

EFFECT OF POTASSIUM FERTILIZER, BIOSTIMULANTS AND EFFECTIVE MICROORGANISMS AS WELL AS THEIR INTERACTIONS ON POTATO GROWTH, PHOTOSYNTHETIC PIGMENTS AND STEM ANATOMY

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ABSTRACT

Increasing potassium fertilizer rate up to 80 kg K₂O/ fed significantly increased shoot fresh and dry weights, photosynthetic pigments concentration as well as stem anatomical characters. The highest weight was obtained due to application of 40 kg K₂O/ fed in the first and second seasons as compared with untreated plants. Exogenous application of biostimulants, in particular, seaweed extract significantly increased shoot fresh and dry weights, photosynthetic pigment concentration as well as increased the diameter of stem, pith and thickness of collenchyma layers, and xylem tissue as well as the length and width of large vascular bundle beside the thickness of external phloem as compared to control in both seasons. Addition of effective microorganisms to the soil significantly increased shoot fresh and dry weight, photosynthetic pigments concentration as well as the diameter of stem, length and width of vascular bundle, and the thickness of xylem.

As for the interactions, application of biostimulants, in particular, seaweed extract, significantly increased fresh and dry weights of potato shoot, photosynthetic pigments concentration as well as improved anatomical characteristics of potato stem grown under all potassium fertilizer rates with or without an addition of effective microorganisms. The highest value was obtained under the treatment of 40 kg K₂O/fed with addition of effective microorganisms and spraying plants with 500 mg/l seaweed extract as compared with control plant in the first and second growing seasons.

INTRODUCTION

Potato (*Solanum tuberosum* L.) rates fourth among the world's various agricultural products in production volume, after wheat, rice and corn (Fabeiro *et al.*, 2001). It contains high levels of carbohydrates and significant amounts of vitamins B and C and other minerals (Pondey and Chadha, 1996). Potato demands high level of soil nutrients due to relatively poorly developed and shallow root system in relation to yield (Perrenoud, 1993). This high rate of dry matter production results in large amounts of nutrients removed per unit time, which generally most of the soils are not able to supply. Hence, nutrient application from external sources as fertilizers becomes essential. According to Perrenoud (1993), a crop yielding 37 ton ha⁻¹ removes 113 kg N, 45 kg P₂O₅ and 196 kg K₂O ha⁻¹. Potato crop is a heavy remover of soil potassium and is the nutrient taken up in the greatest quantity; the tuber removes 1.5 times as much potassium as nitrogen and 4-5 times the amount of phosphate (Perrenoud, 1993). The importance of potassium (K) fertilization in the Egyptian agriculture has risen since the completion of the High Dam that resulted in the deposition of the suspended Nile silt in the upstream of the

formed lake. This Nile silt enriched the Egyptian soils with K during the seasonal floods (Abd El-Hadi *et al.*, 1997). However, continuous cropping without replenishing nutrients can cause an irreparable damage to soil fertility. A K deficiency may affect respiration, photosynthesis, chlorophyll development, and water content of leaves (Sangakkara *et al.*, 2000). Potassium increases the photosynthetic rates of crop leaves and carbon dioxide (CO₂) assimilation, and facilitates carbon movement (Sangakkara *et al.*, 2000). Potassium nutrition has pronounced effects on carbohydrate partitioning by affecting either phloem export of photosynthates (sucrose) or growth rate of sink and/or source organs (Cakmak *et al.*, 1994). Furthermore, K plays an important role in the translocation of photosynthates from source to sink (Cakmak *et al.*, 1994).

Using biofertilizers in potato production in Egypt to produce safety yield and free of harmful chemicals and toxic materials is well recommended to take place in European market, and to have the consumer who is willing to pay high rate price for healthy safe product. Effective microorganisms' stock solution "EM" is one of many biofertilizers used in this concern. EM as a biofertilizer was first used in Japan by Higa (1995), it contains a group of beneficial microorganisms (primary photosynthetic and lactic acid bacteria, yeast, actinomycetes and fermenting fungi) which are cultured and used for many purposes; a) promotes germination, flowering, fruiting and ripening in plants, b) improves physical, chemical and biological environments of the soil and suppresses soil borne pathogens and pests, c) enhances the photosynthetic capacity of crops. EM can be used by two ways; watering into the soil and foliar spray.

Natural products, which contain phytohormones or exhibit hormone-like activity, have received increasing attention for use as nutrients supplements in agriculture and horticulture (Seadh *et al.*, 2008, Ezzat *et al.*, 2011). Seaweed extracts (SW) (*Ascophyllum nodosum* Jol.) and humic acid (HA) are in common use as major components of vegetable and crop biostimulant formulations. Auxine and cytokinin-like activities of humic acids have been reported (Piccolo *et al.*, 1992). Cytokinins and auxine have been identified and quantified in SW (Sanderson *et al.*, 1987). These natural products have been shown to enhance plant growth under normal or stressed condition (Seadh *et al.*, 2008; Ezzat *et al.*, 2011). Humic acid is considered to increase the permeability of plant membranes and enhance the uptake of nutrients. Moreover, humic acid is also considered to improve soil nitrogen uptake and encourage the uptake of potassium, calcium, magnesium and phosphorus, making these more mobile and available to plant root system (Piccolo *et al.*, 1997; Pascual *et al.*, 1999).

The aim of the present study was to clarify the effect of various biostimulators (Seaweed extract and Humic acid) and effective microorganisms as well as potassium rates on potato growth, photosynthetic pigment concentration and stem anatomy. It is the main hope to find out the most favorable treatments producing more growth and pigment concentration in potato plant. It was further hoped that the results of this work finally may lead to the utilization of one or more of these chemicals for large scale cultivation of potato under field condition.

MATERIALS AND METHODS

Two field experiments were conducted during the two successive winter seasons of 2007/2008 and 2008/2009 at the EL-Maniel village, Dakhlia Governorate to study the effect of effective microorganisms, biostimulants, and potassium levels as well as their combinations on the potato plant growth, photosynthetic pigment concentration and stem anatomy.

Soil samples and analysis:

Twenty surface samples (0-20 cm depth) were taken at ten different locations. The experimental soil was air dried, grounded, mixed and kept in plastic bags for the analyses. The mechanical and chemical analyses of the soil used were carried out in the two growing seasons and presented in Table (1).

Table (1): Mechanical and chemical soil characteristics at the experimental sites during 2007/2008 and 2008/2009

Physical properties	Value		Chemical properties	Value		Available nutrients (mg Kg ⁻¹)	Value	
	1 st season	2 nd season		1 st season	2 nd season		1 st season	2 nd season
Sand %	21.0	21.1	Field capacity %	32	33	Nitrogen	19	18
Silt%	32.3	33.0	EC(dSm-1)	1.64	1.70	phosphorous	8	7
Clay %	46.0	45.8	pH (Soil paste)	7.82	7.75	Potassium	140	135
Soil texture	clay		Organic matter (%)	2.69	2.80			

Plant Material, EM, Humic acid, and Seaweed Extract

Potatoes tubers; cv Spunta (imported from Holland) were used in the present investigation and obtained from Agric. Res. Center (ARC), Ministry of Agric., Egypt. Tubers were divided to pieces, averaging approximately 50 g weight. As recommended by the Pathology Dept. Ministry of Agric. Egypt, potato tubers pieces were sterilized with Vitavax Kapetan 1% at the rate of 1.25 kg/ton.

Effective micro-organisms were used under the name of EM which consists of a mixed culture of beneficial micro-organisms primarily photosynthetic and lactic acid bacteria, yeast and streptomycetes. The number of each component was recorded in table (2)

Table (2): Components of EM used in the experiments

Total bacterial	Lactic acid bacteria	yeasts	Streptomycetes
2.5-9.6 x 10 ⁸ cfu/ml	6.6-9.9 x 10 ⁶ cfu/ml	10 ⁵ - 10 ⁶ cfu/ml	8.5 x 10 ³ cfu/ml

An extract from brown seaweed (Acadian Seaplants, Dartmouth, Nova Scotia, Canada) prepared by a proprietary process. Seaweed extract is derived by an alkaline hydrolysis procedure from the fresh, intact *Ascophyllum nodosum* and 100% soluble in cold water. Seaweeds contained all the trace elements and plant growth hormones vitamins, amino acids, antibiotic and micronutrients (Crouch and Van Staden, 1993). Also seaweed extract contained protein/amino acids 3–5%, lipid 1%, alginic acid 12–18%, fucose-containing polymers 12–15%, mannitol 5–6%, other carbohydrates

10–15% (Acadian Seaplants Limited, Dartmouth, Nova Scotia, Canada), As described by Fike *et al.*, (2001).

Experimental design:

Farm yard manure has been added during soil preparation in organic fertilization at dose (40 m³/fed.). The experiment comprised of 18 treatments including three different rates of potassium fertilizers used individually or in combinations with EM and biostimulants (Humic acid or seaweed extract). A randomized complete block design in factorial arrangement was used with three replicates. Each plot was 7.2 m² (2.25 x 3.20 m²) included three ridges, each three meters long and 70 cm apart; the distance between hills was 30 cm apart.

Planting procedure:

Potato tuber cv. Spunta was planted in the ridges at 12-15 cm in depth (30-40 cm apart) on October 27th in the first season 2007/2008 and on November 9th in the second season 2008/2009 respectively. Potassium (K) levels occupied the main plots, while the effective microorganisms (EM) were assigned to the sub-plots, in each EM sub-plot the plants were divided into three groups which sprayed with either water (W), seaweed extract (SW) or humic acid (HA). The plot area was 7.2 m² ridged 70 cm apart.

As recommended by the Agric. Res. Center, Egypt, Nitrogen fertilizer was added at three equal portions, the 1st was applied after emergence (18-21 days from planting), in the form of ammonium sulphate (20.5 %), then two and four weeks later in the form of ammonium nitrate (33.5 %) at the rate of 180 Kg N/ fed. Phosphorous and potassium were applied during the soil preparation in the form of calcium superphosphate (15.5% P₂O₅) and potassium sulphate (48 % K₂O) at rate of 75 kg P₂O₅/fed and 20 kg K₂O fed⁻¹ respectively. The respective EM treated plots received dilute EM solution 2 liter/m² before the first irrigation. Potassium fertilizers applied at three levels 0, 40 and 80 kg K₂O fed⁻¹ using potassium sulphate (48 % K₂O). The quantity was divided into two equal doses to be added before the first irrigation and before the second irrigation. Plants were sprayed with an aqueous solution of SW or HA two times at 60 and 75 days from planting. Irrigation was done immediately. All usual cultural practices of potatoes cultivation were carried out according to the procedures that recommended by the Ministry of Agric. Egypt. Harvesting was done after 115 days from planting dates in both seasons.

Sampling dates and data recorded:

One plant sample was taken throughout the experimental period during the two growing seasons, for the growth characters and photosynthetic pigment determination and stem anatomy coincide as best as possible at the physiological stage of 22 foliage leaves; dated at the active growth period (after 90 days from planting). Growth characters: three plants were chosen randomly and carefully taken out of the soil with the aid of a water stream to insure minimal losses of the root system and the tubers if present. Fresh and dry weight of shoot (g) was recorded. Photosynthetic pigments concentration in the 4th upper leaf (mg/g FW) was extracted in methanol over night. The quantity of photosynthetic pigments in leaves was determined by the equation introduced by Lichtenthaler and Wellburn (1985). For anatomical studies,

small pieces (5mm) from the middle part of the 3rd internode from the plant tip were taken after 90 days from planting. The samples were killed and fixed in formalin aceto alcohol for at least 48 h, then washed and dehydrated in series of ethanol and embedded in paraffin wax (52-54 °C melting point). Cross sections were done at 12-15 µm thick using rotary microtome, stained in Saffranin/light green combination, cleared in cloves oil and mounted in canada balsam.

Statistical analysis:

Data were subjected to statistical analysis of variance according to Norman and Streiner, 2003. LSD value was used to test the difference between treatment means at 5%.

RESULTS AND DISCUSSION

Shoot fresh and dry weight:

The effects of potassium fertilizers rates, biostimulants application or effective microorganisms addition and their interactions on shoot fresh and dry weights (g/plant) are illustrated in figures (1,2) and presented in tables (3,4). Data illustrated in figures (1,2) indicated that increasing potassium fertilizer rate up to 80 kg K₂O/ fed significantly increased shoot fresh and weights. The highest weight was obtained due to application of 40 kg K₂O/fed in the first and second season as compared with untreated plants. As regard to the effect of biostimulants, the data illustrated in the same figures clearly showed that application of either seaweed extract or humic acid as foliar application significantly increased shoot fresh weight as compared to control in both seasons. Seaweed extract was more effective than humic acid in increasing shoot fresh and dry weight as compared to foliar application of water. Also, the same figure revealed that addition of effective microorganisms to the soil significantly increased shoot fresh weight.

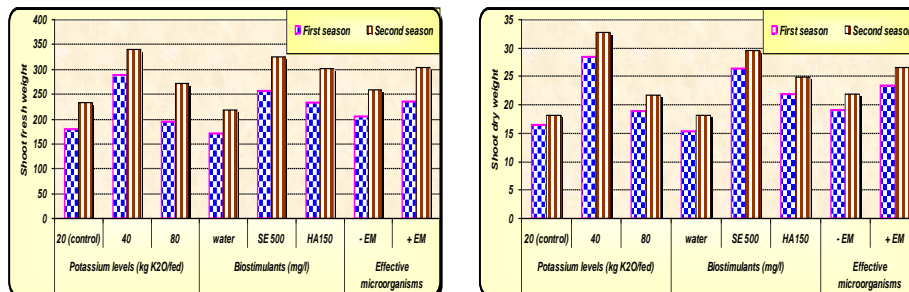


Figure (1,2) Shoot fresh and dry weights (g) of potato plant as affected by potassium fertilizer, biostimulants or effective microorganisms at 90 days from planting in both seasons (SE, Seaweed extract; HA, Humic acid)

As for the interactions, data in the tables (3,4) showed that application of either biostimulants, in particular, seaweed extract, significantly increased fresh and dry weight of potato shoot grown under all potassium fertilizer rates

with or without addition of effective microorganisms. The highest value was obtained under an addition of 40 kg K₂O/fed with addition of effective microorganisms and spraying plants with 500 mg/l seaweed extract as compared with control plant in the first and second growing seasons.

Table (3): Shoot fresh weight (g) of potato plant as affected by the interactions between potassium fertilizer (A), effective microorganisms (B) and biostimulants (C) at 90 days from planting during the first and second season

Treatment	First season					
	- EM			+ EM		
	K ₂₀	K ₄₀	K ₈₀	K ₂₀	K ₄₀	K ₈₀
Water	139.96	190.06	152.10	150.03	217.50	174.96
SE	191.20	320.16	194.20	210.23	390.63	232.33
HA	180.73	293.46	185.63	203.10	307.93	227.33
LSD 5%	A 7.322		B 4.865	C 6.588		ABC 16.137
Second season						
Water	136.16	248.80	215.70	186.00	302.16	221.53
SE	255.60	377.40	278.93	297.60	404.96	340.43
HA	232.20	345.00	247.43	292.13	361.56	326.76
LSD 5%	A 22.71		B 7.519	C 8.165		ABC 20.000

(K₂₀, 20 kg K₂O/ fed; K₄₀, 40 kg K₂O/ fed; K₈₀, 80 kg K₂O/ fed; SE, seaweed extract; HA, humic acid)

Table (4): Shoot dry weight (g/plant) of potato plant as affected by the interactions between potassium fertilizer (A), effective microorganisms (B) and biostimulants (C) at 90 days from planting during the first and second season

Treatment	First season					
	- EM			+ EM		
	K ₂₀	K ₄₀	K ₈₀	K ₂₀	K ₄₀	K ₈₀
Water	12.73	16.96	14.10	13.53	19.83	15.46
SE	17.40	35.40	18.03	19.66	43.16	24.90
HA	15.80	23.86	16.76	19.23	31.80	23.73
LSD 5%	A 1.265		B 0.319	C 0.685		ABC NS
Second season						
Water	13.50	18.66	16.56	14.80	27.46	17.66
SE	19.66	41.20	20.30	22.30	45.20	29.26
HA	18.16	30.66	18.36	20.63	33.36	28.00
LSD 5%	A 3.928		B 0.702	C 1.149		ABC 2.815

(K₂₀, 20 kg K₂O/ fed; K₄₀, 40 kg K₂O/ fed; K₈₀, 80 kg K₂O/ fed; SE, seaweed extract; HA, humic acid)

Potassium application, in particular, 40 kg K₂O/fed improved plant growth represented as shoot fresh and dry weights under the condition of the present investigation. This results are confirmed with many investigations (Moinuddin *et al.*, 2005 and Ahmed *et al.*, 2009); and recently by Bhattacharyya *et al.* (2009) who indicated that application of 180 kg K₂O/ha recorded higher values of growth attributes. It is well known that, potassium is the most important inorganic osmotic component and stimulates growth primarily by its effects on cell extension (Mengel and Arneke, 1982). Moreover, application of K increased the availability of nitrogen and

phosphorus (Sahai, 2004) which resulted in better plant growth and more number of branches per plant.

It is well known from the present investigation that application of humic acid significantly increased plant growth characteristics. The obtained results were confirmed with Seadh *et al.* (2008) on wheat, El-Ghamry *et al.*, (2009) on faba bean, Verlinden *et al.* (2009) on grass, maize, potato and spinach, and Saif El-Deen *et al.* (2011) on sweet potato plants. The mechanism by which humic acid stimulates plant growth are not fully clear, although there are some theories which probably work together. In general, humic acid have two important roles for the development of plants, either directly or indirectly (Nardi *et al.*, 1996) but the mechanism still remain unclear. So far hormone-like substances have been elucidated to understand the mechanism of humic substances in plant metabolism (Muscolo *et al.*, 1999) through their involvement in cell respiration, photosynthesis, oxidative phosphorylation, protein synthesis, and various enzymatic reactions (Zhang *et al.*, 2003). It is possible that the enhancement in the growth of potato plants, after application of humic acid, could be attributed at least partially to increased nutrient uptake by the plants. In this concern, HA has been reported to enhance mineral nutrient uptake by plants, by increasing the permeability of membranes of the root cells (Valdrighi *et al.*, 1996).

The obtained results in the present investigation showed an increase in potato plant growth after seaweed extract foliar application. Similar results have been reported on okra (Zodape *et al.*, 2008) and on tomato (Zodape *et al.*, 2011). In this concern, Zodape *et al.*, (2011) found that foliar application of seaweed extract on tomato shoot increased plant growth represented as plant height, root length over control plants. Besides, their application as farmyard manure, liquid extracts obtained from seaweeds have gained importance as foliar sprays for several crops, because the extract contains growth promoting hormones (IAA and IBA), cytokinins, trace elements (Fe,Cu,Zn,Co,Mo,Mn,Ni), vitamins and amino acids (Sridhar and Rengasamy, 2002). The positive effect of SE on plant growth may be due to its effect on increasing phosphorous uptake and content as recorded in the present investigation. Phosphorous is an essential nutrient and it plays an important role in the biosynthesis and translocation of carbohydrates and is necessary in stimulating cell division and the formation of DNA and RNA (Nijjar, 1985).

The present investigation indicated that an addition of EM increased, in most cases, growth parameters of potato plants. The results in this connection are in agreement with those found by many authors, i.e. Abou-Bakr *et al.* (2005) on potato plant, who indicated that application of EM at (1, 5, and 10 ml/l) induced significant increases in plant height, number of stems per plant, number of leaves per plant, total leaf area per plant in both seasons. The improvement in growth characters may be attributed to the fact that the use of EM enhances the beneficial microbes in the environment, which attributed to the profound effect of a) its ability to release plant growth promoting substances which might be stimulated plant growth, b) synthesis of some beneficial organic acids, bioactive substances and vitamins, c) increasing amino acids content (Schank *et al.*, 1981), d) increasing in the

water and mineral uptake from the soil leading to improving the availability and acquisition of nutrients from the soil (Sarig *et al.*, 1984) due to increases in root surface area, root hairs and root elongation (Sundaravelu and Muthukrishnan, 1993), e) increasing the ability to convert nitrogen to ammonium and thus make it available to plant, f) enhancing the production of biologically active fungistatinal substances which may change the microflora in the rhizosphere and affect the balance between harmful and beneficial organisms (Apte and Shende, 1981), and g) improving the photosynthetic efficiency due to an increase in nutrient availability. Many actual and putative plant growth producing bacteria containing in EM produce phytohormones that are believed to be related to their ability to stimulate plant growth. In most cases, these phytohormones are believed to be changing assimilate partitioning patterns in plants and affecting growth patterns in roots to result in bigger roots, more branched roots, and/or roots with greater surface area. Indole-3-acetic acid is a phytohormone which is known to be involved in root initiation, cell division, and cell enlargement (Salisbury, 1994).

Photosynthetic pigments:

Data presented in Tables (5,6) and Figures (3,4,5) showed that the content of photosynthetic pigments in the leaves of potato plants subjected to potassium fertilizers was higher than control plants. In this concern, an addition of 40 kg K₂O/ fed gave the highest values of photosynthetic pigments. On the other hand, application of potassium fertilizer did not-significantly decreased the ratio between chlorophyll A and chlorophyll B, and the moderate potassium level gave the lowest ratio in this respect.

The data presented in Tables (5,6) revealed that all interactions between potassium fertilizer rates, addition of effective microorganisms and spraying biostimulants (seaweed extract or humic acid) significantly increased the content of chlorophyll B and total chlorophylls in the 3rd upper compound leaf of potato plant at 90 days from planting during the second growing season, but non-significantly increased either chlorophyll A or carotenoid contents in the same leaf as compared with control plants. On the other hand, the ratio between chlorophyll A and chlorophyll B was nonsignificantly decreased in the leaves comparing with the untreated plants. The highest values of photosynthetic pigment contents (Chlorophyll A "1.112", chlorophyll B "0.509", total chlorophylls "1.622", and total carotenoids "0.563" mg/g FW) were obtained due to spraying potato shoot with seaweed extract, and an addition of 40 kg K₂O/fed with addition of effective microorganisms as compared with control plants (0.722,0.129, 0.851 and 0.103 mg/g FW respectively).

The data presented in Table (5) indicated that an addition of EM to potato plants significantly increased chlorophyll B, total chlorophylls, and total carotenoids contents in the leaves whereas; the increase in chlorophyll A content was insignificant. Meanwhile, an addition of EM significantly decreased the ratio between chlorophyll A and chlorophyll B. Regarding the effect of biostimulants on photosynthetic pigments content, the data illustrated in Figures (3,4,5) clearly showed that application of either seaweed extract or humic acid significantly increased photosynthetic pigments,

whereas significantly decreased chlorophyll A/ chlorophyll B ratio. However, seaweed extract was more effective than humic acid on increasing photosynthetic pigments content and decreasing the ratio between chlorophyll A and chlorophyll B.

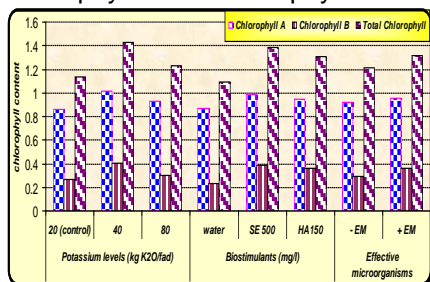


Figure (3): Chlorophyll A, chlorophyll B and total chlorophyll content in the 3rd upper compound leaf of potato plant (mg/g FW) as affected by potassium fertilizer, biostimulants or effective microorganisms at 90 days from planting during the second growing season

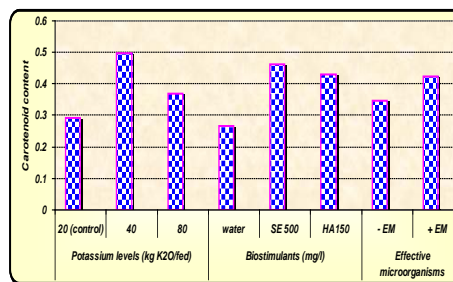


Figure (4): Total carotenoid content in the 3rd upper compound leaf of potato plant (mg/g FW) as affected by potassium fertilizer, biostimulants or effective microorganisms at 90 days from planting during the second growing season (SE, Seaweed extract; HA, Humic acid)

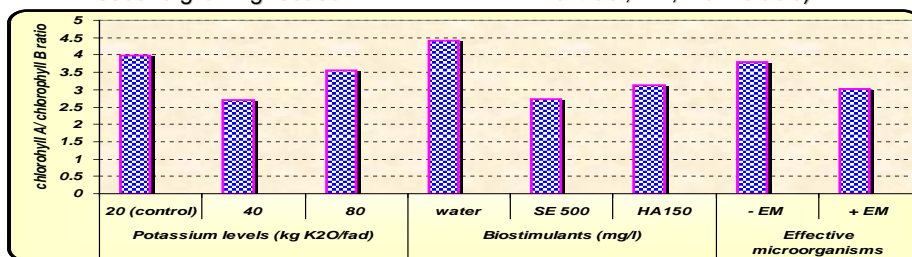


Figure (5):The ratio between chlorophyll A and chlorophyll B in the 3rd upper compound leaf of potato plant (mg/g FW) as affected by potassium fertilizer, biostimulants or effective microorganisms at 90 days from planting during the second growing season(SE, Seaweed extract; HA, Humic acid)

The effect of potassium on photosynthetic pigments was also reported by Shehata and Abo-Sedera (1994). In this concern, El-Sawy (2011) on sweet potato found that increasing potassium levels from 25 upto 75 kg/fed significantly increased total chlorophyll content compared with untreated plants. Moreover, Rai *et al.* (2004) observed that the chlorophyll content in leaves (0.235 to 0.425 mg/100 g) increased significantly with increasing levels of potassium.

Increasing of photosynthetic pigments due to application of HA may be due to increasing the uptake of magnesium and iron, which are required for chlorophyll biosynthesis. This result was reported previously, where, application of potassium humate significantly increased chlorophyll content in

leaves (Seadh et al. 2008, El-Ghamry et al. 2009 and Saif El-Deen et al. 2011).

Table (5): Chlorophylls content in the 3rd upper compound leaf of potato plant (mg/g FW) as affected by the interactions between potassium fertilizer (A), effective microorganisms (B) and biostimulants (C) at 90 days from planting during the second growing season

Treatment	Chlorophyll A					
	- EM			+ EM		
	K ₂₀	K ₄₀	K ₈₀	K ₂₀	K ₄₀	K ₈₀
Water	0.722	0.945	0.865	0.839	0.948	0.894
SE	0.850	1.108	0.936	0.926	1.112	1.028
HA	0.960	0.941	0.940	0.866	1.058	0.920
LSD 5%	A 0.018		B NS	C 0.019		ABC NS
Treatment	Chlorophyll B					
	- EM			+ EM		
	K ₂₀	K ₄₀	K ₈₀	K ₂₀	K ₄₀	K ₈₀
Water	0.129	0.264	0.213	0.145	0.369	0.270
SE	0.343	0.416	0.332	0.375	0.509	0.361
HA	0.196	0.484	0.235	0.422	0.389	0.417
LSD 5%	A 0.019		B 0.014	C 0.020		ABC 0.049
Treatment	Total Chlorophylls					
	- EM			+ EM		
	K ₂₀	K ₄₀	K ₈₀	K ₂₀	K ₄₀	K ₈₀
Water	0.851	1.209	1.079	0.984	1.318	1.131
SE	1.227	1.524	1.269	1.303	1.622	1.386
HA	1.157	1.426	1.174	1.289	1.448	1.337
LSD 5%	A 0.002		B 0.004	C 0.006		ABC 0.015

(K20, 20 kg K2O/ fad; K40, 40 kg K2O/ fad; K80, 80 kg K2O/ fad; SE, seaweed extract; HA, humic acid)

Table (6): Total carotenoid content(mg/g FW) and the ratio between chlorophyll A and chlorophyll B in the 3rd upper compound leaf of potato plant as affected by the interactions between potassium fertilizer (A), effective microorganisms (B) and biostimulants (C) at 90 days from planting during the second growing season

Treatment	Total carotenoids					
	- EM			+ EM		
	K ₂₀	K ₄₀	K ₈₀	K ₂₀	K ₄₀	K ₈₀
Water	0.103	0.352	0.218	0.193	0.440	0.286
SE	0.344	0.559	0.370	0.417	0.563	0.513
HA	0.294	0.530	0.343	0.400	0.537	0.477
LSD 5%	A 0.022		B 0.010	C 0.012		ABC NS
Treatment	Chlorophyll A/ Chlorophyll B					
	- EM			+ EM		
	K ₂₀	K ₄₀	K ₈₀	K ₂₀	K ₄₀	K ₈₀
Water	5.644	3.579	5.151	5.819	2.575	3.756
SE	2.787	2.869	2.886	2.571	2.267	2.910
HA	4.914	2.122	4.333	2.188	2.834	2.284
LSD 5%	A NS		B 0.176	C 0.245		ABC NS

(K20, 20 kg K2O/ fad; K40, 40 kg K2O/ fad; K80, 80 kg K2O/ fad; SE, seaweed extract; HA, humic acid)

The obtained results indicated that application of SE increased total chlorophyll and carotenoids contents of potato leaves. Our findings coincide with some earlier finding on *Vigna sinensis* (Sivasankari et al., 2006), *Cyamopsis tetragonoloba* (L.) Taub and *Abelmoschus esculentus* (L.) Moench (Thirumaran et al. 2009) and tomato (Zodape et al., 2011). Earlier studies by Whapham et al. (1993) had shown that the betaines present in extracts of *A. nodosum*, when used in the cucumber cotyledon bioassay

devised for cytokinins (Fletcher, 1982), resulted in enhanced chlorophyll levels in comparison to the controls. These data strongly indicated that the effects on leaf chlorophyll contents produced by the use of seaweed extracts are due to the betaines contained in them.

It is well noted from the present results that EM had a stimulative effect on all photosynthetic pigments fraction concentrations during the two growing seasons. The enhancing effects of bio fertilizers on chlorophylls concentration and their content may be attributed to their effects on increasing not only mineral uptake (Hauka, 2000) but also the production of growth substances especially cytokinins (Omay *et al.*, 1993). Cytokinins are known to stimulate chlorophyll synthesis and delay chlorophyll destruction and senescence (Daiziel and Lawrence, 1984). Subba Rao, (1993) added that, the beneficial effects of bacterization on chlorophylls may be attributed to N₂-fixation process, and/or to the production of growth promoting substances like gibberellins and other compounds of auxin type which gave a positive effect of plant growth, chlorophyll content nutrient uptake (Bashan and Holguim, 1997). Moreover, the increase of chlorophylls and carotenoids due to EM treatment may be attributed to the effects of phosphate-dissolving bacteria on decreasing soil pH, increasing the availability of some nutrients such as Fe, Zn, Mn and Cu to plant uptake, potassium content (El-Shahawy, 2003), stimulating and surviving nitrogen fixing bacteria such as *Azospirillum* (Algawady and Gaur, 1988).

Stem anatomy :

The data presented in table (7) and illustrated in figures (6,7,8) showed, in most cases, that addition of potassium fertilizer rates from 20 (control) to 80 Kg K₂O/fed increased stem and pith diameter, collenchyma thickness, chlorenchymatous layer thickness, length and width of vascular bundle, as well as the thickness of phloem and xylem. The results also indicated that the best treatment for increasing the thickness of either stem or collenchyma as well as the width of vascular bundle was addition of 40 Kg K₂O/fed. On the other hand, an addition of 80 kg K₂O/fed gave the highest values of thickness of chlorenchymatous tissue, length of vascular bundle, thickness of internal and external phloem as well as thickness of xylem.

As regard to the effect of effective microorganisms, the data in the same table and figures clearly showed that inoculation of potato plants with EM increased the diameter of stem, number of parenchymatous layer, length and width of vascular bundle, and the thickness of external phloem and xylem, meanwhile decreased the diameter of pith, and the thickness of epidermis, collenchyma layers, and, *internal* phloem tissue. Foliar application of both biostimulants, in particular, seaweed extract increased, in most cases, all studied anatomical characteristics of potato stem, where its application increased the diameter of stem, pith and thickness of collenchyma layers, and xylem tissue as well as the length and width of large vascular bundle beside the thickness of external phloem. On the other hand, the thickness of internal phloem tissue was decreased. The same Table and figures indicated that, in most cases, all interactions increased all anatomical characteristics of potato stem.

The highest values for thickness of epidermis, collenchyma and external phloem as well as stem diameter were obtained due to inoculated potato plants with effective microorganism and foliar spraying with seaweed extract under moderate potassium fertilizer rate (40 kg K₂O/fed). Meanwhile, foliar spraying with seaweed extract without effective microorganisms inoculation under 40 kg K₂O/fed showed the highest diameter of pith. The data also indicated that the highest values of large vascular bundle width and thickness of xylem tissue were obtained due to foliar application of humic acid in the absence of effective microorganisms inoculation under 80 kg K₂O/fed. On the other hand, foliar application of either humic acid or seaweed extract under 40 kg K₂O/fed without inoculation with effective microorganisms gave the highest values of length of large vascular bundle and thickness of internal phloem tissue. Regarding the thickness of parenchyma tissue, the data in the same table and figure proved that the highest thickness of chlorenchymatous tissue could be attained after foliar application of humic acid under 40 kg K₂O/fed with effective microorganisms inoculation.

The increase in stem diameter under experimental factors may be attributed to its effects on enhancing nitrogen uptake and content in plant tissues (unpublished data). These nutrients induced meristematic activity as well as cell division and its elongation through auxin production (Salem 2000). El-Rewainy, Hamdia and Galal, Anaam (2004) who reported that, nitrogen not only increased the growth substances but also increase their translocation in the plant. In addition, phosphorus is a component of RNA and DNA (Marschner 1995), therefore it play an important role for cell division activity. Moreover, the increase in stem diameter due to the inoculation with mixed three strains of used bacteria may be attributed to their ability to release plant growth substances, mainly IAA, GA3 and cytokinins (Omay *et al.* 1993). Auxins and cytokinins increased cell division and cell enlargement (Arteca 1996). He added that the exogenous application of cytokinins promote cell enlargement caused by an increase of water uptake as a result of an increase in the osmotic potential of the cells. Moreover, the increase in vascular bundle dimensions may be due to stimulation in cell division in the procambium and extension growth.

It could be concluded that application of 40 kg K₂O/fed with addition of effective microorganisms and spraying plants with 500 mg/l seaweed extract proved to be more effective in improving plant growth, photosynthetic pigments as well as stem anatomy as compared with control plant.

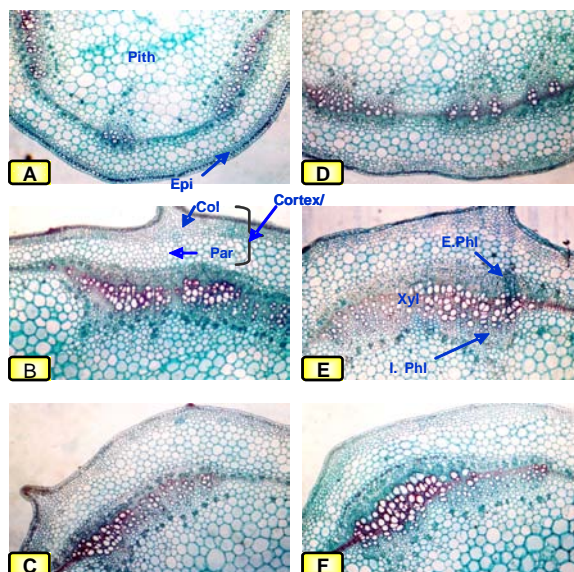


Figure (6): Cross section in the 3rd internode of potato stem as affected by effective microorganisms, biostimulants under 20 Kg K₂O/fed at 90 days from planting in the second season (E.Phil, External phloem; I. Phl, Internal phloem; Epi, Epidermis; Col, Collenchyma; Par, Parenchyma; Xyl, Xylem; A,1; B, 2; C,3; D,4; E, 5; F, 6)(40x)

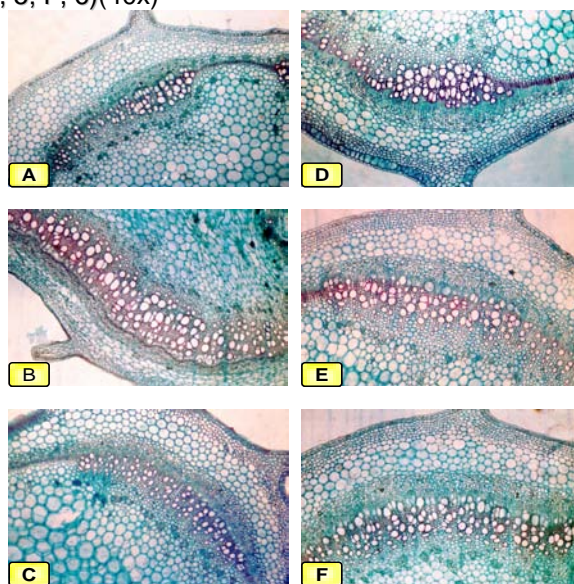


Figure (7): Cross section in the 3rd internode of potato stem as affected by effective microorganisms, biostimulants under 40 Kg K₂O/fed at 90 days from planting in the second season (A,7; B, 8; C,9; D,10; E, 11; F, 12) (40x)

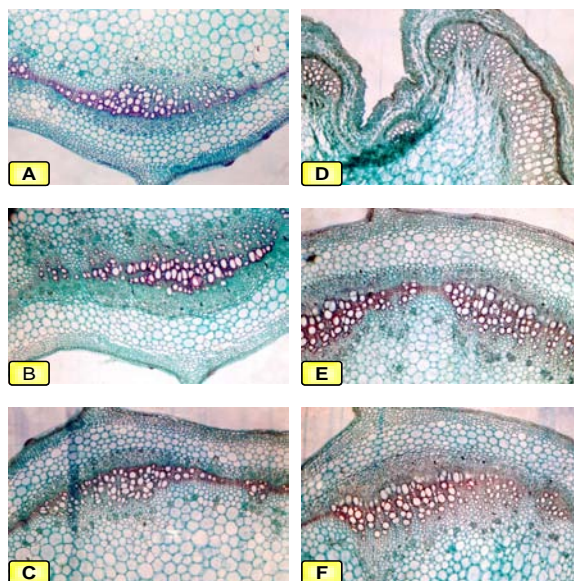


Figure (8): Cross section in the 3rd internode of potato stem as affected by effective microorganisms, biostimulants under 80 Kg K₂O/fedat 90 days from planting in the second season (A,13; B, 14; C,15; D,16; E, 17; F, 18) (40x)

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**تأثير التسميد البوتاسي، المنشطات الحيوية، الكائنات الدقيقة الفعالة وتداخلاتهم
علي نمو نبات البطاطس، محتوى صبغات البناء الضوئي وتشريح الساق
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اجريت تجربتان حقليتان خلال الموسم الشتوي لموسمي 2008/007 و 2009/2008 لدراسة تأثير التسميد البوتاسي، المنشطات الحيوية، الكائنات الدقيقة الفعالة وتداخلاتهم علي الوزن الطازج والجاف للمجموع الخضري، محتوى الأوراق من صبغات البناء الضوئي وتشريح الساق في نبات البطاطس.

زيادة التسميد البوتاسي حتي 80 كجم فوق أكسيد البوتاسيوم/فدان تزيد معنوياً الوزن الطازج والجاف للمجموع الخضري، محتوى صبغات البناء الضوئي بالإضافة زيادة غالبية القراءات التشريحية للساق ممثلة في قطر كل من الساق والنخاع، سمك الكولنكيما وبارنكيما البناء الضوئي، طول وعرض الحزمة الوعائية الكبيرة، وكانت أحسن نتائج يمكن الحصول عليها من خلال 40 كجم فوق أكسيد البوتاسيوم/فدان مقارنة بالكنترول. استخدام المنشطات الحيوية وبصفة خاصة مستخلص الطحالب البحرية أو التلقيح بالكائنات الدقيقة الفعالة يزيد جميع الصفات السابقة. بالنسبة للتداخل بين عوامل الدراسة، لوحظ أن استخدام المنشطات الحيوية خاصة مستخلص الطحالب يزيد معنوياً الوزن الطازج والجاف للمجموع الخضري، محتوى الأوراق من صبغات البناء الضوئي، تحسين الصفات التشريحية المدروسة للساق. يمكن التوصية أنه للحصول علي أفضل نتائج للصفات المدروسة يجب تسميد النباتات بـ 40 كجم فوق أكسيد البوتاسيوم/فدان، مع رش المجموع الخضري بـ 500 ملليجرام/لتر مستخلص طحالب بعد تلقيح النباتات بالكائنات الدقيقة الفعالة.

قام بتحكيم البحث

**كلية الزراعة – جامعة المنصورة
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**أ.د / محمد نصر الدين هلاي
أ.د / محمد عبد العزيز نصلر**

Table (7): Anatomical characteristics of potato stem as affected by potassium fertilizer rate, effective microorganism and biostimulants as well as their interactions at 90 days from planting during the second growing season

Treatment			Stem Diameter (μ)	Pith Diameter (μ)	Epidermis Thickness (μ)	Cortex thickness (μ)				Large Vascular Bundle Dimension (μ)		External Phloem Thickness (μ)	Internal Phloem Thickness (μ)	Xylem Thickness (μ)
						Collenchyma		Parenchyma		Length	Width			
						Number	Thickness	Number	Thickness					
K20	-EM	W (1)	1850	838	12	6	110	4	220	164	168	42	50	72
		SE (2)	2530	1142	12	6	150	4	200	332	368	50	78	204
		HA (3)	2320	1288	8	5	120	5	230	158	324	44	46	68
	+EM	W (4)	2100	1104	8	5	90	5	180	220	320	30	26	156
		SE (5)	2650	1026	12	5	160	5	300	340	348	90	78	172
		HA (6)	2650	1462	4	6	120	3	250	220	328	50	58	112
K40	-EM	W (7)	2450	1200	8	5	130	4	250	252	260	66	62	124
		SE (8)	3400	1568	8	5	160	7	380	368	320	114	94	160
		HA (9)	3250	1558	8	5	150	5	260	368	252	98	114	156
	+EM	W (10)	2820	1408	12	7	130	4	300	264	248	70	62	132
		SE (11)	3370	1538	16	6	160	5	420	320	292	100	64	156
		HA (12)	3250	1498	12	4	150	5	430	284	260	60	50	174
K80	-EM	W (13)	2160	676	12	6	120	5	310	300	208	74	70	156
		SE (14)	2560	1204	8	4	100	5	310	260	340	78	50	132
		HA (15)	2400	1092	4	5	100	8	250	300	380	48	52	200
	+EM	W (16)	2250	978	8	9	150	4	250	228	260	50	66	112
		SE (17)	3200	1532	12	5	90	8	400	332	360	88	90	154
		HA (18)	2950	1438	8	5	120	5	300	328	288	78	110	140

(K₂₀, 20 kg K₂O/ fed; K₄₀, 40 kg K₂O/ fed; K₈₀, 80 kg K₂O/ fed; SE, seaweed extract; HA, humic acid; EM, Effective microorganisms)