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Mitigatory effect of Fertigation with Humic, Fulvic, Phosphoric Acids and Seaweeds Extract on Heat Stressed Snap bean Plants under Delta Region Conditions

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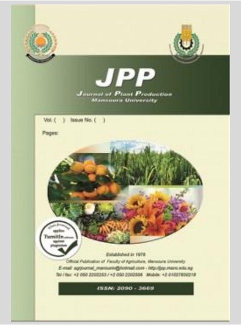

ABSTRACT

Abiotic stress as heat stress (extreme temperatures) cause unavertable conditions for plant growth lead to increase losses in yield and poor fruit quality. The intent of this study is to focus on the role of some bio-stimulants as soil application (fulvic and humic acids, seaweed extract and phosphoric acid) on snap bean productivity under natural heat stress. The results showed that soil application with falvic acid (1 L/fed) markedly improved snap bean plants vegetative growth behavior, minerals content and photosynthetic pigments of leaves, yield and its quality in the two seasons. The lowest values of all the above mentioned parameters were observed with the control plants as a result of heat stress damages under the experiment conditions.

Keywords: Heat stress - Bio-stimulants – Fulvic acid – Snap bean

INTRODUCTION

Snap bean (*Phaseolus vulgaris* L) is considered one of the most important protein sources to human feeding specially in the developing countries. As much as 60 % of snap bean world production occurs under abiotic heat stress and drought conditions (Franca *et al*., 2000). Because the world temperature is day by day rising due to climatic changes, the crops physiological performance is also changing and adaptation (tolerance) for heat is getting minimized. During seed germination, heat stress may slow down or totally inhibit germination and in advanced stages of plants growth, heat stress adversely affects carbon dioxide exchange rate, photosynthesis, the level of hormones, water relation and metabolites which reflected negatively in the end on yield. In the same manner, high temperature affects plants development and the economic yield, resulted in unfavorable pre- and post-harvesting damages as sunburns on plant parts (leaves, branches and stems), scorching of leaves, root and shoot inhibition, leaf abscission and senescence, fruit damage and discoloration and finally the yield was reduced (Wahid *et al*., 2007).

The bio-effectors application which known as plant bio-stimulants, become a common agricultural practice and have many benefits for stimulating protection against stresses as well as plants growth. A bio-stimulant is defined as microorganism and/or organic materials (including extracts of seaweed, humic substances, amino acids, and microbes) that used to improve nutrients uptake, stimulate plants growth, reduce fertilizer consumption and enhance stress resistance (Van-Oosten *et al.,* 2017). Moreover, the use of bio-stimulants may also decrease fertilizer requirements by enhancing the assimilation of micro- and macro-nutrients (Nardi *et al*., 2009 and Ertani *et al*., 2012).

Humic substances (HSs) are natural organic compounds of the soil organic matter formed by plants and animals residues decomposition under the action of micro-organisms (Morales *et al*., 2012). HSs can classified to humin, fulvic acid (FA) and humic acid (HA) due to their solubility at different pH conditions (Suh*et al*., 2014) . Fulvic acids (FAs) are humic acids with carbon-poor functional groups, higher oxygen contents and lower molecular weight which range only few hundred Daltons and can pass through micro-pores of artificial or biological membranes system, but humic acids can't, with a larger molecule weight range to few thousands Dalton (Weng *et al*., 2006 and Bulgari *et al*., 2015). The application of HSs increase root growth, nutrients uptake and enhance resistance to abiotic heat stress and salinity. The differences in HSs effects are due to their sources, the receiving plant, the environmental conditions as well as doses and manner of application (Rose *et al*., 2014 and Canellas *et al*., 2015). Soil aggregation, aeration, microbial growth, water holding capacity, organic matter mineralization and transport of micro and macronutrients are improved by humic substances indirectly (Saruhan *et al*., 2011 and Daur and Bakhashwain 2013). Humic acid rates required by the effective role on plant growth and metabolism is greater when applied with soil than those required when supplied by foliar spray (Anjun *et al*., 2011). Soil amendment with HA and FA together with mineral fertilization creates a great contribution to soil fertility and stability causing exceptional plant development and nutrients uptake (Khaled and Fawy 2011 and Yang *et al*., 2013).

Fulvic acids (FAs) have higher adsorption and cation exchange capacities, greater numbers of carboxyl groups and greater total acidity than HA and may play roles as a

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natural chelator in both transportation and mobilization of microelements (Bocanegra *et al*., 2006). FA can remain in the soil solution at a wide range of pH, high salt concentration and have long-lasting potentials interact with roots (Zimmerli *et al*., 2008). FA confers benefits to plants by improving nutrients uptake, stabilizing soil pH, enhancing drought resistance in addition to reducing fertilizers leaching (Suh *et al.*, 2014).

Seaweed extracts (SE) are rich in macro and micro nutrients, proteins, polysaccharides, phyto-hormones, poly unsaturated fatty acids, osmolytes and polyphenols. The previous compounds enhance multiple beneficial responses on plants as enhancing seeds germination and establishment, plants development and productivity, increasing post-harvest shelf life, resistance against abiotic and biotic stresses as well as their elements content (Du-Jardin, 2015). Also, SE contain prominent amounts of active plant growth substances as cytokinins, auxins and their derivatives (Du-Jardin, 2015). These growth hormones interact with the physiological and the biochemical mechanisms of plants, thus improving crop productivity (Hernández-Herrera *et al*., 2014).

Phosphorus (P)is an essential component of phospholipids, nucleic acids and energy-rich phosphate compounds, so it plays a vital role in fruit, seed and root development, and diseases tolerance. P deficiency can inhibit plant growth and reduce both yield and fruit quality. Over doses application of P fertilizers will increase P losses to water resources and impair water quality through eutrophication (Von-Wandruszka, 2006). Consequently, appropriate P management is required to maintain crop yield and minimize environmental impacts. P fertilizer plays a vital role in photosynthesis as well as stimulating roots development by improving nutrients uptake and water use efficiency (Zhu *et al*., 2017).

So, the current study was designed to examine the effect of some bio-stimulants on productivity of snap bean under natural heat stress conditions. We hypothesized that one of treated bio-stimulant could be an effective bio stimulant to improve productivity of snap bean.

MATERIALS AND METHODS

Experimental location: Two field experiments were carried out during two consecutive late summer seasons at private vegetable farm at Shabraweysh near El-Mansoura, Dakahlia, Egypt. The experimental soil was clay loamy with the following chemical parameters: pH 8.12, electrical conductivity (EC) 0.612, dS m^{-1} and soluble elements (cations and anions) (meq L^{-1}) were Ca^{+2} 12.9, K^{+} 0.12, Mg^{+2} 11.91, Na^{+} 3.58, CO_3^{-2} 1.35, SO_4^{-2} 0.82 and Cl^{-1} 4.13.

The seeds of Giza 4 cultivar were obtained from Garaa Seed Company and sown on 30 March 2017 and 2018 seasons. The experimental plot contained 6 rows with 5 m length and 0.8 m width with spacing 0.10 m between plants making total area 24 m^2/plot. The experiment was applied under drip irrigation system in the two seasons.

Meteorological data of experimental location during 2017 and 2018 growing seasons were presented in Table 1.

Experimental design and treatments:

The experimental layout was complete randomize block design with three replicates. The applied bio-stimulators were arranged as followed:

1) Humic acid (1.5 L/fed).
2) Fulvic acid (1 L/fed).
3) Seaweed extract (1 L/fed).
4) Phosphoric acid (2 L/fed).
5) Control.

The applied fertigation treatments (irrigation with tested substances) were assigned in three times, the first one with sowing seeds, the second and third were after 20 and 45 days from sowing seeds with tested concentrations.

Recorded Data: Two of the six rows was customized for vegetative growth parameters and chemical measurements, two for green pods and quality parameters and the last two for dry seeds yield.

Vegetative growth parameters(at 60 days from sowing seeds)

Plant height,
Total fresh weight/plant,
Total dry weight/plant and
Total chlorophyll: according to Mackinny (1941).

Chemical content of leaves: Leaves were collected 60 days after sowing were oven dried at 70°C until constant weight, 0.5 gm of dried mater were digested using H_2SO_4 and H_2O_2 as described by Cottenie (1980). The extracts were used to determine the following chemical contents:

Total nitrogen: It was determined by the method as described by Plummer (1978).

Total phosphorous: It was determined calorimetrically according to Jackson (1967).

Total potassium: It was determined using flame photometer as described by Piper (1950).

Yield parameters: Green pods of all plants from each plot were harvested at the proper maturity stage, counted and weighed in each harvest, following parameters were estimated:

Total fresh green pods yield (ton/fed.).
Marketable green pod yield (ton/fed) and
Dry seed yield (kg/fed).

Green pods quality parameters: Representative samples from mature green pods from each experimental plot were randomly taken for determining the following characteristics:

Protein content: It was determined according to Piper (1947).

Carbohydrates content: It was determined according to Shaffer and Hartman (1921).

Titratable acidity: It was determined according to method described in AOAC. (1975).

Fibers content: It was determined according to method described in AOAC. (1984).

Statistical analysis: The collected data of the two growing seasons were arranged and statistically by Co-Stat statistical software program according to Gomez and Gomez (1984) and the means were compared by Duncan's Multiple Range Test (Duncan 1965).

RESULTS AND DISCUSSION

Vegetative growth parameters:

Data presented in Table 2 show that all treatments were significantly different in their effects on all studied vegetative growth characteristics in both seasons of the study. Soil application with FA resulted in the highest means values of all studied traits compared with control treatment in the two growing seasons.

These results may be due to the fact that application of FA have many positive effects on soil properties, one of which may be contribute to soil cation exchange capacity (Malan 2015 and Moradi *et al*., 2017). Also, when Fulvic acid applied to soil, it already converted into the available known humic substances that either directly or indirectly enhance plants performance (Lotfi *et al*., 2015) which reflected positively on plant growth, photosynthesis pigments and development stages. In addition, application of FA and HA through irrigation water may increase organic matter in the soil which directly improve nutrients retention by stimulating the activity of soil microbes causing conversion of the nutrients (macro and micro) from the organic to the mineralized form Stevenson, 1994. In this connection, FA increased root elongation and enhanced number of root initials on hypocotyl sections of plants (Eyheraguibel *et al*. 2008), number and length of lateral roots and micro-nutrients (Dobbss et al. 2007). These results are in the same line with those of Kamel *et al*., (2014) on cucumber plants and Suh *et al*., 2014 on tomato plant.

Table 1. Meteorological data of experimental location during 2017 and 2018 growing seasons.

Data	Max. Temp. (°C)		Min. Temp. (°C)		Relative humidity (%)	
	2017	2018	2017	2018	2017	2018
Mar.	23.95	23.71	11.17	11.46	71.45	70.56
Apr.	28.51	26.71	15.35	13.07	65.87	63.54
May.	31.48	31.25	19.75	18.96	62.98	67.77
Jun.	33.68	31.98	20.06	21.04	69.21	65.88
Jul.	34.16	34.21	23.46	22.03	69.10	69.85

Table 2. Vegetative growth parameters of Snap bean plants as affected by soil application of some bio-stimulants treatments in late summer seasons of 2017 and 2018.

Treatments	Plant height (cm)		Total fresh weight (g)		Total dry weight (g)		Total chl. (a+b) (mg/gm F.Wt.)	
	I	II	I	II	I	II	I	II
Control	42.1 c	44.8 e	96.8 e	90.9 d	18.6 c	17.6 d	8.95 e	8.40 c
PA	55.6 b	48.2 c	101.6 c	93.6 c	19.6 b	18.1 bc	10.50 c	9.54 b
SE	54.4 b	46.9 d	99.7 d	91.8 d	19.6 b	17.9 cd	9.92 d	9.37 b
HA	58.1 a	49.6 b	104.8 b	95.6 b	20.3 ab	18.5 ab	10.87 b	9.90 b
FA	59.7 a	50.2 a	107.1 a	98.6 a	21.0 a	18.6 a	12.54 a	10.61 a

PA: phosphoric acid 2 L/fed, SE: Seaweed extract 1 L/fed, HA: Humic acid 1.5 L/fed and FA: Fulvic acid 1 L/fed.
Means followed by the same letter in the same column are significantly different at the 5% level according to Duncan's multiple range test.

Negative response to heat stress condition was clearly observed with the the control plants as they were physiologically stressed (Table 1). This response might be due to the fact they were developed low physiological or metabolical mechanisms by which they can be protected against the prevailing higher temperature stress (Bita and Gerates, 2013). In addition, these external and related internal metabolical conditions of control plants might be associated with expression of reactive oxygen radicals in toxic levels, inducible oxidative stress (Cakmak and Marschner, 1992 and El-Stener and Osswald, 1994).

Chemical contents of leaves:

Data in Table 3 show the effect of irrigation with some bio-stimulants treatments on the mineral contents of snap bean leaves. The obtained results indicated that all applied treatments significantly differed on their effects on mineral contents in both seasons of study. FA recorded the highest values of mineral contents leaves followed by HA and SW in the two seasons compared with check plants.

Table 3. Chemical content of Snap bean leaves as affected by soil application of some bio-stimulants treatments in late summer seasons of 2017 and 2018.

Treatments	N(%)		P(%)		K(%)	
	I	II	I	II	I	II
Control	2.43 e	1.89 e	0.30 d	0.20 e	2.18 e	1.85 e
PA	2.99 c	2.45 c	0.44 b	0.33 c	2.42 c	2.09 c
SE	2.56 d	2.02 d	0.38 c	0.23 d	2.29 d	1.96 d
HA	3.23 b	2.69 b	0.48 a	0.38 b	2.52 b	2.19 b
FA	3.64 a	3.10 a	0.50 a	0.40 a	2.18 a	2.31 a

PA: phosphoric acid 2 L/fed, SE: Seaweed extract 1 L/fed, HA: Humic acid 1.5 L/fed and FA: Fulvic acid 1 L/fed.
Means followed by the same letter in the same column are significantly different at the 5% level according to Duncan's multiple range test.

The Enhancement of FA on N, P and K were in agreement with the previous findings of Yakhin *et al*., 2017 and Geng *et al*., 2020. Also, FA play important roles as a natural chelator in both transportation and mobilization of micronutrients (Bocanegra *et al*. 2006) increased chlorophylls content in soybean plants (Chen *et al*. 2004) and photosynthesis rate in maize (Anjum *et al*. 2011).

The obtained results are in accordance with the studies conducted by Lee (2007) on tomato and Cimrin and Yilmaz (2005) on lettuce. FA acid may increase N and Mg (structural component of Chl.) uptake, enhanced Chl. accumulation causing greater rate of photosynthesis as well as retarding senescence. These results may be contributed to the favorable role of fulvic acid that easily chelate minerals (calcium, magnesium, iron, zinc and copper) and it can directly provide plants with these elements (Malan 2015 and Lotfi *et al*., 2015).

Furthermore, under high temperatures (Table 1) and lowest values of stressed plants (control) may be due to chlorophyll a and b degradation was greater in the young leaves as compared to the developing leaves (Karim *et al*., 1997 and 1999). Similar effects on photosynthetic apparatus or chlorophyll pigment contents were suggested to be related to the active oxygen species production (Camejo *et al*., 2006 and Guo *et al*., 2006). In addition, heat stress effects on photosynthesis and respiration, thus leads to diminish plant productivity and shorten life cycle (Barnabas *et al*., 2008).

Green pods and seed yield parameters:

Data presented in Table 4 show that the applied bio-stimulants were significantly differed in all fruit yield parameters of snap bean plants in the two growing seasons. The bio-stimulant FA gave higher values of all fruit yield parameters followed by HA in the two seasons of this study.

Our findings were in agreement with Suh *et al*., (2014) on tomato; Kamel *et al*., (2014) on cucumber and Moradi *et al*., (2017) on safflower. Promotional effects of falvic and/or humic acid on fruit weight have been mentioned in several studies. Either foliar or soil application of HA has led to significant increment in fruit weight and total yield in pepper plants (Karakurt *et al.,* 2009) as well as early and total yield in tomato plants (Yildirima 2007). These previous results and those of this present study demonstrate that both humic and fulvic acids could increase snap bean yield. Similarly, FA enhanced pods quality, including antioxidant activity, total soluble solids, carotenoids, total phenolic, capsaicin and carbohydrates (Aminifard *et al.,* 2012).

Table 4. Green pods and dry seed yield parameters of Snap bean plants as affected by soil application of some bio-stimulants treatments in late summer seasons of 2017 and 2018.

Treatments	Green Pods Yield (ton./fed.)		Marketable yield (ton./fed.)		Dry seed yield (kg./fed.)	
	I	II	I	II	I	II
Control	4.986 d	4.799 b	4.238 d	4.079 c	695.3 d	689.3 c
PA	5.519 c	5.347 ab	5.077 bc	5.053 ab	868.3 b	840.3 b
SE	5.453 c	5.116 b	4.962 c	4.655 bc	800 c	807.3 b
HA	6.028 b	5.546 ab	5.455 b	5.157 ab	835.3 bc	871 b
FA	6.712 a	6.131 a	6.376 a	5.824 a	1083 a	1068 a

PA: phosphoric acid 2 L/fed, SE: Seaweed extract 1 L/fed, HA: Humic acid 1.5 L/fed and FA: Fulvic acid 1 L/fed.
Means followed by the same letter in the same column are significantly different at the 5% level according to Duncan's multiple range test.

These results might be contributed to the favorable impact of fulvic acid that enhanced transportation of minerals, affected cell membrane, improved plant hormone-like activity, protein synthesis, promoted photosynthesis, modified enzyme activities, solubilized micro and macro elements, reduced active levels of toxic minerals and increased microbial population (Malan, 2015).

Yield is a result of integration of physiological and metabolic performance of plants, any factor that influences these activities at any stage of plant growth actually affect the yield. The increment in green pods and dry seeds yield characteristics may be contributed to the increment in vegetative growth parameters, number and weight of pods which have great impact on total yield and quality. These obtained results are in agreement with the results obtained by Awad *et al.,* (2006); Rathore *et al.,* (2009) and Abd El-Baky *et al.,* (2019). This finding was of great benefits because it enables farmers to sell considerable part of common bean pods earlier with high prices. Again, the enhancing effects of such treatment on snap bean plant yield related to the promotional impact on fruit setting, number and weight of fruits per plant. This also may be due to the previous mentioned favorable impacts of the same treatment on vegetative growth behavior, minerals content, metabolic activity (chlorophyll and carbohydrates content) and the antioxidant bio-constituents, *i.e.* corotenoids and phenols content (Abd El-Basir, 2010).

The treatment with bio-stimulants might be exported sufficient sugars, amino acids, stimulator hormones and defense materials at early stages of flowering and fruiting, which essentially required for the fruit setting activities specially under stress conditions Yang *et al.,* (2002). Moreover, the induce able effect of the internal and/or external antioxidants on early fruit setting under stress conditions may be also due to their protective effect on the most sensitive reproductive in plants organs (pollen grains and ovules) and their viability which in turn enhanced the efficiency of fertilization process and hormonal stimulation (Fathy *et al.,* 2003).

Green pods quality parameters:

Data presented in Table 5 showed that soil application of some bio-stimulants were significantly affected green pods quality parameters of snap bean in the two growing seasons. Also, fulvic acid as a soil treatment gave the highest values for all studied parameters. In contrary, the control plants exhibited significantly the lowest values for all the studied traits in two seasons.

Table 5. Green pods quality parameters of Snap bean plants as affected by soil application of some bio-stimulants treatments in late summer seasons of 2017 and 2018.

Treatments	Protein (%)		Carbohydrate (%)		Acidity (%)		Fibers (%)	
	I	II	I	II	I	II		
Control	3.45 b	3.53 b	16.92 e	15.23 d	0.53 d	0.42 c	6.31 a	6.33 a
PA	3.88 b	3.53 b	18.29 c	16.03 c	0.61 bc	0.49 bc	4.89 d	4.82 d
SE	3.58 b	3.53 b	17.14 d	15.61 cd	0.58 cd	0.46 c	5.24 c	5.13 c
HA	4.72 ab	4.50 ab	18.62 b	16.67 b	0.66 b	0.54 b	6.15 b	6.11 b
FA	6.65 a	6.53 a	18.96 a	17.42 a	0.77 a	0.65 a	4.26 e	4.28 e

PA: phosphoric acid 2 L/fed, SE: Seaweed extract 1 L/fed, HA: Humic acid 1.5 L/fed and FA: Fulvic acid 1 L/fed.
Means followed by the same letter in the same column are significantly different at the 5% level according to Duncan's multiple range test.

Promotional effects of both fulvic and humic acids on pod weight have been reported in several studies. Either foliar or soil applications of humic acid cause significant increment in early yield and total yield in tomato (Yildirima, 2007) as well as the mean fruit weight and total yield in peppers (Karakurt *et al*., 2009). Previous results and those of the present one illustrate that humic and fulvic acids may increase snap bean yield. In the same manner, FA improved pepper fruit quality including antioxidant activity, total soluble solids, total phenolic, capsaicin, carbohydrates, and carotenoids (Aminifard *et al*., 2012). Bio-stimulants enhance the primary metabolism of plants, increasing the levels of free amino acids, carbohydrates, various enzymes, proteins biosynthesis and pigments (Yakhin *et al*., 2017). Also

This results are too far extent proved that the control plants were greatly affected by the prevailing temperature extremes (Table 1) in severe and harmful way during their reproductive stage and probably inducible oxidative stress. Similar responds were reported by Bita and Gerats, 2013. Temperature extremes known to impacts directly on fruit productivity, causing aggressive abortion in flowers and buds as well as impair pollen viability and germination, which in turn cause poor fruit setting (Aloni *et al.,* 2001 and Erickson and Markhart, 2002).

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التأثير التخفيفى للرى التسميدى بأحماض الهيوميك والفولفيك والفوسفوريك ومستخلص الطحالب البحرية لنباتات الفاصوليا المجهدة حراريا تحت ظروف منطقة الدلتا

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تسبب الأجهادات البيئية وخاصة درجات الحرارة المرتفعة ظروف غير ملائمة للنمو مما يؤدي الى زيادة الفاقد من المحصول وتدهور صفات جودة الثمار في محاصيل الخضر. تهدف هذه الدراسة الى معرفة دور اضافة بعض المنشطات الحيوية (أحماض الهيوميك والفولفيك ومستخلص الطحالب وحمض الفوسفوريك) مع ماء الري بالتنقيط علي انتاجية الفاصوليا تحت ظروف الحرارة العالية الطبيعية. أوضحت النتائج تفوق معاملة الاضافة الأرضية لحمض الفولفيك حيث سجل اعلى النتائج لصفات النمو الخضري والمحتوى الكيماوي للأوراق من المعادن وصبغات البناء الضوئي وكذلك المحصول وصفات الجودة. سجلت نباتات معاملة الكنترول أقل النتائج بالنسبة للصفات محل الدراسة نتيجة التأثر بالحرارة المرتفعة تحت ظروف التجربة وذلك في موسمي الدراسة.

الكلمات الدالة: الحرارة المرتفعة – المنشطات الحيوية – حمض الفولفيك - الفاصوليا