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Evaluation of *Spirulina Platensis* as Bio.Stimulator for Organic Farming Systems

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ABSTRACT

The exploitation of *Spirulina platensis* as bio-stimulator for organic- farming systems was studied. Mass production of *Spirulina platensis* biomass was done in polethylene transparent containers (1000 LTRS. capacity) under continuous aeration, at 30 °C, pH9.00 and replacement of Na+ salts by K+ (13 g/L) in the standard medium was more effective for production of bio-stimulator yield. Chemical analysis of *Spirulina platensis* bio-stimulator revealed that it contains: 6.7%N, 2.47%P and 2.14%K as well as adequate amounts of minor elements needed for plant nutrition. It was found that cultivation of *Spirulina platensis* in the modified medium increased its nitrogen and potassium level ten and sixteen times respectively compared to standard medium. Lead was totally absent in *Spirulina platensis* biomass in either standard or modified medium. The bioreactor design was modified to reduce the composting process time. Recorded results for *Spirulina platensis* application as biostimulator revealed that the yield of pepper inoculated with bio-stimulator is recommend than that of NPK.

Key Words: *Spirulina*, organic farming, bio-stimulation.

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INTRODUCTION

Several greenhouses and field studies have been carried out to evaluate the effect of inoculating different crops with cyanobacteria bio-fertilizer *(El-Nawawy and Hamdi 1975, El- Nawawy et al. 1958, Taha and El – Refai, 1963, El-fadl et al. 1964, Shakeeb, 1970, El-Borollosy, 1972, Taha et al. 1973, Khadr, 1975, Ghazal, 1980, Yanni et al. 1988 and Kandil, 1997).* These studies showed that the effect of biofertilizer varied according to the strains used, chemical fertilizers added and crop rotation. In general; researches showed that cyanobacteria under appropriate conditions could be served as useful nitrogen source for crops production *(Littler et al. 2006 and Kemka et al. 2007)*. *Spirulina platensis* is a planktonic blue - green algae found in warm water and alkaline volcanic lakes. It could be cultivated on marginal, unusable and unfertile soils. Wild *Spirulina* sustains hunger flocks of filamentous in the alkaline East African lakes. *Spirulina* biomass consists of about 62% amino acids and it contains also the whole spectrum of natural mixed carotene and Xanthophyl phytopigments which is considered as the richest natural source of vitamin B-12 *(Hendrickson, 1989 and kemka et al. 2007)*. Biomass yield of *Spirulina* species are changed by variation in the culture conditions. Various environmental factors- such as nutrients, light intensity and pH directly affect the growth of organisms *(Danesi et al. 2004, Colla et al. 2007 and Ogbonda et al. 2007)*. The calcium content in the microalga is relatively lower than the other ingredients. Some studies show that a calcium polysaccharide in *Spirulina*, called the Calcium Spirulan (Ca-SP), can inhibit the invasion and metastasis of tumor as well as the replication of enveloped virus *(Hayashi et al. 1996)*. Therefore, researchers have paid much attention to the improvement of the calcium content in *Spirulina*. Algae bio-fertilizer could be used all over the world for inoculating soils to increase crops productivity along desert coast limes, near alkaline lakes and in villages. Algae offer yet another way to help people through soil renewal, providing economic opportunity without resenting to the vicious cycle of chemical fertilizers, soil exhaustion and depending on imports *(Ladygina and Gurevich, 2000). Spirulina* is more efficient at converting resources into protein than conventional grain and meat production as well as conserving soil. Hopefully, future *Spirulina* farms can exploit this advantage and produce inexpensive food from algae *(Postal Sandra, 1989 and kemka et al. 2007).* Protein production from *Spirulina* requires coast about 20 times less than soybeans, 40 times less than corn and 200 times less than beef production this means increased food production, could be achieved returning cropland to forest, consequently reducing the greenhouse effect *(The Hunger project, 1982).*

AIM OF THE STUDY

The aim of the present study is to evaluate the fertilization value of *Spirulina platensis* as biostimulator in a semi pilot plant compared with the recommended dose of NPK and organic fertilization by using of compost on the yeild of sweet pepper in newly cultivated sandy soils.

MATERIALS AND METHODS

Growth Conditions:

Nährlösung für medium

Solution 1 (g/l): $NaHCO_3$ 13.61, Na_2CO_3. 10 H_2O, K_2HPO_4 0.50, distilled water 500 ml.

Solution 2 (g/l): Na NO_3 2.50, K_2SO_4 1.00, Na Cl 3.00, $MgSO_4$ $7H_2O$ 0.30, $CaC_{12}2H_2O$ 0.04, $FeSO_4$ $7H_2O$ 0.01, EDTA 0.08, microelement solution 5ml, distilled water 500 ml.

Autoclave solution 1 and 2, each separately, unite after cooling and add 5ml of applied microelemnt solution .

Spirulina plantensis standard straim Obtained from the********

Microelement solution	Stock solution l (g/L)	Applied solution (ml/L)
$ZnSO_4$ $7H_2O$,	0.1	2.0
$MnSO_4$ $4H_2O$,	0.1	2.0
H_3BO_3	0.2	5.0
CO (NO3)6 H_2O,	0.02	5.0
Na_2MoO_4 $2H_2O$	0.02	5.0
$CuSO_4$ $5H_2O$,	0.0005	1.0
Na_2SeO_3,	0.0005	1.0
distilled water,		981.0
$FeSO_4$ $7H_2O$		0.7
EDTA		0.8g

Spirulina platensis starter inoculum was produced in batch culture under sterile conditions using standard Nährlösung für medium at pH 9.00 *(Von shak, 1987)*. The sodium salts content of the medium was 250 mM, mostly sodium bicarbonate. The previous standard medium was modified to be more effective for the growth of *Spirulina* by replacing the Na^+ salts by k^+ salts in the same concentrations. The flasks of 5 L capacity containing the medium inoculated with *Spirulina platensis* standerd strain obtained from the culture collection of agric. Microbiol. Dept. NRC which was isolated and purified by *Aly (2000)* were incubated at 30°C on a rotary shaker at 3000 rpm and; continuously illuminated with white fluorescent lamps providing 80 M photons /m^2 at the surface of the flasks for 30 days.

Propagation of *Spirulina platansis* biomass. For mass production semi pilot plant was carried out in the field in open yard using 40 tanks each of 1000 litres capacity using modified media. The tanks were filled with the modified medium and arranged in 8 groups each contains five tanks connected together with sub main plastic pipes as shown in Figure (1). The eight plastic pipes were connected separately to main plastic pipe which connected to an air pump compressor. The compressed air was sterilised through air filter before going to pass through growth tanks .The tanks were inoculated by the standard inoculums at the rate of 5 litres each.. The system was operated for 30 days. After the incubation period *Spirulina* biomass was harvested by draining tanks to open basins and left to dry (air drying) for 7 days. The dried biomass was crashed, packed and kept for further use. Macro and microelement as well as amino acids contents of the net *Spirulina* biomass were determined (Tables 3 and 4a,b). The collected dry material was used for preparation of the foliar fertilizer at the rate of 80 g/l. This liquid was sprayed on the grown pepper variety BG - 11 seedlings directly after transplantation. After that the experiment was sprayed every 15 days.

Figure 1: Diagram of the used system for mass production of *Spirulina platensis* bio-fertilizer.

Table 3: Chemical analysis of *Spirulina platensis* under different growth conditions.

Sample	%			ppm					
	N	P	K	Na	Ni	Fe	Zn	Mn	Pb
Standard Cultures	0.08	0.04	0.02	59.7	0.6	5.1	3	2	Nil
Modified medium	0.88	0.06	0.32	63.5	0.12	4.5	7	4.5	Nil
Dry of material Splrulina	6.7	2.47	1.14	11.6	0.75	12.4	292	11.5	Nil

Table 4-a: Amino acids composition of *Spirulina platensis*.

Essential amino acids	Per 10 gram	Total (%)
Isoleucine	350mg	5.6%
Leucine	540mg	8.7%
Lysine	290mg	4.7%
Methionine	140mg	2.3%
Phenylalanine	280mg	4.5%
Threonine	320 mg	5.2%
Tryptophane	90 mg	1.5%
Valine	400 mg	6.5%

Table 4-b

Non-Essential amino acids	Per 10 gram	(%) Total
Alanine	470mg	7.6%
Arginine	430mg	6.9%
Acid Aspartic	610mg	9.8%
Cystine	60mg	% 1.0
Acid Glutamic	910mg	%14.6
Glycine	320mg	5.2%
Hstidine	100mg	1.6%
Proline	270mg	4.3%
Serine	320mg	5.2%
Tyrosine	300mg	4.8%
Acids Amino Total	6200Mg	100%

A field experiment was carried out in sandy soil Table (2). At wadi-El- Natroun area in Egypt. For evaluation of *Spirulina* bio-stimulator as foliar application at the rate of 40 L/ fed in comparison to both compost and the recommended dose of NPK using sweet pepper (Varity BG-11) as test plant and the treatments were as follows:

1. Recommended dose of NPK for pepper as fallows; a 800 kg/ fed as Ammonium sulphate (15.5 % N); 300 kg super phosphate 15% P_2 O_5 and 100 kg potassium sulphate (48 % k_2O).
2. Agricultural wastes compost was used at the rate of 20 T/ fed. The chemical composition of the used compost is illustrated in Table (1).
3. The previously prepared *Spirulina* solution.

Table 2: chemical properties of soil used for cultivation of pepper.

Parameters	Values before cultivation
Organic matter (%)	0.96
pH (1 :2.5)	7.05
EC dSm^{-1} (1:5)	2.7
Soluble cations (meq./L)	
Ca^{++}	16.0
Mg^{++}	5.0
Na^{+}	5.45
K^{+}	2.0
Soluble anions (meq./L)	
CO_3^{--}	1120.0
HCO_3^{-}	1.6
Cl^{-}	10.0
SO_4^{--}	16.85
Total N (ppm)	1120

The experiment was carried out in a complete randomized block design for the 3 treatments with 4 replicates; each treatment comprises 8 rows, each 10 m long and 60 cm width.

Irrigation system:

Drip irrigation system was used at the rate of 4 litres per hours for each plant. Three times per week.

Table 1: Chemical Analysis of compost used for filed experiments.

Parameters	Values
pH	6.88
EC dSm^{-1}	11.82
Solube Cations (meq/L)	
Ca^{++}	42.0
Mg^{++}	42.0
Na^{+}	488.7
K^{+}	97.2
Soluble anions (meq/L)	
CO_3^{--}	-
HCO_3^{-}	38.0
Cl^{-}	369.0
SO_4^{--}	282.9
Total N%	1.63
Total C%	30.6
C/N ratio	18.88:1
OM%	52.6
P%	0.64
K%	0.73
Fe ppm	202.4
Zn ppm	533.0
Mn ppm	141.0
Pb ppm	6.0

Irrigation system:
Drip irrigation system was used at the rate of 4 litres per hours for each plant. Three times per week.

RESULTS

The *Spirulina platensis* isolated from El khadra lake Wadi-El- Natron., has the ability to live under extreme conditions of pH and salt concentration. *Spirulina platensis* showed a pronounced increase in biomass yields by replacing Na^+ salts by k^+ salts in the modified medium at the same concentrations (250 mM). The optimized condition for biomass production was at 30°C and pH 9. The chemical analyses of *Spirulina platensis* produced under different growth conditions (Table 3) clearly showed that the major nutrients (NPK) in the product varied according conditions and preparation. The cultivation of *Spirulina platensis* in the modified medium increased its nitrogen and potassium content ten and sixteen times respectively compared to standard medium. Data in Table (3) revealed that lead is totally absent in *Spirulina* biomass in either standard or modified medium. Analysis of *Spirulina* dry biomass revealed that it contains 6.7; 2.47, 1.14% on dry base of nitrogen, phosphorus and potassium, respectively. Results of the chemical analysis of the soil in this experiment showed that it is very poor in its nutrients especially N and K, which are highly needed for the production of vegetable crops (Table 4a,b). The yield of pepper was gathered 4 times as shown in Table (5). The first collection of pepper was higher in the presence of *Spirulina platensis* compared to the other two treatments which reached 9, 6 and 7 Kg for compost and NPK, respectively. Also, the second collection after two weeks behaved the same trend. On the other hand, the third week revealed that the yields due to both NPK and *Spirulina platensis* fertilization were nearly the same (30 and 28 kg), respectively.

Table 5: The crop yield of pepper (Kg/60 m2) as affected by different fertilization treatments.

Treatment / Yield@	NPK	Spirulina	Compost
First collection	7.00	9.00	6.00
Second collection	6.00	8.90	4.00
Third collection	30.0	28.0	20.0
Forth collection	70.0	50.0	36.0
Total yield collection	113	95.9	66.0

The fourth collection behaved differently as the NPK treatment yielded the highest (70 Kg), while the other two treatments were only 36 and 50 Kg for compost and *Spirulina*, respectively.

DISCUSSION

Spirulina sp. is planktonic photosynthetic filamentous cyanobacterium *(Vonshak et al. 1982)*, identified by the main morphological feature of the genus, i.e. the arrangement of multicellular cylindrical trichomes in a helix along the entire length of the filaments. The *Spirulina platensis* models have contributed significantly to different field applications *(Grawish, 2008 and Yang et al. 2008)*. It is well known that biomass of *Spirulina* has been performed to removal unwanted materials such as excess fertilizer, heavy metals, textile dyes and pesticides from wastewaters *(Chojnacka et al. 2005, Solisio et al. 2006 and Pane et al. 2008)*. So the aim of the study was to exploit *Spirulina platensis* as bio.stimulator for organic farming systems. The *Spirulina platensis* isolated from Wadi- El- Natron., this strain was characterised by extreme conditions of pH and salt concentration. The k^+ salts showed great influence on biomass of *Spirulina platensis*. *Yang et al. (2008)* reported that K^* is a very important element for algae. On the contrary, *Celekli and Yavuzatmaca (2009)* reported that the highest biomass yields were observed at 1.5 g L^{-1} NaCl. The optimized condition for biomass production was at 30°C and pH9. Same result was reported by *Kemka et al. (2007)*. The chemical analyses of *Spirulina platensis* produced under different growth conditions clearly showed that the major nutrients (NPK) in the product varied according conditions and preparation. This result was coincided with *Sassano et al. (2007)*. It was shown that standard culture of *Spirulina* contains lowest amounts of macro and micro elements compared to the modified medium. The cultivation of *Spirulina platensis* in the modified medium increased its nitrogen and potassium content ten and sixteen times, respectively compared to standard medium. *Spirulina* is also attractive for its highly binding ability to many elements *(Mosulishvili et al. 2002 and Zheng and Gao, 2008)*, so it is possible for *Spirulina* to enhance its microoelements content by the strong bioaccumulation. Also biomass production by *Spirulina* species are changed by variation in the culture factors such as nitrogen source *(Danesi et al. 2002 and Colla et al. 2007)*. Results also, revealed that lead is totally absent in *Spirulina* biomass in either standard or modified medium, which is a good indicator for safety use of *Spirulina* as plant growth promoter. The pollution of aquatic ecosystems caused by heavy metals from industrial and domestic sources leads to the bioaccumulation of these toxicants in cyanobacteria and microalgae with damage or inhibition of specific enzymes and transfer of metals through the food web *(He et al. 1998)*. According to the above mentioned results modified medium was chosen for propagation of *Spirulina* biomass. Analysis of *Spirulina* dry biomass revealed that it contains nitrogen, phosphorus and potassium, respectively. These obtained results are in harmony with the findings of *(Ross and Doming, 1990)* who stated that *Spirulina* was used safety along history of safe human consumption. The poverty of essential nutrients from the soil especially N and K, which is highly needed for vegetable crops production. Therefore the use of the bio-fertilizer *Spirulina platensis* may cover this shortage in these nutrients. The first collection of pepper was higher in the presence of *Spirulina platensis* compared to those compost and NPK, respectively. This result could due to high content of the free amino acids of the *Spirulina* product, as well as its content of macro and micro elements. In addition to the presence of some growth promoting substances directly absorbed by the leaves. These substances are absorbed rather faster than the nutrients from the soil by using mineral fertilization or compost. On the other hand, the third week the yields of both NPK and *Spirulina platensis* were nearly the same. The NPK treatment showed the highest yield in the fourth collection, compared to the compost and *Spirulina platensis* respectively. This could due to the effect of NPK addition after every collection of the yield, which was more effective. Foliar compost application showed that *Spirulina* solution stimulated growth and achieved good quality of green pepper plants. This result recommended *Spirulina platensis* to be used as a successful organic fertilizer. According to our knowledge none report in *Spirulina platensis* as organic fertilizer yet. A number of workers observed an increase in rice seed germination, root and shoot growth, weight of rice grains and their protein content with use of cyanobacteria

(Misra and Kasushik, 1989, Singh and Trehan, 1973 and venkataraman and Neelakantan, 1969) have expressed different views regarding the nature of these substances. Some have described them as hormones *(Singh and Trehan, 1973)* while others have described them either as vitamins *(Misra and Kasushik, 1989)* or as amino acids *(Watanabe, 1951 and watanabe and Roger, 1984)*. *Karthikeyan et al. (2007)* clearly indicated that these cynobacteria excrete IAA, amino acids and other growth promoting compounds into their immediate environment, which in turn can stimulate the growth of microbial populations in soil. This study clearly reveals the agronomic potential of these strains, providing the support as ideal candidates for development of inoculants. The simultaneous stimulation of *spirulina* solution could be attributed to its contents of nutrients which easily absorbed by plant leaves during the plant growth compared to the other treatment which were add as soil fertilizer.

CONCLUSION

It could be concluded that the yield of pepper inoculated with bio-stimulator is recommend than that of NPK, using organic farming system, as the decrease in the total yield could be recovered by the high price of crops produced by organic farming: as well as it is more safe from the health point of view.

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