

The beneficial effects of bio-fertilizers combinations and humic acid on growth, yield parameters and nitrogen content of broccoli grown under drip irrigation system

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Abstract

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Using the organic materials such as humic acid and Bactrian fertilizer has been reported positively effect on soil health and enhancing the productivity of broccoli in open file condition under drip irrigation. Developing a suitable strategy for combination of the humic acid and bio-fertilizer mixed for agricultural application under open filed is important for enhancing soil fertility, productivity and reducing the use of heavy inorganic fertilizer for higher production. The aim of this study was determining the effect of Bactrian fertilizer (*Pseudomonas*, *Bacillus*, *Azospirillum* (and humic acid on growth and yield of broccoli (*Brassica oleracea* L. var. *Italica*) under drip irrigation and determine the ideal combination of both organic materials (humic acid and bio-fertilizer mixed) in soil condition during the experiment periods 1/10/2016 to 1/2/2017. Randomized complete block design designing the experiment; the first factor was Bactrian fertilizers with three levels (0, 10, 20 mg/L), while the second factor was humic acid with three levels (0, 5, 10 ml/L). The results showed a significant difference in combinations between bio fertilizers mixed and humic acid applications on vegetative yield parameters, the best treatments was interaction bio fertilizer and humic acid (T9 and T6) for plant height and leaves number. For chlorophyll contents the best interaction was for T9 and T5. While, the best treatment for yield parameters such as for head diameter and circumference was T3. For nitrogen content the best treatments were T9, T5 and T3, while for protein content T9 was the best treatment for higher content of crudes. It was concluded that the treatments with high concentration of Bactrian mixed have highest value for yield parameters, protein content as well as nitrogen.

Keywords: broccoli; *Brassica oleracea* L.; bio stimulators; humic acid; organic fertilizer; nutrition

Introduction

The agriculture sectors used high technology such as soilless culture, hydroponic, aquaponics for producing veg-

etables and herbs (Al-Tawaha et al., 2016; Edaroyati et al., 2017; Al-Tawaha et al., 2018a; Makhadmeh et al., 2007; Jones, 2005; Raviv & Lieth, 2008; Resh, 2013). But to overcome the soil problem the researchers are still looking for

clean technique characteristics for sustainable and environmental systems in modern agriculture to increase the production of vegetables such as broccoli in soil fields with reduced inputs of fertilizers (e.g. inorganic fertilizer) through preparing of programs focused on product quality and sustainability. Nowadays, finding alternative methods for high quality and quantity of broccoli is very important for reducing the high levels of accumulations of nitrates (NO₃⁻) because broccoli is a nitrogen demanding vegetable (Vågen et al., 2007) and other inorganic fertilizers have negative effect on human health (Luo et al. 2006; Parks et al. 2008). Thus, intruding clean techniques such as bio-fertilizer and humic acid are interesting for optimization.

Bio-fertilizer is natural substance which is composed of many strains of bacteria and fungus for decreasing the chemical fertilizers in fertilization applications. In addition, bio-fertilizer has a positive role in helping the plants through contain of microorganisms, which are capable of mobilizing nutrient elements from unavailable form to available form through different biological processes (Ekta et al., 2017). Also, bio fertilizers offer an economically attractive and ecologically sound means of reducing external inputs and improving quality and quantity of vegetable produce. Many investigators reported and approved that the bio-fertilization increased yield of cruciferous vegetables such as broccoli (Selim et al., 2009; Salim et al., 2018; Zaki et al., 2009; El-Nemr et al., 2011, Al-Taey & Majid, 2018).

Broccoli (*Brassica oleracea* L. var. *Italica*) is a member of the crucifer family which is found in the Mediterranean region. Moreover, broccoli is native to the Mediterranean region (Gray, 1982; Yildirim, 2007, Zaki et al, 2012). Sprouting broccoli (*Brassica oleracea* var. *italic*) popularly known as broccoli is an important 28 vegetable crop. Nowadays, the Iraqi agriculture committee introduce broccoli crop to Iraq for its suitability to grow there because it is one of the winter vegetables belonging to the 29 family of *Cruciferae*. Broccoli is more nutritious than any other vegetables of the same 30 genus (Yoldas et al., 2008). It contains 3.3% protein and has 31 high content of vitamin A and C, iron and calcium. The United States Department of Agriculture (USDA) nutrient database (USDA Database, 2018a) reported that the content of 100 g broccoli florets are protein, total lipid, and carbohydrate 2.82, 0.37, and 6.64 g, respectively. In addition, the contents of the minerals Ca, Fe, Mg, P, K, and Zn are 47, 0.73, 21, 66, 316, and 0.41 mg per 100 g broccoli florets, respectively. Furthermore, broccoli, content of vitamin A and C, thiamine, niacin, riboflavin, high concentrations of carotenoids, which are believed to have 33 preventative qualities about human cancer (Michaud et al., 2002). Broccoli in both parts stalks and florets have almost the equivalent nutrient values.

However, there are no studies on the responses of growth and yield parameters in broccoli to the bio-fertilizers combinations and humic acid application under drip irrigation system. The objectives of this study were to determine the effects of different levels of Bactrian fertilizers and humic acid on broccoli plant growth and yield, and the relationships of interaction between bio-fertilizer and humic acid on with drip irrigation on the soil with plant growth and yield.

Material and Methods

Experiment site

This experiment was carried out at the field of the Faculty of Agriculture, University of Al-Qasim Green, Babylon, Iraq, during the period 1/10/2016 to 1/2/2017.

Physical and chemical properties of soil filed

Prior to the start of the experiment random soil samples were obtained from 0-30 cm and analyzed at the Department of Horticulture Faculty of Agriculture to determine physical and chemical properties of the soil (Table 1). The experiment was conducted on soil content: sand 36.3%, silt 33.6% and clay 30.1%. The pH was 7.1 and EC 3.51 ds.m⁻¹.

Table 1. Physical and chemical properties of the soil

Physical properties of soil component	Texture	Percent
Sand		36.3
Silt		33.6
Clay		30.1
Chemical properties of soil component	Units	Value
EC	ds.m ⁻¹	3.51
PH		7.1
Organic matter		1.2

Rainfall, temperature and humidity

The averages of rainfall, temperature and humidity are presented in Table 2. The rainfall during the experiment period from October-December in 2016 was approximately in the range (0.0-28.9 mm) - the rainfall peaks in December (28.9.1 mm), while the lowest occurs in October (0.0 mm). The mean minimum and maximum temperatures range from 25.81°C to 10.19°C during the experiment period, while the average relative humidity ranged from 52.90 to 82.72%.

Table 2. The average of rainfall, temperature and humidity

Rainfall mm	Hm %	HM %	T C°	Tm C°	TM C°	Study period
0.0	12.49	52.90	25.81	16.93	35.72	Oct.
0.7	17.86	63.52	15.60	7.63	24.91	Nov.
28.9	39.43	82.72	10.19	4.24	16.78	Dec.

Experimental treatments

The treatments in this study consisted of nine different humic acid and bio-fertilizer mixed levels during the experiment period from 1/10/2016 to 1/2/2017 under drip irrigation system. A list of treatments is presented in Table 3.

Table 3. The treatments applied for the experiment

Treatments	Symbol	Dose
T1	A0B0	control
T2	A1B0	Bactrian mixed 10 gm/L only
T3	A2B0	Bactrian mixed 20 gm/L only
T4	A0B1	humic acid 5 ml/L only
T5	A1B1	Bactrian mixed 10 gm/L + humic acid 5 ml/L
T6	A2B1	Bactrian mixed 20 gm/L + humic acid 5 ml/L
T7	A0B2	humic acid 10 ml/L only
T8	A1B2	Bactrian mixed 10 gm/L + humic acid 10 ml/L
T9	A2B2	Bactrian mixed 20 gm/L + humic acid 10 ml/L

Experimental design

Randomized complete block design designing the experiment; the first factor was Bactrian fertilizers (0, 10, 20 mg/L), while the second factor was humic acid with three levels (0, 5, 10 ml/L).

Plant materials and irrigation system

Seedlings were developed by placing seed of the cv. Max (select seed. Holland) in cells in seedling trays containing peat moss on 22 Aug. 2016. Tray dimensions were 68×40 cm containing 209 cells. The seedlings were cultured in 1 Oct. 2016. The seedlings sowed inside lines, the distance between them - 40 cm and between the plants - 20 cm, each treatment has 30 plants and 10 plants to each replicate. The field was irrigated by drip irrigation system. The bio-fertilizers were added with peat moss.

Statistical analysis

The results were analyzed by the Statistical Analysis Software (SAS) (Version 9.4) with an analysis of variance (ANOVA) and according to least significant difference (LSD) at 0.05 level significant, the treatments means compared.

Results and Discussion

The results of applications for interactions high concentrations of Bactrian mixed and humic acid have been often recommended as a method to improve crop production in broccoli by enhancing and controlling physiological processes. The main aims of this study is to determine the effect of Bactrian fertilizer (*Pseudomonas*, *Bacillus*, *Azospirillum*) and humic acid on growth and yield of broccoli (*Brassica oleracea* L. var. *Italica*) under drip irrigation condition. Analysis of variance indicated that the investigated effect of Bactrian fertilizer and humic acid for vegetative and yield components were statistically significant ($p \leq 0.05$. LSD). The general review of the results in Table 4 showed the treatments have high concentrations of Bactrian mixed (20 gm/L) with interaction humic acid (5 and 10 ml/L) such as treatment 9 (Bactrian mixed 20 gm/L + Humic acid 10 ml/L), treatment 6 (Bactrian mixed 10 gm/L + Humic acid 10 ml/L) and treatment 8 (Bactrian mixed 20 gm/L + Humic acid 5 ml/L) have high plant height, leaf area, leaves number, stem diameter and dry weight of leaves. Also, treatment 3 with high concentration of Bactrian 20 ml/L has high for plant height, leaf area, leaves number, stem diameter, dry weight of leaves.

Vegetative growth parameters and dry weight of leaves

According to the results that are presented in Table 4, the tallest broccoli was achieved for treatments T6, T9 (37.83,

Table 4. Effect of bio fertilizer mixed and humic acid applications on vegetative growth parameters

Treatments	Plant height (cm)	Leaf area (cm ²)	Leaves number	Stem diameter (mm)	The dry weight percentage of leaves (%)
T1	27.9 ^c	666	13.67 ^e	30.49 ^b	9.517 ^b
T2	33.3 ^b	862	19.33 ^{bcd}	32.48 ^{ab}	9.810 ^{ab}
T3	35.23 ^{ab}	965 ^{a*}	21.67 ^{ab}	43.42 ^{a*}	9.690 ^{ab}
T4	32.73 ^b	723	16.33 ^{cd}	36.21 ^{ab}	9.520 ^b
T5	32.6 ^b	906 ^a	20.33 ^{abc}	37.23 ^{ab}	9.843 ^{ab}
T6	37.83 ^{a*}	1037 ^{a*}	23.33 ^{a*}	38.48 ^{ab}	9.807 ^{ab}
T7	34.2 ^{ab}	749	17.00 ^{cd}	36.56 ^{ab}	9.877 ^{ab}
T8	36.3 ^{ab}	996 ^{a*}	22.33 ^{ab}	40.83 ^{a*}	9.840 ^{ab}
T9	37.73 ^{a*}	1059 ^{a*}	23.67 ^{a*}	38.59 ^{ab}	9.917 ^a
LSD	3.923	160.3	3.472	7.931	0.3952

Mean values followed by the same letter in the same column are not significantly different at $P < 0.05$, based on least significant difference test (LSD)

37.73 cm) followed by T8, T3 and T7 respectively with values (36.3, 35.23 and 34.2 cm). While the highest leaves area was obtained under T9, T6 followed by T8 and T3 (1059, 1037, 996, 965 cm²) respectively. The maximum number of leaves was recorded as (23.67, 23.33) for T9 and T6 followed by (22.33, 21.67) for treatments T8 and T3 respectively as presented in Table 4. However, the highest value of stem diameter was achieved for T3 and T8 (43.42, 40.83) and the lowest stem diameter was achieved at control treatment. However, there is no significant difference in the dry weight of leaves for all treatments.

Chlorophyll contents

The results showed that there are significant differences among the treatments by interaction of bio-fertilizers applications with humic acid in chlorophyll contents. According to Fig. 1, the highest chlorophyll contents in mg/g were achieved under T9, T5 (86.93, 86 mg/g) followed by T6, T8 and T3 with values (85.03, 84.77 and 83.63) respectively. While the lowest chlorophyll contents in mg/g showed are under control. The highest chlorophyll contents were recorded under treatment with high concentrations of Bactrian mixed 20 gm/L + humic acid 10 ml/L and the second treatment was Bactrian mixed 10 gm/L + humic acid 5 ml/L and this result agrees with what author reported.

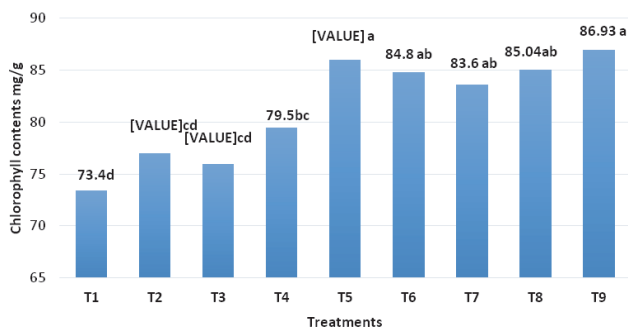


Fig. 1. Effect of biofertilizer and humic acid applications on chlorophyll contents (mg/g); values are expressed as means; bars followed by the same letter are not significantly different at $p \leq 0.05$. LSD; chlorophyll contents = 5.79; T1 - control; T2 - Bactrian mixed 10 gm/L only; T3 - Bactrian mixed 20 gm/L only; T4 - humic acid 5 ml/L only; T5 - Bactrian mixed 10 gm/L + Humic acid 5 ml/L; T6 - Bactrian mixed 20 gm/L + humic acid 5 ml/L; T7 - humic acid 10 ml/L only; T8 - Bactrian mixed 10 gm/L + humic acid 10 ml/L; T9 - Bactrian mixed 20 gm/L + humic acid 10 ml/L; Mean values followed by the same letter in the same column are not significantly different at $P < 0.05$, based on least significant difference test (LSD)

Yield parameters and nitrogen content

The results showed that there are significant differences among the treatments by interaction of bio-fertilizers applications with humic acid in chlorophyll contents, heads diameter, heads weight, heads dry weight, marketable yield while the humic acid achieved a significant difference among the treatments in all of the study parameters.

On the other hand, the results in Table 5 showed that the treatment 3 has highest head diameter (24 cm) and head circumference (67 cm) while the lowest values for both parameters were (14 cm) and (39 cm) respectively under control treatments.

Protein contents

According to Fig. 2, the highest protein content in heads was achieved under T9 (2.7) followed by T5, T3, T8 and T2 with values (2.58, 2.49, 2.48 and 2.36) respectively. While the lowest protein content in heads showed under control and T4.

This study showed a significant indication in vegetative parameters with the bio-fertilizer application in broccoli plants especially in marketable yields which is the most important factor for producers and growers. However, the application of bio-fertilizers (Azotobacter, Azospirillum and phosphorus solubilising bacteria) in concentration ranged

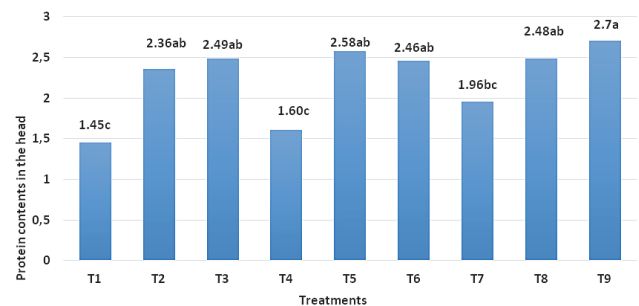


Fig. 2. Effect of biofertilizer and humic acid applications on protein content of heads; values are expressed as means; bars followed by the same letter are not significantly different at $p \leq 0.05$. LSD; chlorophyll contents = 0.723; T1 - control; T2 - Bactrian mixed 10 gm/L only; T3 - Bactrian mixed 20 gm/L only; T4 - humic acid 5 ml/L only; T5 - Bactrian mixed 10 gm/L + humic acid 5 ml/L; T6 - Bactrian mixed 20 gm/L + humic acid 5 ml/L; T7 - humic acid 10 ml/L only; T8 - Bactrian mixed 10 gm/L + humic acid 10 ml/L; T9 - Bactrian mixed 20 gm/L + humic acid 10 ml/L; mean values followed by the same letter in the same column are not significantly different at $P < 0.05$, based on least significant difference test (LSD)

Table 5. Effect of bio fertilizer and humic acid applications on curds and yield parameters

Treatments	Head diameter (cm)	Head circumference (cm)	Head weight (kg)	Dry weight percentage %	Marketable yield t/h	N. contents in heads
T1	14.00 ^d	39.00 ^c	0.469 ^d	9.72 ^b	11.39 ^b	2.23 ^c
T2	17.67 ^{cd}	46.30 ^{cde}	0.674 ^{bc}	10.36 ^{ab}	15.49 ^{ab}	3.43 ^{ab}
T3	24.00 ^{a*}	67.30 ^{a*}	0.798 ^{ab}	10.52 ^{ab}	17.96 ^a	3.70 ^a
T4	16.00 ^{cd}	45.00 ^{cd}	0.615 ^c	10.92 ^a	14.31 ^{ab}	2.45 ^c
T5	18.67 ^{bc}	52.30 ^{bcd}	0.713 ^{abc}	11.01 ^a	16.26 ^{ab}	3.70 ^a
T6	19.67 ^b	57.70 ^{ab}	0.734 ^{abc}	11.00 ^a	16.69 ^a	3.59 ^{ab}
T7	19.00 ^{bc}	55.00 ^{bc}	0.663 ^{bc}	10.70 ^a	15.27 ^{ab}	2.82 ^{bc}
T8	19.33 ^b	54.00 ^{bcd}	0.768 ^{ab}	10.93 ^a	17.36 ^a	3.60 ^{ab}
T9	19.00 ^{bc}	57.70 ^{ab}	0.816 ^a	11.15 ^a	18.31 ^a	3.88 ^a
LSD	3.105	9.73	0.1361	0.827	4.692	0.866

Mean values followed by the same letter in the same column are not significantly different at $P < 0.05$, based on least significant difference test (LSD)

(10-20 gm/L) led to positive effect on utilization of nutrients available in the soil which increased growth and activity of microbial saprophytes which influenced the yield of broccoli/ha. Moreover, Azospirillum particularly play a major role in plant growth and increases the yield of crops by improving root development, mineral uptake. This positive role of these bio-fertilizers must be reported to growers and producers for more attention to decrease the inorganic fertilizer for broccoli and other vegetables. New report by Salim et al (2018) agrees with the result for positive effect of bio-fertilizers Azotobacter chroococcum and Pseudomonas fluorescens on growth of Broccoli (*Brassica oleracea L. var. Italica*) - improved growth performance, nutrition, and increase in plants growth of broccoli and sustain yield. Another study by Manea and Abbas (2018) reported positive effect of bio-fertilizer on growth and yield broccoli. These results agreed with Al-Taey and Majid (2018) for lettuce. Moreover, many authors supported these findings (Singh & Akhilesh, 2000; Sharma, 2000; Sharma et al., 2008). Yadav et al. (2012) showed that the increase in the number of broccoli leaves per plant due to the application of bio-fertilizers (Azotobacter) with a combination with NPK might increase the availability of nutrients through direct addition in the soil. Bhardwaj et al. (2007) reported that application of bio-fertilizer such Azotobacter helps in secretion of substances of growth promotion which leads to uptake and enhance the decomposition of nutrients, transportation of water through root development. Wang and Kale (2004) showed that using Azotobacter + Azospirillum with 150 kg/ha increased significantly broccoli vegetative parameters such as plant height, branches number per plant and fresh yield of leaves and stems as well as dry weight of leaves and stems. The significant increase in plant height, number of leaves per plant, and leaf area were observed due to inoculation of bio-fertilizers. Moreover, the application of Azotobacter improved

nitrogen status of the soil because this is free nitrogen fixer. Application of efficient and healthy strain of Azotobacter in rhizosphere have resulted in greater fixation of atmospheric nitrogen for use by the plant resulting in vigorous growth of plants (Manivannan & Singh, 2004; Yadav et al., 2012).

The results showed significant differences among treatments with high concentration of humic acid and especially for the application achieved highest values in all vegetative parameters these results agreed with Yilmaz et al. (2013) who find increasing in growth parameters with humic acid application in broccoli, similarly with Salt et al. (2001) who found increasing of the yield and quality of various oil seed crops. Lower dose of humic acid is equally effective to the higher levels in increasing plant growth and enhancing the nutrient uptake; humic acid increases chlorophyll content, accelerates the cell metabolism and hormonal growth responses etc. Selim and Mosa (2012) reported that application of humic acid via fertigation increased broccoli yield and quality parameters. By using humic acid, the yield was increased for the NPK 75% was higher than NPK 100% without humic acid (HA), while no significant difference was found between the HA-amended NPK 50% treatment and the unamended NPK 100% treatment. While no statistically significant differences were found for TSS, fertigation with humic acid improved head diameter and protein concentration. Also, in NPK 100% treatment higher values for head diameter, protein, and vitamin C were found compared to NPK 50% treatment. The increase in the productivity of the HA-amended treatments most probably was due to the increase in moisture retention, and the improvement of nutrients supply in the root zone (Selim et al., 2009). The role of humic acids in improving broccoli yield and quality parameters could be attributed to direct or indirect effects on plant growth. Concerning the direct effects, it has been demonstrated that humic acid could induce an increase in the root

surface by affecting root morphology (Schmidt et al., 2007). Regarding the indirect effects of humic acid in improving yield and quality, it was reported that the humic acid application increased soil enzyme activity and promoted the growth of microorganisms in the rhizosphere (Soil- Root interface) (Sellamuthu & Govindaswamy, 2003; Burhan and AL-Taey, 2018). The effects of interaction between bio-fertilizer and humic acid showed a significant difference in all vegetative parameters (Table 3, Table 4, Fig. 1, and Fig. 2). Treatments have high concentration of humic acid (10 ml) with Bactrian mixed 20 gm/L. It is probably due to the fact that humic acid in combination with bio-fertilizers help for better root proliferation and growth which facilitate more uptake of nutrients with water, the higher leaf area and more chlorophyll responsible for improved photosynthesis process and enhanced carbon synthesis, reversely effected in dry weight (Ekta et al., 2017; Ohta et al., 2004; Ramesh et al., 2010; Al-Taey et al, 2017; Rajpar et al., 2011; Mohamed et al., 2009) due to its effect in endorsing plant growth through improving root growth and nutrient uptake (Canellas et al., 2002; Zandonadi et al., 2007). In addition, soil application of humic acid and inoculated cucumber plants with Azotobacter along with 3/4 recommended N chemical fertilizer dose were the best treatment for maximizing the growth, yield and nutritional status of cucumber plants and low NO₃ content in fruits.

Conclusions

Based on this finding it could be concluded that the best treatment on basis of marketable yield and content of nitrogen is form NO₃⁻ with safety rage for human nutrition. However, using the high concentrations of humic acid combined with bio-fertilizers is proposed to modify soil texture, soil structure integrity, aeration and endorsing plant growth through improving root growth and nutrient uptake and this led to positive effect of vegetative growth of the total yield. Therefore, humic acid and bio-fertilizer as a product improves the soil fertility and increases the availability of nutrients to plants and thus it influences enhancing the vegetative growth such as N content, protein content and marketable head biomass.

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