sup>3</sup>) significantly increased the antioxidants content and decreased protein content in both seasons. Application of kaolin was significantly increased in all antioxidants and protein content in both seasons in comparison with other treatments (Table 4).

The lowest level of irrigation water quantity (800.34 m<sup>3</sup>/fed) with kaolin application had significant increases in vitamin C, anthocyanin, phenolic compounds and total flavonoids content. On the other hand, the application of

high level of irrigation water quantity (2400.40 m<sup>3</sup>) and kaolin antitranspirant had a significant effect on protein content, in both seasons (Table 4).

Phenolic compounds are divided in two major groups, non-flavonoid phenolics, and flavonoids (reviewed by Teixeira *et al.*, 2013). Two major secondary metabolic biochemical pathways underlie the synthesis of a wide range of important phenolic and flavonoid compounds, including anthocyanin. They found performed a thorough molecular and biochemical analysis to assess how spray application of Kaolin influences major secondary metabolism pathways associated with berry quality-traits, leading to biosynthesis of phenolics and anthocyanins, with a focus on the phenylpropanoid, flavonoid (both flavonol- and anthocyanin-biosynthetic) and stilbenoid pathways (Dinis *et al.*, 2016). Kaolin-treated vines demonstrated generally enhanced phenolic-biosynthetic molecular mechanisms that ultimately resulted in higher concentration of phenolics, including anthocyanin. These metabolic pathways are tightly associated with berry quality, and better grape berry quality translates into better wine quality, so, into added value to the winemaking industry, as these compounds are responsible for wine organoleptic properties, like color, flavor, astringency, and bitterness. The conjugation of kaolin application with other mitigation strategies based on viticultural practices or the application of other protective compounds with same characteristics can also be potentially explored in the future (Conde *et al.*, 2016).

The increasing water quantity applied to plants, the antioxidant decreased because both of free and total water in the leaf tissues were higher under the highest water quantity level (2400 m<sup>3</sup>/fed) (Ezzat *et al.*, 2009) followed by

(1600.73 m<sup>3</sup> water/fed) Similar results are obtained by Hussein *et al.* (2011). Crude protein of broccoli was lower at water stress during both seasons compared with normal irrigation because water dificate is one of the most important limitations to photosynthesis (Tezara *et al.*, 2005).

Concerning antitranspirants which are biodegradable organic film formulated to protect plants from injury of shock caused by excessive transpiration of water stress through different vegetative plant organs, consequently enhancing the vegetative growth (Table 2). This positive effect on vegetative growth has reflection on total head yield and its quantity (Tables 3 and 4). Many other investigators had results which in good harmony with those obtained here Abd El-Aal-Faten *et al.*, (2008). The obtained results are in agreement with the most previous investigation which pointed out the similar direct correlation between antitranspirants materials and some elemental nutrition in tissues of plant (Moftah, 1997; Yadov and Dashora, 2003; Al-Moftah *et al.*, 2005).

Additionally, there is real role of humic acid in enhancing the stability of soil aggregates and in reducing the disaggregating impact of wetting-and-drying cycles on soil structure. The formation of these aggregates was explained in terms of the formation of clay-humic complexes through bridging polyvalent cations adsorbed on clay surfaces (Table 1) (Piccolo and Mbagwu, 1994). Moreover, humic acid, its favorable effect on antioxidant content of heads might be referred to the increase in soil micro-organisms which enhance nutrient cycling, especially in adequate amount of

water (Sayed *et al.*, 2007), and reduced soil pH (Osman and Ewees, 2008; Osman and Rady, 2012), thus increased the availability of nutrients to be absorbed by plant roots. Humic acid also promotes plant growth through its effect on ion transfer at the root level by activating the oxidation reduction state of the medium and improving the absorption of nutrients by preventing their precipitation in the nutrient solution. In addition, humic would act as chelating agent, through -OH and -COOH as active groups for micronutrients and water molecules (Sayed *et al.*, 2007).

**4. Effect of irrigation water quantities, foliar application with antitranspirants and humic acid as soil amendments and their interactions on water use efficiency of broccoli plants.**

The interactions between water quantity and reducing irrigation water substances, the results in Table 5 show significant effect among the different combinations. The highest value of water use efficiency 18.34 and 24.17 was obtained in plants grown under the lowest level of irrigation (800 m<sup>3</sup>/fed) along with application of humic acids in both seasons, respectively.

Regarding the effect of irrigation water quantity on water use efficiency WUE the results presented in Table 5 show that, the best value of water use efficiency was obtained under the lowest water quantity (800 m<sup>3</sup>/fed) compared to 1600 and 2400 m<sup>3</sup>/fed, in both seasons of study. Kaolin and humic acid had a significant effect on WUE compared with calcium carbonate and control treatments, in two seasons (Table 5).

**Table 5. Water use efficiency of broccoli heads as affected by water quantities, antitranspirants, humic acid and their interactions in 2014/15 and 2015/16 seasons.**

Irrigation quantities (m <sup>3</sup> )	Treatments	Irrigation No.		Water quantity at 1 <sup>st</sup> irrigation (m <sup>3</sup> /fed.)		Seasonal rainfall (mm)		Soil water contribution (mm)		Seasonal applied (m <sup>3</sup> /fed.)		Water Use Efficiency WUE (kg/m <sup>3</sup> )	
		2014 /15	2015 /16	2014 /15	2015 /16	2014 /15	2015 /16	2014 /15	2015 /16	2014 /15	2015 /16	2014 /15	2015 /16
2400.40	Control (tap water)							30.55	32.14			5.433 d	6.594 f
	Kaolin	7	7					41.26	40.25	2450.07	2435.28	9.071 c	8.796 e
	Calcium carbonate							35.66	34.78			8.158 c	8.229 e
	Humic acid							52.16	50.18			9.051 c	8.458 e
1600.73	Control (tap water)							28.44	28.22			8.096 c	9.022 e
	Kaolin	5	5	250.32	265.11	12.6	8.5	38.25	37.78	1650.40	1635.60	13.681 b	13.288 d
	Calcium carbonate							33.18	31.11			12.404 b	12.596 d
	Humic acid							48.26	46.36			13.563 b	13.308 d
800.34	Control (tap water)							22.45	20.14			14.759 b	16.602 c
	Kaolin	3	3					35.28	35.33	850.01	835.21	18.510 a	24.087 a
	Calcium carbonate							30.11	30.35			17.270 a	21.733 b
	Humic acid							42.36	43.14			18.346 a	24.178 a
2400.40 m <sup>3</sup>		--	--	--	--	--	--	39.91	39.33	--	--	7.928 C	8.0192 C
1600.73 m <sup>3</sup>		--	--	--	--	--	--	37.03	35.86	--	--	11.936 B	12.053 B
800.34 m <sup>3</sup>		--	--	--	--	--	--	32.55	32.24	--	--	17.221 A	21.650 A
	Control (tap water)	--	--	--	--	--	--	27.14	26.83	--	--	9.420 C	10.739 C
	Kaolin	--	--	--	--	--	--	38.26	37.78	--	--	13.754 A	15.390 A
	Calcium carbonate	--	--	--	--	--	--	32.98	32.08	--	--	12.611 B	14.186 B
	Humic acid	--	--	--	--	--	--	47.59	46.56	--	--	13.653 A	15.314 A

Means of each column for every separate factor and interaction followed with the same letters are not significantly different according to Duncan multiple range test at the probability of 0.05 levels

The value of WUE gradually decreased with improving water quantity up to the highest level and showed opposite trend to that of total yield (Table 3).

The results are in accordance with those obtained by Anwar (2005), Youssef (2007) and Ezzat (2009) on potato. They found that, the efficiency of water use was increased by applying deficit water irrigation.



On the basis of the forced results, it could be recommended that irrigation of broccoli plants with 1600.73 m<sup>3</sup>/fed with application of humic acid or spray with Kaolin "antitranspirant" was the most efficient treatment for growth, yield, quality and WUE of broccoli plants grown in clay loam soil (Dakahlia Governorate).

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## استجابة البروكلي لكميات مياه الري والرش الورقي بمضادات النتج وحمض الهيومك كإضافة أرضية على المحصول والجودة وبعض مضادات الأكسدة

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أجريت تجربتان حقليتان بمحطة بحوث البرامون، المنصورة - محافظة الدقهلية، مصر خلال الموسمان الشتويان 2015 و 2016 لدراسة تأثير ثلاث كميات من مياه الري (800.34، 1600.73 و 2400.40 م<sup>3</sup> / فدان كعمالة شائعة الاستخدام) وبعض مضادات النتج مثل محلول الكاولين 4% في صورة سيلكات الألومونيوم، كربونات الكالسيوم (6%) وكذلك حمض الهيومك في صورة هيومات البوتاسيوم (80% حمض هيومك، 11-13% بوراً) بالإضافة إلى التفاعل بينهما وتأثير ذلك على النمو والإنتاج والجودة وبعض مضادات الأكسدة على البروكلي. أدى استخدام المستوى الثاني من الري المائي (1600.73 م<sup>3</sup> / فدان) مع استخدام الكاولين أو حمض الهيومك إلى تأثير معنوي في ارتفاع النبات، عدد الأوراق / النبات، الوزن الجاف / النبات، المساحة الورقية / النبات، متوسط وزن الرؤوس الرئيسية والجانبية، ومحصول الرؤوس الرئيسية والجانبية والمحصول الكلي للرؤوس بالنسبة للفدان في كلا الموسمين من الدراسة، دون اختلافات كبيرة بينهما. أدى زيادة استخدام مياه الري من 800.34 إلى 2400.40 م<sup>3</sup>/فدان إلى زيادة معنوية في صفات النمو الخضري في كلا الموسمين. لم تسجل أي اختلافات معنوية في صفات النمو الخضري والمحصول بين كميات مياه الري (1600.73 و 2400.40 م<sup>3</sup> / فدان) في كلا الموسمين. أدى لإضافة الكاولين أو استخدام حمض الهيومك إلى زيادة معنوية في معظم الصفات الخضرية والمحصول مقارنة بالكمية التي استخدمت. أدى استخدام مضاد النتج الكاولين إلى زيادة معنوية في المواد المضادة للأكسدة (فيتامين ج، الإنثوسيانين، المركبات الفينولية، الفلافونيد). ومن ناحية أخرى أدى الري النباتيات بالمستوى الأعلى (2400 م<sup>3</sup> / فدان) إلى زيادة معنوية في محتوى البروتين في كلا الموسمين. أدى استخدام المستوى المنخفض من مياه الري (800 م<sup>3</sup>) إلى زيادة محتوى المواد المضادة للأكسدة، ونقص محتواها من البروتين، كما أدى استخدام الكاولين إلى زيادة المواد المضادة للأكسدة والبروتين في كلا الموسمين مقارنة بالمعاملات الأخرى. بصفة عامة أدى استخدام الكاولين أو حمض الهيومك تحت كمية مياه الري بمعدل 1600.73 م<sup>3</sup> / فدان كأفضل المعاملات لإنتاج مثالي من محصول البروكلي والذي يهدف إلى تحقيق أقصى قدر من كفاءة استخدام المياه تحت ظروف هذه الدراسة. وهذا بدوره يشجع استخدام هذه الإضافات للحد من استهلاك المياه بنسبة 33.3%.