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Effect of bio stimulants on some physiological parameters of *Psium satvium*

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ABSTRACT **: Bio stimulants are gaining a more attention as being ecofriendly to human and environment. This study was conducted to evaluate the impact of two bio stimulants (*Spirulina platensis and Trifolium alexandrinum*) on some physiological parameters of one leguminous plant (*Psium satvium L.*).To achieve this goal four treatments were done in this experiment: Control without any treatment, Soil treated with 10 g of powdered *Spirulina*, Soil treated with 10 g of powdered *Trifolium* and finally soil treated with two bio stimulants (10 g *Spirulina* +10 g *Trifolium*).The obtained results clarified that all tested growth parameters were increased .Total pigments were increased with special references to combination of two bio stimulants. Parellel, Total carbohydrates were generally enhanced in all treatments if compared with control Water soluble vitamins as vitamin B Complex ,biotin, choline, folate, vitamin A,E and K were enhanced as a result of application of bio stimulants. On the other hand, No changes were revealed in Vitamin A, B2, B3, B5, B6, Cartenoid (α,B.Carotene, Cryptoxanthin, Leutin and zeaxanthin).**

KEYWORDS: Bio stimulants , Carbohydrates, Psium satvium, Vitamins-Total pigments.

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I. INTRODUCTION

The overuse of synthetic agrochemicals have resulted in massive ecological degradation throughout the world leading to ocean dead zones , eutrophication , soil infertility and biodiversity loss (Kohler and Triebskorn, 2013,Chagnon et al .,2014,Hallmann et al .,2014). Chemical fertilizers are needed to get good crop yields but due to their abuse, they can be harmful for the environment and their cost can make them not economic agricultural products (Bobade et al.,1992). Chemical fertilizers are expensive; cause pollution to the environment .These problems may be avoided by the use of bio fertilizers (AL-Khiat, 2006). There are attempts have been undertaken to substitute chemical fertilizers with bio fertilizers such as cyanobacteria (blue green algae) (Asari et al.,2008). And Trifolium which rich in nutrients (Fulkerson et al.,2007). The role of bio fertilizers in sustainable agriculture recorded special significance , particularly in the present context of high cost of chemical fertilizers (Kannaiyan , 2002). Bio fertilizers are containing living microorganisms or natural compounds derived from organisms improve soil chemical and biological properties , stimulate plant growth and restore soil fertility(Raouf et al .,2012).

Bio fertilizers such as Cyanobacteria (blue green algae) that are capable of fixing atmospheric nitrogen (Asari et al.,(2008). It have many advantages than chemical bio fertilizers , they are non-polluting , inexpensive, utilize renewable resources , in addition to their ability of using free available solar energy to fix atmospheric nitrogen and carbon dioxide.besides they supplying N2 to crops and supply other nutrients such as vitamins and growth substances (Wagner , 1997).

The use of algae as a bio fertilizers provide a possible solution to the previous problems. The bio fertilizers not only enhance agricultural production but also diminish environmental pollution (Kawalekar, 2013).

Cyanobacteria can play a major role in the sustainable agriculture by increasing the soil fertility, crop growth and yield and improvement of the environmental quality (Singh et al., (2016) & Osman et al., (2016). It has been proven that cyanobacteria have the ability to fix atmospheric nitrogen into usable forms for plants and hence, affects rice fields(Pereira et al.,(2008),Sharma et al.,(2010).Also, several reports showed that the beneficial effects of higher plant bio stimulants on the growth and yield of many crops such as (Trifolium) (Elzaawely et al.,2017),garlic cloves(Elzaawely et al.,2008).It well known that legume application as bio stimulant not only increases total soil organic carbon and nitrogen content but also reduces negative

environment impacts (Blesh,2019).Soil incorporated with plant residues from green manure showed activation of the physic-chemical protection of organic carbon(Garcia-Franco et al.,2015)and this triggers plant health by improving the plant nutrition. Trifolium alexandrinum (Egyption clover) is a highly nutritious forage due to its high amounts of nutrients, particularly protein (15-25%DM), minerals (11-19%) and carotene (Fulkerson et al.,2007).Additionally, it is rich in trace elements such as Fe, Zn, Mn and Se.

Materials and Methods

1-Materials

Healthy and viable seeds free from visible infection, with uniform size of pea (Psium satvium L) were obtained from Horticultural Research Institute Center of Egypt.

2-Experimental program

Before cultivation, the field was plowed and laid out into four rows. Each row with 16 lines, each line has 3.5m×80 cm). Each line was treated with powders of Spirulina platensis (A) and Trifolium alexandrinum (F), in addition to control. Three weights from each treatment were applied (2.5, 5, 10 g) for Spirulina platensis (A) and Trifolium alexandrinum (F) separately and in combinations.in three replicates. Then the field was irrigated.

After the irrigation, the seeds were cultivated during winter season (October 2018/march 2019).The time of the experiment was lasted for 4 months, during this period, the phonological parameters were taken and examined weekly. At the end of incubation peroid, the seeds were collected, dried, and ground to fine powder, and then the biochemical analyses were done.

3-Growth characteristic and yield:

Growth parameters like shoot length (cm), leaf area (cm2), number of leaves were recorded in table (1-3).Samples were taken randomly after 45 days from sowing. At the harvest period, Pea samples were taken to determine the yield components like the number of pods/plant, pod length (cm) according to (Rao et al., 1976).

4-Determination of biochemical components:

The plant photosynthetic pigments (Chlorophyll a, Chlorophyll b and Cartenoid) were determined according to the method adapted by Metzner et al.,(1965). Total carbohydrate determined as method described by Said and Naguib(1946). Vitamins were determined according to the method of AOAC (2005).

Results

1-Vegetative growth characteristics:

Data in Tables (1, 2, 3,4,5,6 and 7) clarified that all the studied growth parameters were significantly increased by raising the concentration of the bio stimulants either Spirulina sp or Trifolium sp if compared with control. The same trend was occurred under application of the two fertilizers.

2-Total pigment contents:

Table(2-1) clarified that application of two bio fertilizers (Spirulina platensis (A3) & Trifolium alexandrinum (F3) to soil led to a marked increases in the total pigments in soil treated with F3 , followed by A3 if compared with control since it was 74.342 &34.676 mg/g in F3 ,A3 respectively .The previous increase in total pigment was due to the highly increase in carotenoid (56.047 & 26.035 mg/g) in F3 and A3 respectively .Moreover, The combination of two bio fertilizers resulted in a highly increase in total pigments 76.901 mg/g if compared with control (7.118 mg/g).

3- Total carbohydrate fractions

The analysis of carbohydrate fractions including DRV (Direct reducing value), polysaccharides and TRV (total reducing value) Table (2-2). The application of bio fertilizers led to general increase in total carbohydrate fractions in all treatments if compared with their corresponding control. The highest percentage of increase was recorded in F3 since it was 71.128 mg/g, followed by combination A3F3, since it was 29.312 mg/g followed by A3, since it was 24.534 mg/g. Such increase in total carbohydrate fractions may be attributed to the increase in total reducing sugar with a decrease in polysaccharides.

4- Vitamins Contents

Evaluation of water soluble vitamins such as vitamin B-complex , biotin , choline , folate , pantothenic acid ,Vitamin A,E and K ,Data in Table (2-3) showed that the treatment of Psium satvium by two selected bio fertilizers (Trifolium alexandrinum(10 g) & Spirulina platensis (10 g) resulted in an increase in the amount of vitamin B1 since it was 8.94 mg/g , 7.11 mg/g if compared with control (6.22 mg/g). also Biotin vitamin B7 was also increased since it was 17.46 mg/g , 12.45 mg/g in A3 & F3 respectively if compared with control(7.43). Moreover, vitamin B9 was also increased 4.81mg/g , 3.73 mg/g in A3 & F3 respectively . the same trend was also observed in vitamin B12 ,4.23 mg/g & 3.11mg/g in A3 & F3 respectively .Choline (14.41 mg/g & 12.31 mg/g) respectively , Folate (11.81 mg/g & 9.31mg/g) respectively , vitamin C (13.16 mg/g , 11.27mg/g), vitamin E (11.11mg/g , 8.32mg/g) finally vitamin k (10.52 mg/g , 8.74 mg/g) in A3 & F3

respectively . On the other hand no changes were observed in vitamin B2 , B3 , B5 , B6 ,Folate (DFE) , Folate (Food) and pantothenic acid , vitamin A , Carotenoid (α , B , Carotene , cryptoxanthin , lutein and zeaxanthin).

Discussion

Bio fertilization is an important tool to enhance the yield. It becomes an alternative biotechnology to chemical fertilizers. It has many merits; it is safe for human and environment. It gained significance in sustainable agriculture as it enhancing crop productivity to friendly environment and reducing polluting effects of synthetic fertilizers (Singh et al., 2011). Algae considered as photosynthetic organisms, including prokaryotic cyanobacteria and euokaryotic microalgae as well as a macro forms such as sea weeds (marine forms) (Lee, 2008). The algae either micro or macro- forms have a significant role in environmental carbon sequestration and are responsible for 50% of the total photosynthesis on the earth (Moroney and Ynalvez, 2009). The utilization of cyanobacteria and eukaryotic green microalgae in the Mineralization, mobilization of organic compounds with the production of bioactive compounds such as (polysaccharides, growth hormones, antimicrobial compounds, etc.) can improve the plant growth and thus makes them suitable as bio fertilizers options (Gayathri et al., 2015 and Prasanna et al., 2016a). They have a key role in maintaining the productivity of terrestrial and aquatic ecosystems through photosynthesis and N fixation, and improving the availability of nutrients through cycling and transformations (Moroney and Ynalvez, 2009). Application of nitrogen fixing cyanobacteria called algalization which not only enhanced the nitrogen status of the soil and plant but also minimizes the use of chemical nitrogen fertilizer (Etesami and Alikhani, 2016). Microalgae, especially cyanobacteria are also considered as potential bio control agents as they exhibit antagonistic effect against many plant pathogens such as bacteria, fungi and nematodes, mainly as a result of production of hydrolytic enzymes and biocidal compounds such as benzoic acid, etc. (Gupta et al., 2013). These antimicrobial compounds can suppress pathogenic microbes through the disruption of the cytoplasmic membrane, and inhibition of protein synthesis, etc. (Swain et al., 2017). The inoculation of these organisms influences various metabolic processes in plants as they elicit the activity of plant defense enzymes, thereby transporters, chelating agents etc. that lead to enhanced plant immunity to pathogens, and increase in plant growth and crop yields (Gupta et al., 2013).

The potential use of microalgae as bio fertilizer, for enhancing soil fertility, plant growth, fruit quality and nutritional characteristics and grain yield (Coppens et al., 2016). However, recent studies showed that the inoculation of cyanobacteria could also increase the availability of other micro-nutrients (Zinc, Copper Iron, etc.) and macronutrients (carbon, nitrogen, phosphorus, potassium) in soil and their translocation inside plants, up to grains (Coppens et al., 2016).

The present study highlights the prospects of cyanobacteria (Spirulina platensis) as options for bio fertilization, bioremediation, and as agents for improving soil structure and functioning, and enhancing plant growth and yields. Furthermore, It is known that Trifolium alexandrinum contains high amounts of trace elements like Cu , Fe , Se , Mn , Zn which are important in cell division and enlargement as well as photosynthesis resulting increased shoot growth (Lopez et al.,2008).The yield of pea were significantly affected by using bio stimulants , our results are consistent with (Badr et al.,2014; Zaghloul et al.,2015) who indicated that inoculated pea significantly surpassed on un-inoculated ones in a number of pods/plant , seed yield/plant , leaf area/plant, stem length/plant and pod length/plant. Additionally, Mishra et al.,(2010) said that application of bio stimulants led to a significant increase in the number of pods .This increase may be due to bio stimulants adding organic matter to the soil thus improving soil structure and thus enhancing plant growth and crop yield (Maqubela et al.,2009). By the way, Trifolium as gteen manure ,intercropped with oats in maize, increased maize yield by 10 % and returned 43 kg N/ha(Ghaffarzadeh,1997).The beneficial role of trifolium in plant growth resulting in higher yield due to enhance photosynthesis activity by nitrogen fixation and consistent with (Giambalvo et al., 2011).

Regarding the phenological characters of Psium satvium during different growth stages it was cleared that the addition of two biofertilizers (Spirulina platensis &Trifolium alexandrinum) to the test plant led to a marked increase in all tested parameters (Area of leaves, number of leaves, length of stem, number of pods and length of pods).the tested plant was highly responded to high dose of algae A3, plant F3 and their combinations (A3F3). These results might be due to the addition of biostimulants to plants modi¬fies the morphology of plant roots in a similar way to IAA, suggesting that they induce a “nutrient acquisition response” that favors the uptake of nutrients via an in¬crease in the absorptive surface area (Ertani et al., 2012).Also, they enhance nutrient use efficiency, stimulate plant development and growth (Kunicki et al., 2010; Calvo et al., 2014; Halpern et al., 2015; Le Mire et al., 2016), and eventually enhancing crop quality and yield (Ziosi et al., 2013; Van Oosten et al., 2017).The enhancement effect of algae extract on pea plant growth characteristics may be attributed to the auxin content of the algae extract which has an effective role in cell division and enlargement. This leads to increase the shoot growth, leaves number, and plant dry weight (Gollan and Wright 2006). Moreover, Spirulina

platensis is a rich source of potassium and contains considerable amounts of Ca, Cu, Fe, Mg, Mn, P and Zn(Marrez et al., 2014), which have a great role in cell division and enlargement and induce the photosynthesis and this in turn reflected on a great shoot growth (Lopez et al. 2008). The stimulative effect of algae extract might be attributed to that it contains trace elements and plant growth hormones (required for plant regulator) and high levels of organic matters and fatty acids available to plant which enhances yield parameters (Erulan et al., 2009). Microalgal extract used as a foliar spray application showed an increased plant growth. In particular, a high plant height and a great number of flowers and branches per plant were recorded when plants were sprayed with Arthrospira spp [Garcia et al., 2016] and Shalaby et al., 2014).

In case of pigment contents, addition of the two bio fertilizers (Spirulina platensis(A3) & Trifolium alexandrinum (F3) to soil led to a marked increases in the total pigments in soil treated with F3 , followed by A3 if compared with control since, it was 74.342 &34.676 mg/g in F3 , A3 respectively .The previous increase in total pigment was due to the highly increase in carotenoid (56.047 &26.035 mg/g) in F3 &A3 respectively .Moreover, the combination of two biofertilizers resulted in a highly increase in total pigments 76.901 mg/g ; if compared with control (7.118 mg/g).These results are in harmony with Paudel et al.,2012& Czeczko & Mikos-Bielak ,2004). stated that the most important of biofertilizers or biostimulants as Spirulina platensis and Trifolium alexandrinum is increasing content of chlorophyll in leaves , intensifying photosynthesis , Moreover, a significant increase in the carotenoid content under the influence of biostimulant application was also obtained by (Grabowska et al .,2012). Latique et al., 2013 reported that algae extract increased significantly total chlorophyll content in pea leaf tissues, Haroun and Hussein et al., (2003) said that using of algal bio fertilizers resulted in marked increase in Chl.a ,Chl.b , total chlorophylls and total pigments content of leaves. Higher sugar levels in plants treated with bio stimulants have been found in several species, associated with higher chlorophyll accumulation, net photosynthesis (Abbas and Akladious, 2013; Abdalla, 2013). Bio stimulants improve the primary metabolism of plants, increasing the levels pigments as reported by Yakhin et al. (2017).

With respect to the effect of selected bio fertilizers on carbohydrate contents of Psium sativum seeds, it could be stated that soil amended with two bio fertilizers Trifolium alexandrinum and Spirulina platensis either separately or in combination led to general increase in total carbohydrate fractions if compared with control plant. The highest increase was recorded in F3 followed by A3&F3 and finallyA3.This may be attributed to the increase in total reducing sugar and the decrease in in polysaccharides. These results are in harmony with Grabowska et al.,(2012)& Parallel,A.EBIC.,(2012) said that the application of bio stimulants resulted in the highly significant increase of total and reducing sugars .Moreover, (Adam.1999).Mohamed ELanwar et al.,(2010) said that the inoculation of soil with cyanobacterial species increased carbohydrates of produced pea seeds. Bio stimulants can be associated with an increase in carbohydrate concentration in leaves (Abdalla, (2013). Ghallab and Salem (2001) stated that due to using algal bio fertilizers led to increase in growth characters, nutrients, sugar of tested plant. Bio stimulants improve the primary metabolism of plants, increasing the levels of carbohydrates as reported by Yakhin et al. (2017). Also , The application of bio stimulants were led to an increase in the content of reducing sugars of the plant (Gurav et al,, 2013).Moreover, Dawa etal.,(2014) detected that application of bioferilizers had a significant increase on total carbohydrates, reducing sugar ,non – reducing sugar and total sugar .

Regarding the impact of two selected biofertilizers on vitamins content of Psium satvium Data in table (2-3) showed that the treatment of Psium satvium by high doses two selected bio fertilizers 10 g in case of Trifolium alexandrinum & 10 g in Spirulina platensis resulted in an increase in the amount of vitamin B1 also Biotin, vitamin B7 was also increased . Moreover, vitamin B9 was also increased. The same trend was also observed in vitamin B12 , .Choline, Folate, vitamin C vitamin E finally vitamin k in A3 & F3 respectively . On the other hand, No changes were observed in vitamin B2 , B3 , B5 , B6 ,Folate (DFE) , Folate (Food) and pantothenic acid , vitamin A , Carotenoid (α , B , Carotene , cryptoxanthin , lutein and zeaxanthin).These results agreed with(Nada et al .,2011) detected that a positive influence of bio stimulant on yield parameters was observed as well as on the vitamin C. Similar results have also been reported by Sendur et al. (1998) and Meena et al. (2013) Who indicates that the application of bio fertilizers showed maximum vitamin-C of plants.

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Tables:

Table (1): Phenological characters of *Psium* sativum treated with two selected biofertilizers (*Spirulina platensis & Trifolium alexandrinum*) during vegetative stage.

Treatments	Leaf area	No .of leaves	Length of stem
	No.= 3	No.= 3	No.= 3
Control	5.85±0.85l	4.5 ±0.5i	16.5±1.5i

A1	13.6 ±0.6k	7.5 ±0.5h	29.95 ±0.05h
A2	15.25 ±0.25j	8.5±0.5g	30.5±0.5h
A3	16.4±0.4i	9.5 ±0.5f	31.75 ±0.25g
F1	16.65 ±0.15hi	9.5 ±0.5f	32.5 ±0.5g
F2	17.15 ±0.15gh	10 ± 0.00f	33.75±0.25f
F3	17.55±0.05g	10.5±0.5e	35.5 ±0.5e
A1F1	18.25 ±0.25f	9.5 ±0.5f	35.1 ± 0.4e
A1F2	18.6 ±0.1ef	10.5±0.5de	35.25 ±0.25e
A1F3	19.15±0.15de	11.5±0.5c	36.75±0.25d
A2F1	19.7 ±0.21d	11± 0.00cd	36.5 ± 0.00d
A2F2	20.85 ±0.15c	11.5 ±0.5c	36.75 ±0.25d
A2F3	21.75 ±0.25b	12.5 ±0.5b	37.75 ±0.25c
A3F1	22.25 ±0.25b	11.5 ±0.5c	37.75 ±0.5c
A3F2	23.5 ±0.5a	12.5 ±0.5b	39.5 ±0.5b
A3F3	23.5 ±0.5a	13.5 ±0.5a	40.5 ±0.5a
f	426.235**	65.368**	348.102**
Test of Homogeneity of Variances	1.382 ns	0.512 ns	1.604

One- way ANOVA
L.S.D (Less Significant deference)
(a- a- Non significant difference , a- b significant difference)
Means with different letters within column are significant difference , P ≤ 0.05-0.01
Means with the same letters within column Non significant difference , P ≤ 0.05-0.01
***Significant at 0.05 ** Significant at 0.01.**

A1 :*Spirulina platensis* (2.5 g). A2:*Spirulina platensis*(5 g)
A3: *Spirulina platensis*(10 g). F1:*Trifolium alexandrinum*(2.5 g).
F2:*Trifolium alexandrinum*(5 g). F3:*Trifolium alexandrinum*(10 g).
A1F1: *Spirulina platensis*(2.5 g)and*Trifolium alexandrinum* (2.5 g).
A1F2: *Spirulina platensis*(2.5 g) and (5 g*) Trifolium alexandrinum.*
A1F3: *Spirulina platensis*(2.5 g) and (10 g*) Trifolium alexandrinum*
A2F1: *Spirulina platensis*(5 g) and (2.5 g*) Trifolium alexandrinum*
A2F2: *Spirulina platensis*(5 g) and (5 g*) Trifolium alexandrinum*
A2F3: *Spirulina platensis*(5 g) and (10 g*) Trifolium alexandrinum*
A3F1: *Spirulina platensis*(10 g) and (2.5 g*) Trifolium alexandrinum*
A3F2: *Spirulina platensis*(10 g) and (5 g*) Trifolium alexandrinum*
A3F3: *Spirulina platensis*(10 g) and (10 g*) Trifolium alexandrinum*