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EFFECT OF BIOSTIMULANTS EXTRACTS ON MORPHO-PHYSIOLOGICAL AND AGRONOMIC TRAITS OF SOYBEAN INOCULATED WITH *RHIZOBIUM* AND MYCORRHIZA

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ABSTRACT: *There is no doubt that decreasing production of vegetable oils became a dire in Egypt, which the ratio of our self-sufficiency not extends beyond 15%. Two field experiments were carried out in the Experimental Farm, Sadat city, Minufiya to study the effect of biostimulants extracts (seaweed and yeast) and bioinoculants (Rhizobium japonicum and/or mycorrhiza) on nodulation, growth performance, nutrients uptake and productivity of soybean (Giza 22 cultivar) plants grown in sandy soil during 2012 and 2013 seasons. The obtained results could be summarized as follows:*

1- Foliar application with yeast extract 1% significantly enhanced nodulation (nodules number and dry weight), microbial activities (nitrogenase, dehydrogenase and mycorrhiza spores populations), growth aspects (plant height, numbers of branches and leaves per plant and shoots dry weight), yield and its components (number of pods plant^{-1}, 100-seed weight, seed yield plant^{-1} and seed and straw yields ha^{-1}) and nitrogen content in seeds and straw compared with the other treatments. However, the plants treated with seaweed extract 2% produced the highest values of total chlorophyll, phosphorus content and oil percentage.

2- Co-inoculation with Rhizobium and mycorrhiza reflected a marked increase on nodulation, microbial activities, growth, yield and chemical constituents over single inoculation with either Rhizobium or mycorrhiza and compared to uninoculated plants depending on symbiotic relationships between mycorrhiza fungi and N_2-fixing bacteria.

3- The interactions between the biostimulants and bioinoculants were found to be significant for all traits studied. Foliar application of yeast extract combined with dual inoculation was the most effective treatment for increasing most abovementioned traits with exception of total chlorophyll, phosphorus content and oil percentage which recorded the highest values by seaweed extract in presence of the same inoculation. On the other hand, the lowest values of all traits were obtained with untreated plants in both seasons.

Key words: *Yeast, Seaweed, Mycorrhiza, nodulation, Yield, Soybean.*

INTRODUCTION

There is no doubt that decreasing production of vegetable oils became a dire in Egypt, especially our local production not exceed 150,000 ton (according to data issued by Ministry of Agriculture, Egypt). The ratio of our self-sufficiency not extends beyond 15% (Osman and Salem, 2011). Arid lands in Egypt represents 97% of the total area, characterized by high temperature, low relative humidity, high rate of evaporation, and little rainfall, leading to degraded soils. Legumes are considered important crops for maintaining soil fertility and crop productivity in degraded soils (Hafeez *et al.,* 2001). Among legumes, soybean [*Glycine max* (L.) Merrill] is an important N_2 fixing crop, cultivated throughout the world. New reclaimed sandy soil is suitable for soybean sowing to avoid the severe competition with other strategic crops in Delta. Soybean is the world's leading source of vegetable oil and its seed contains about 20% oil on a dry weight basis provides approximately 30% of the world's supply of oil. Soybean seeds contain about 40% proteins provides approximately 60% of the world's supply of vegetable protein (Abbasi *et al.,* 2008).

Soybean plants obtains nitrogen and phosphorus directly from the soil and indirectly form symbiotic N_2-fixation and P

dissolving when inoculated with effective strains of *Bradyrhizobium japonicum* and vesicular arbuscular mycorrhiza (AM). Soils in nontraditional areas of soybean production seldom contain sufficient population of naturalized *Bradyrhizobium japonicum* to ensure satisfactory nodulation. In recent years, increased interest in low-input agriculture has seen the growing development and use of commercial biological inoculants to increase the mobilization of key nutrients and enhance their availability to crop plants (Owen *et al.,* 2015). Soybean roots make use of two main important symbiotic systems, *Rhizobium* and mycorrhizal systems. Soybean growth and yield can be increased by the increase N and P supplies from symbiotic root systems (Amiri *et al.,* 2013). Almost all of the nitrogen fixed goes directly into the plant, which improves the nutritional status than uninoculated plants (Unino.). Arbuscular mycorrhiza is symbiotic associations that play a key role in plant nutrition by absorbing and translocating mineral nutrients from soil to host plants. AM fungi can improve the nutritional status of the host plant through uptake of nutrients, especially P, based on extensive mycelium in the soil. The mycelium is accumulate and translocate nutrients and water to the roots, where the fungus grows between and within cortical cells forming arbuscules or intracellular coils and vesicles involved in nutrient transfer and storing (Smith and Smith, 2011). AM can play an important role by provide a direct link between soil and plant roots. Mycorrhiza can increased absorption surface area, and can even act to protect the host plant from pathogens and other environmental stress (Jeffries *et al.,* 2003).

Seaweeds form an integral part of marine coastal ecosystems. It has been estimated that there are about 9,000 species of macroalgae broadly classified into three main groups based on their pigmentation, nutrient values and chemical composition (Dawczynski *et al.,* 2007 and Khan et al., 2009). The benefits of seaweeds as sources of organic matter and fertilizer nutrients have led to their use as soil conditioners and foliar application. Seaweed extract is the rich source of several primary nutrients like K, P; secondary nutrients like Ca, Mg; trace elements like Zn, Cu, Fe, Mn and beneficial elements like Ni and Na. Seaweed extracts stimulate various aspects of growth and development resulting in around good health of the plants (Pramanick *et al.,* 2013). About 19.9 million tonnes of seaweeds and other aquatic algae products are produced annually (FAO, 2010), a considerable portion of which is used for nutrient supplements and as biostimulants or biofertilizers to increase plant growth and yield. The seaweed extract is biodegradable, non-toxic, non-polluting and non-hazardous to humans, animals and birds (Dhargalkar and Pereira, 2005). Seaweeds provide for an good source of bioactive compounds such as carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals, also it contains gibberellins, cytokinins and auxin like growth promoting substances (Osman and Salem, 2011). Numerous studies have revealed a wide range of beneficial effects of seaweed extract applications on plants, such as early seed germination and establishment, increase nutrient uptake from soil (Turan and Köse, 2004), improved crop performance and yield (Rathore *et al.,* 2009 and Osman and Salem, 2011) and elevated resistance to biotic and abiotic stress (Khan *et al.,* 2009).

Yeast extract is a natural component contains many of the nutrient elements, amino acids, carbohydrates, reducing sugars, enzymes and vitamins B1, B2, B6 and B12 which is safe and non-pollutant compounds (Mahmoud, 2001). Also it is a source of cytokinins and protein that enhance cell division and enlargement (Barnett *et al.,* 2000). Moreover, Yeo *et al*. (2000) indicated that yeast extract contains trehalose-6-phosphate synthase which is a key enzyme for trehalose bio synthesis. They suggested that the production of trehalose not only affects plant development but also improves stress tolerance. Many investigators reported that spraying plants with yeast extract improved plant growth (Mahmoud *et al.,* 2013), chlorophyll formation (Hammad and Ali, 2014) and crop

yield and its quality (Al-Tawaha and Ababneh, 2012).

Improving tolerance of soybean plants to the environmental stress by using bioinoculants and biostimulants is aim of this study. Therefore, the present investigation was designed to disclose the influence of different biostimulants extracts from seaweed and yeast on nodulation, enzymes activity, vegetative growth and productivity of soybean plants inoculated with *Rhizobium* and mycorrhiza under sandy soils conditions.

MATERIALS AND METHODS

1.Experimental site and procedures

Two field experiments were carried out during the summer seasons of 2012 and 2013 at the Experimental Farm, Sadat city, Minufiya, Egypt (latitude 30°37' N, longitude 30°50' E). Soil samples of experimental site were randomly collected from depth 0–30 cm using an auger before sowing to analyzed for determination of some physical and chemical properties of the soil according to Jackson (1973) and Chapman and Pratt (1978) as shown in Table (1).

The field experiments were conducted to examine the effect of synergistic interactions between *Rhizobium* bacteria (*Bradyrhizobium japonicum* L.) and mycorrhiza fungi (*Glomus mosseae* L.) on nodulation, growth, NP uptake and yield attributes of soybean under applying seaweed (2%) and yeast (1%) extracts in sandy soil. Foliar applications of extracts were applied at two plant stages; second trifoliolate (V2) and beginning bloom (R1). The experiment included twelve treatments which were the combination of three biostimulants and four bioinoculants. The experimental design was a split plot with three replications. Stimulants extracts were arranged at random in the main plots, whereas bioinoculants treatments were assigned at random in the sub-plots.

2. Preparation of seaweed extract

The seaweed (*Ulva lactuca*) as a chlorophyta (green algae) was used in this study. The seaweed was collected from the Mediterranean Sea coasts of Alexandria city, Egypt. The collected samples were washed with seawater to remove unwanted impurities and transported to the laboratory immediately, then washed using distilled water. The seaweed samples were cut into small pieces, homogenized by grinder with stainless steel blades at ambient temperature, filtered with muslin cloth and stored (Eswaran *et al.,* 2005). The liquid filtrate was taken as 100% concentration of the seaweed extract and diluted to obtain the concentration of 2% using distilled water. Chemical analysis of seaweed extract is presented in Table (2).

3. Preparation of yeast extract

The bread yeast powder was activated by using sources of carbon and nitrogen with the ratio of 6:1. This ratio is suitable to get the highest vegetative production of yeast. Each ml of activated yeast contained about 12000 yeast cells (Barnett *et al.,* 2000). Such technique allowed yeast cells to be grown and multiplied efficiently during conductive aerobic and nutritional conditions. To produce beneficial bioconstituents i.e., phytohormones, carbohydrates, proteins, amino acids, fatty acids, vitamins, enzymes, minerals ….etc, hence allowed such constituents to release out of yeast cells in readily form. The media subjected to two cycles of freezing and thawing for releasing their bioconstituents. The plants were treated with yeast extract at 1%. Chemical analysis of yeast extract is presented in Table (2).

Table 1. Mechanical and chemical properties of the experimental site.

Properties	Particle size distribution (%)			Texture class	pH	E.C. ds/m	O.M. %	Available nutrients (ppm)		
	Sand	Silt	Clay					N	P	K
2012	91.8	3.5	4.7	Sandy	7.3	0.42	0.38	9.4	4.3	19.4
2013	91.5	4.0	4.5	Sandy	7.2	0.46	0.33	9.8	3.5	18.5

Table 2. Chemical analysis of yeast and seaweed extracts.

Yeast extract (according to Mahmoud, 2001)			
Amino acids (mg /100g dry weight)		Vitamins and carbohydrates (mg⁄100g dry weight)	
Arginine	1.99	Vit.B1	2.23
Histidine	2.63	Vit.B2	1.33
Isoleucine	2.31	Vit.B6	1.25
leucine	3.09	Vit.B12	0.15
Lysine	2.95	Thimain	2.71
Methionine	0.72	Riboflavin	4.96
Phenyl alanine	2.01	Insitol	0.26
Threonine	2.09	Biotin	0.09
Tryptophan	0.45	Nicotinic acid	39.88
Valine	2.19	P anthothenic acid	19.56
Glutamic acid	2.00	P amino benzoic acid	9.23
Serine	1.59	Folic acid	4.36
Aspartic acid	1.33	Pyridoxine	2.90
Cystine	0.23		
Proline	1.53	Total carbohydrates	23.20
Tyrosine	1.49	Glucose	13.33
Seaweed extract (according to Kumar and Kaladharan, 2007 and Kavitha *et al.,* 2008)			
Amino acids (%)		Nutrients	
Arginine	0.89	Nitrogen	0.18 %
Histidine	0.31	Phosphorus	0.48 %
Isoleucine	0.38	Potassium	1.89 %
leucine	0.72	Calcium	0.11 %
Lysine	0.46	Magnesium	0.01 %
Methionine	0.19	Sodium	0.13 %
Phenyl alanine	0.60	Iron	256.0 ppm
Threonine	0.99	Zinc	11.87 ppm
Tryptophan	0.13	Copper	15.62 ppm
Valine	0.66	Manganese	13.12 ppm
Glutamic acid	1.40		
Serine	0.94		
Aspartic acid	1.59		
Cystine	0.10		
Proline	0.41		
Tyrosine	0.39		

4. Inoculation with *Rhizobium* and mycorrhiza

The N_2-fixing bacteria (*Bradyrhizobium japonicum* L.) was obtained from microbiological department, Soil, Water, Environ. Research Institute, ARC. For inoculation soybean seeds were coated with Arabic gum solution (20%) as an adhesive agent and rolled into the suspension of bacteria (10^8 cfu / ml) before sowing. The AM fungi (*Glomus mosseae* L.) used was isolated originally from the experimental site. The AM inoculant was built up in pots with *Zea mays* L as the host plant to allow AM fungi to colonize roots and complete their life cycle by forming chlamydospores in the soil. After 60 days of growth the infected sand soil, containing about 45 spores per g soil together with infected root fragments chopped into approximately 0.5 cm pieces and extra radical mycelium, was used as inoculant. The inoculum was previously weighted for each mycorrhizal plot (150 g m^{-2}), spread congenial at the soil surface of each plot, and then mixed into the top 5 cm of soil before sowing.

5. Crop management

The experimental field was prepared after faba bean harvesting. The area of each experimental plot was 14.4 m^2, including six rows, four meters long and 60 cm apart. Seeds of Giza 22 cultivar were sown on April 25^{th} and 27^{th} in first and second seasons, respectively in hills 10 cm apart. Eighteen days after sowing (DAS), plants were thinned to two plants /hill, i.e. 333333 plants ha^{-1}. The experimental soil was cultivated with soybean in several times previously. During soil preparation P_2O_5 and K_2O fertilizers were added at the rates of 74 and 57 kg ha^{-1}. After plant emergence, 48 Kg N ha^{-1} was added to plants in the form of ammonium nitrate (33.5 N%) as a starter stimulant dose. Irrigation water was supplied from a deep well with pH 7.1 and EC 0.31 dS m^{-1}. Each row of soybean had a drip lateral established for irrigation. The distance between laterals is 60 cm and 20 cm spacing between drippers with 4 L h^{-1} discharge rate.

6. Measurements

- Nodulation and microbial activities:

At 60 days after sowing, eight plants were uprooted by mattock at random in each experimental plot. The roots were dipped in water to remove the soil carefully then washed with distilled water. The following data were recorded:

1- Number of nodules /plant.

2- Dry weight of nodules /plant (g.).

3- Mycorrhiza spore populations (count / g. soil).

Mycorrhizal spores were extracted from the rhizosphere soil by the method of Allen *et al.* (1979). Soil samples were randomly collected using an auger. Samples were carefully mixed and saturated amount of 25 g of soil with distilled water for 15 minutes to make 45 ml of solution in a 50 ml centrifuge tube. Centrifuge the suspension at 2000 rpm for 10 minutes. Pouring off the supernatant and resuspending the soil in a 2M sucrose- calgon solution and centrifuged again at 2000 rpm for 10 minutes. The supernatant, containing the spores, was poured into a separatory funnel and let sit for 10 minutes. At this time, mycorrhizal spores will be attached to the glass walls of the separatory funnel. The liquid was then slowly drained out (approximately 10 min.). Spores were washed carefully from the funnel walls with 2 ml of distilled water into a petri dish. The number of spores was counted immediately under electron-microscope. The total number of spores was expressed per gram dry soil.

4- Nitrogenase activity (µmole C_2H_4/ g D.Wt nodules/hr): It was measured in the root samples, chromatography by acetylene reduction assay technique as described by Hardy *et al.* (1973) and Somasegaran and Hoben (1985).

5- Dehydrogenase activity (µg TPF/ g dry soil /24 hours): It was measured in rhizosphere soil, spectrophotometry for triphenyl formazan (T.P.F) produced from the reduction of 2,3,5 triphenyl tetrazolium chloride (T.T.C) using acetone for extraction according to Thalmann (1967).

- Vegetative growth and chlorophyll

Eight guarded plants from each plot were taken randomly at 75 days after sowing to estimate plant height, numbers of branches and leaves /plant, shoots dry weight /plant. Total chlorophyll was measured at 75 DAS with a hand-held chlorophyll meter (SPAD-502, Konica Minolta Company, Japan).

- Yield and its components:

At harvest, eight guarded plants were taken to determine number of pods per plant, seed index and seed yield per plant. Seed and straw yields of inner three rows were determined and converted in ton ha^{-1}.

- Chemical constituents

At maturity, dried seeds and straw samples were collected from each plot for chemical analysis. Oil % in the seeds was determined by Soxhlet extraction apparatus as described by AOAC (2000). The nitrogen (N%) was determined by the micro Kjeldahl method (AOAC, 2000). Phosphorus (P%) was determined by the vanado-molybdate phosphoric acid yellow color method outlined by Jackson (1973).

7. Statistical analysis

All measurements data during the two seasons in this study were analyzed according the methods described by Snedecor and Cochran (1980). Duncan's multiple range test (Duncan, 1955) was used to compare between the treatments mean at probability 5%. Statistical analysis was done using the CoStat package program, version 6.311 (cohort software, USA).

RESULTS AND DISCUSSION

1 Nodulation and microbial activities

Nodulation and enzymes activity as influenced by biostimulants extracts and *Rhizobium* and mycorrhiza inoculation are given in Table (3). Yeast application exhibited the highest nodulation, nitrogenase and dehydrogenase activities and mycorrhiza spores followed by seaweed application**.** Variability in the number and dry weight of nodules among the extracts was higher as compared to that recorded by unsprayed plants. Yeast extract was suggested to participate in a beneficial role during vegetative and reproductive growths due to enhancement promoting hormones (auxin and cytokinins) content in leaves (Abou EL-Yazied and Mady, 2012), carbohydrates accumulation (Barnett *et al.,* 2000) and/or the fact that this substance enhanced cell division and nutritional status. Seaweed extracts stimulate various aspects of growth and nodulation development resulting in around good health of the plants, due to the presence of good amount of P in it, the liquid seaweed fertilizers proliferate root development, enhance root to shoot ratio, thereby, making the plants more able to uptake adequate nutrients from the deeper layer of soil (Pramanick *et al.,* 2013). Significant increases in number of nodules, nodules dry weight and N_2-activity were observed when plants treated with yeast extract at a rate of 3 L fed^{-1} (Zaghloul *et al.,* 2015).

Plants treated with *Rhizobium* nodulated well. The number and dry weight of nodules and enzymes activities in plants inoculated with mycorrhiza plus *Rhizobium* were significantly greater than those of plants inoculated with *Rhizobium* only. Stimulation of N_2-fixing activity is believed to be related to an improvement in P uptake by the plants.

Mycorrhiza has strong mycelia, which expand the area of roots available for absorption of nutrients (Shockley *et al.,* 2004), and then stimulate *Rhizobium* infection. The enhancement of nitrogenase activity and leghaemoglobin content could be attributed to that AM improves the uptake of iron and other nutrients involved in biosynthesis of nitrogenase and leghaemoglobin (Abd-Alla *et al.,* 2014). The diffusion coefficient of phosphate ion in the soil is very small, so the uptake of this nutrient is extensively dependent on the concentration gradient and the diffusion conditions (e.g. water content) of the soil. Therefore, plants with a large root system are superior to other plants with regard to phosphate uptake (Yadav *et al.,* 2007). It is evident that the maximum dehydrogenase activity and mycorrhiza spore count in dual inoculated plants may be due to the fact that both AM fungus and *Rhizobium* are active in

root cortical cells. That means the presence of one symbiont may be influencing the activity of the other (Devi and Reddy, 2001 and Rahman *et al.,* 2010).

With regard to nodulation and changing in enzymes activities and mycorrhiza spores, data in the same Table show that the highest values were obtained from plants sprayed with yeast extract in combined with seed inoculated by *Rhizobium* and mycorrhiza in the two seasons. However, the lowest values of these traits were obtained by plants which were uninoculated and untreated with biostimulants. Such results came along with those obtained by (Zaghloul *et al.,* 2015).

Table 3. Nodulation, enzymes activities and mycorrhiza spores as influenced by biostimulants extracts and bioinoculants.

Treatment	2012					2013				
	Unino.	Rhi	AM	Rhi+AM	Mean	Unino.	Rhi	AM	Rhi+AM	Mean
	Nodules (number plant^{-1})									
Control	2.50 f	30.67 d	7.25 ef	64.83 b	26.31 C	3.08 f	36.33 d	8.92 ef	50.33 c	24.67 C
Seaweed	5.75 ef	42.25 c	11.33 e	82.25 a	35.40 B	4.33 f	39.58 d	8.50 ef	76.83 b	32.31 B
Yeast	4.00 f	60.33 b	9.17 ef	87.83 a	40.33 A	8.08 ef	71.33 b	13.17 e	94.67 a	46.81 A
Mean	4.08 C	44.42 B	9.25 C	78.30 A		5.16 CD	49.08 B	10.20 C	73.94 A	
	Nodules dry weight (g. plant^{-1})									
Control	0.012 g	0.126 e	0.034 fg	0.229 d	0.100 C	0.017 f	0.135 d	0.068 def	0.117 de	0.084 C
Seaweed	0.072 ef	0.308 c	0.080 ef	0.408 b	0.217 B	0.038 ef	0.332 c	0.116 de	0.414 b	0.225 B
Yeast	0.025 fg	0.286 c	0.058 fg	0.728 a	0.274 A	0.028 f	0.300 c	0.087 def	0.697 a	0.278 A
Mean	0.036 D	0.240 B	0.057 C	0.455 A		0.028 D	0.256 B	0.090 C	0.409 A	
	Mycorrhiza spores (number 100 g^{-1} soil)									
Control	8.00 i	12.00 hi	28.00 de	32.00 cd	20.00 C	12.00 e	16.00 de	36.00 c	40.00 bc	26.00 C
Seaweed	20.00 fg	20.00 fg	36.00 bc	40.00 b	29.00 B	16.00 de	16.00 de	44.00 bc	48.00 b	31.00 B
Yeast	16.00 gh	24.00 ef	40.00 b	48.00 a	32.00 A	24.00 d	20.00 de	40.00 bc	60.00 a	36.00 A
Mean	14.67 D	18.67 C	34.67 B	40.00 A		17.33 C	17.33 C	40.00 B	49.33 A	
	Nitrogenase activity (µmole C_2H_4 g. D.Wt^{-1} nodules hr^{-1})									
Control	0.22 l	11.05 e	0.43 i	15.67 d	6.84 C	0.19 k	9.28 f	1.21 j	11.19 e	5.47 B
Seaweed	1.24 hi	16.87 c	2.05 h	24.97 b	12.58 B	3.54 h	18.37 c	4.58 g	28.24 b	13.68 A
Yeast	3.64 g	14.61 d	6.02 f	38.67 a	14.54 A	2.45 i	15.34 d	3.05 hi	35.11 a	13.99 A
Mean	1.70 D	14.18 B	2.83 C	26.43 A		2.06 D	14.33 B	2.95 C	24.85 A	
	Dehydrogenase activity (µg TPF g.$^{-1}$ dry soil 24 h^{-1})									
Control	25.21 l	35.42 g	37.29 ef	39.84 d	34.44 C	22.05 j	34.84 i	40.33 fg	42.61 e	34.96 C
Seaweed	32.54 h	36.74 fg	40.32 d	46.57 b	39.04 B	37.25 h	41.35 ef	39.20 g	47.22 c	41.25 B
Yeast	35.61 g	38.25 e	44.91 c	50.26 a	42.26 A	40.42 fg	44.62 d	50.63 b	53.77 a	47.36 A
Mean	31.12 D	36.80 C	40.84 B	45.56 A		33.24 D	40.27 C	43.38 B	47.86 A	

2. Vegetative growth and chlorophyll

Data in Table (4) show that significant increases in plant height, number of branches and leaves per plant and shoots dry matter in plants treated with yeast extract followed by seaweed extract as compared to unsprayed plants at 75 DAS. The beneficial effect of yeast on plant growth may be attributed to that is a source of plant growth hormones, carbohydrates, amino acids and vitamins which influence on plant metabolism and enzyme activity which in turn encourage vegetative growth (Abou EL-Yazied and Mady, 2012; Abbas, 2013 and Mahmoud *et al.,* 2013). The algae extraction effects may refer to algae extraction contains the nutrient elements and growth hormones that improve nutrients uptake and increased the plant growth (Abd El-Moniem and Abd-Allah, 2008). With regard to photosynthetic pigments, Sridhar and Rengasamy (2011) mentioned that groundnut plants treated with 1 % seaweed liquid enhanced the concentrations of photosynthetic pigments. In this case, the increments of chlorophyll a and chlorophyll b on 30 DAS were more than 105% and 45%, respectively when compared to control.

Co-inoculation with Mycorrhiza and *Rhizobium* significantly produced the tallest plants, larger branches and leaves numbers per plant and the heaviest shoots dry weight compared to uninoculated plants and plants inoculated with either Mycorrhiza or *Rhizobium.* Optimum growth of leguminous plants is usually dependent on symbiotic relationships with mycorrhiza fungi and N_2-fixing bacteria (Xavier and Germida, 2003 and Moradi *et al.,* 2013). Mycorrhizal infection of plant roots usually stimulates plant growth through effects on nutrient uptake, nodulation and N_2 fixation and/or water supply. Plants inoculated with *Rhizobium* either alone or in combination with AM fungi brought about significant changes in total chlorophyll (Arumugam *et al.,* 2010).This stimulating effect may be attributed to an increase in N_2 fixation, which might led to an increase in cytokinins content. Cytokinins are known to delay senescence of plant tissue through their effect on reducing the loss of chlorophyll.

Interaction of co-inoculation and yeast or seaweed extracts application significantly increased growth and chlorophyll over single inoculation with either *Rhizobium* or mycorrhiza and compared to untreated plants with bioinoculants and biostimulants (Table 4). Significant increase in growth may be attributed to the improvement in nitrogen and phosphorus nutrition due to mycorrhiza and *Rhizobium* inoculation as well as the effects of nutrition and hormones due to yeast or seaweed extracts application. The increases in shoots dry matter amounted to 36.19 and 33.09% in the first and second seasons, respectively due to sprayed plants with yeast extract under dual inoculation compared to untreated plants. Similar studies have convincingly demonstrated that foliar application of yeast extract provides pea growth especially when plants inoculated with *Rhizobium and* mycorrhiza (Zaghloul *et al.,* 2015).

3. Yield and its components

Data in Table (5) reveal that significant differences were registered in yield and its attributes (number of pods plant^{-1}, 100-seed weight, seed yield plant^{-1} and seed and straw yields ha^{-1}) among the various biostimulants extracts treatments in both seasons. The greatest yield components were observed by foliar application of yeast extract (1%) which represented about 15.72 and 13.32 % increases in seed yield ha^{-1} in the first and second seasons, respectively compared to untreated plants. The positive effect of yeast extract on yield and its components may be attributed to its beneficial effect during vegetative growths through improving nodulation, photosynthesis, carbohydrates accumulation, flower formation and their set due to its high auxin and cytokinins content (Barnett *et al.,* 2000 and Mahmoud, 2001). Other investigators found that seed yield was significantly increased by seaweed extract (Rathore *et al.,* 2009) and yeast extract (Abou EL-Yazied and Mady, 2012 and Al-Tawaha and Ababneh, 2012) more than untreated legume plants.

Table 4. Soybean growth parameters and chlorophyll content as influenced by biostimulants extracts and bioinoculants.

Treatment	2012					2013				
	Unino.	Rhi	AM	Rhi+AM	Mean	Unino.	Rhi	AM	Rhi+AM	Mean
					Plant height (cm)					
Control	72.28 b	79.69 ab	72.61 b	80.33 ab	76.23 C	75.17 d	83.42 bc	79.94 bcd	83.83 bc	80.59 B
Seaweed	76.89 ab	81.58 ab	79.81 b	86.36 a	81.16 B	77.00 cd	86.19 ab	83.77 bc	88.30 ab	86.09 A
Yeast	77.33 ab	83.28 ab	81.50 ab	88.67 a	82.70 A	81.83bcd	87.67 ab	84.19 bc	92.33 a	86.51 A
Mean	75.50 B	81.52 A	77.97 B	85.12 A		78.50 C	85.76 A	82.63 B	88.15 A	
					Branches (number plant^{-1})					
Control	3.67 c	4.25 abc	4.42 bc	4.50 abc	4.21 C	3.92 b	4.64 ab	4.08 b	4.58 ab	4.31 C
Seaweed	3.92 bc	4.50 abc	4.67 abc	4.83 ab	4.48 B	4.50 ab	4.50 ab	4.58 ab	4.67 ab	4.56 B
Yeast	4.00 bc	4.83 ab	4.92 a	4.92 a	4.67 A	4.42 ab	4.58 ab	4.83 a	5.00 a	4.71 A
Mean	3.86 D	4.53 C	4.67 B	4.75 A		4.28 C	4.57 B	4.50 B	4.75 A	
					Leaves (number plant^{-1})					
Control	12.83 f	13.50 ef	13.17 ef	13.58 ef	13.27 C	12.75 c	14.08 bc	14.08 bc	14.42 abc	13.83 C
Seaweed	14.83bcd	16.08 bc	16.28 abc	17.50 a	16.17 A	14.67abc	14.67 abc	15.00 ab	16.33 a	15.17 A
Yeast	14.08cde	15.33bcd	16.33 abc	16.92 ab	15.67 B	14.25 bc	14.75 ab	15.17 ab	15.75 ab	14.98 B
Mean	13.91 C	14.97 B	15.26 B	16.00 A		13.89 C	14.50 B	14.75 B	15.38 A	
					Shoots dry matter (g. plant^{-1})					
Control	22.05 d	26.15bcd	24.51 cd	28.16 abc	25.22 C	20.79 d	23.75 a-d	21.07 cd	23.89 a-d	22.38 C
Seaweed	25.03 cd	28.76 ab	28.92 ab	29.02 ab	27.93 B	22.95bcd	25.50 ab	25.98 ab	26.17 ab	25.15 B
Yeast	27.53abc	29.25 ab	28.46 ab	30.03 a	28.82 A	23.70 a-d	26.06 ab	24.50 abc	27.67 a	25.48 A
Mean	24.87 D	28.05 B	27.30 C	28.59 A		22.48 D	25.10 B	23.85 C	25.91 A	
					Total chlorophyll (SPAD value)					
Control	29.15 b	33.32 ab	32.66 ab	39.87 a	33.75 C	27.91 d	32.61 a-d	31.45 bcd	38.21 ab	32.55 C
Seaweed	30.85 b	36.83 ab	35.72 ab	41.45 a	36.21 A	32.41 a-d	37.82 abc	33.91 a-d	39.75 a	35.97 A
Yeast	32.51 ab	35.28 ab	34.94 ab	40.41 a	35.79 B	30.92 cd	36.22 abc	35.46 abc	38.51 ab	35.28 B
Mean	30.84 D	35.14 B	34.44 C	40.58 A		30.41 D	35.55 B	33.61 C	38.82 A	

Table 5. Soybean yield as influenced by biostimulants extracts and bioinoculants.

Treatment	2012					2013				
	Unino.	Rhi	AM	Rhi+AM	Mean	Unino.	Rhi	AM	Rhi+AM	Mean
					Pods (number plant^{-1})					
Control	31.00 e	36.75 cd	34.33 de	38.58 a-d	35.17 C	29.42 c	36.08 bc	33.67 c	36.25 bc	33.86 B
Seaweed	37.25 bcd	40.92 ab	39.92abc	41.08 ab	39.79 B	34.83 bc	40.17 ab	38.33 abc	43.25 a	39.15 A
Yeast	40.08 abc	42.75 a	41.92 a	43.17 a	41.98 A	35.00 bc	40.50 ab	41.00 ab	44.58 a	40.27 A
Mean	36.11 D	40.14 B	38.72 C	40.94 A		33.08 C	38.92 B	37.67 B	41.36 A	
					100-seed weight (g.)					
Control	12.69 b	14.05 a	13.35 ab	14.37 a	13.62 B	12.43 c	13.12 b	13.08 b	14.20 ab	13.21 B
Seaweed	13.22 ab	14.32 a	14.49 a	14.59 a	14.16 A	13.90 ab	14.29 a	14.47 a	14.65 a	14.33 A
Yeast	14.13 a	14.41 a	14.57 a	14.96 a	14.52 A	13.95 ab	14.48 a	14.43 a	14.92 a	14.45 A
Mean	13.35 C	14.26 B	14.14 B	14.64 A		13.43 C	13.96 B	13.99 B	14.59 A	
					Seed yield (g plant^{-1})					
Control	10.37 c	13.03 ab	11.13 bc	13.27 ab	11.95 C	11.61 c	13.10 abc	12.44 bc	13.36 abc	12.63 B
Seaweed	11.55 abc	13.36 ab	13.77 ab	14.30 a	13.25 B	13.51 abc	14.82 ab	14.07 abc	15.04 a	14.35 A
Yeast	12.10 abc	14.13 a	13.40 ab	14.34 a	13.49 A	13.06 abc	14.69 ab	14.33 ab	15.40 a	14.37 A
Mean	11.34 D	13.51 B	12.77 C	13.97 A		12.73 D	14.20 B	13.61 C	14.60 A	
					Seed yield (ton ha^{-1})					
Control	2.326 d	3.026 abc	2.705 cd	3.157 abc	2.804 C	2.580 c	3.418 ab	2.821 bc	3.457 ab	3.069 C
Seaweed	2.762 bcd	3.227 abc	3.033 abc	3.310 ab	3.083 B	3.098 b	3.457 ab	3.473 ab	3.551 ab	3.395 B
Yeast	2.848 bc	3.317 ab	3.222 abc	3.589 a	3.244 A	3.212 b	3.540 ab	3.489 ab	3.670 a	3.478 A
Mean	2.645 D	3.190 B	2.986 C	3.352 A		2.964 D	3.472 B	3.261 C	3.560 A	
					Straw yield (ton ha^{-1})					
Control	4.435 c	5.494 ab	4.906 b	5.549 ab	5.096 B	4.277 c	5.019 ab	4.890 bc	5.201 ab	4.847 B
Seaweed	5.178 b	5.617 ab	5.635 ab	5.993 a	5.606 A	5.050 ab	5.402 ab	5.321 ab	5.477 ab	5.313 A
Yeast	5.471 ab	5.600 ab	5.615 ab	6.084 a	5.693 A	5.128 ab	5.387 ab	5.521 ab	5.676 a	5.428 A
Mean	5.028 D	5.570 B	5.385 C	5.875 A		4.818 C	5.269 B	5.244 B	5.451 A	

The data in the same Table show that yield and its components were significantly increased by inoculation with *Rhizobium* and mycorrhiza either separately or together as compared with uninoculated plants in the two growing seasons. The yield increases with co-inoculation of *Rhizobium* and mycorrhiza were much higher than with a single inoculation using *Rhizobium* or mycorrhiza. The results from this experimental show that mixed inoculants can be an efficient biological fertilizer that maximizes soybean yields. As an average of the two seasons, the increase in seed and

straw yields ha^{-1} due to dual inoculation amounted to 23.23 and 15.03 % more than the uninoculated plants, respectively. This superiority may be attributed to a greater amount of assimilates and nutrients uptake over the single inoculation which contributed to dry matter accumulation and this in turn to increase the yield. The increase in seed yield obtained herein is well agrees with the increases in the number and dry weight of nodules, enzymes activity (Table 3) and growth and photosynthetic pigments (Table 4). These findings are in harmony with those of other researchers who found that yield and its components were increased by dual inoculation with *Rhizobium* and mycorrhiza (Rahman *et al.,* 2010; Ngakou *et al.,* 2012 and Amiri *et al.,* 2013) compared to uninoculated legume plants.

The interaction between biostimulants and biofertilizers inoculation on yield attributes had significant effect. The data in Table (5) show that co-inoculation with *Rhizobium* and mycorrhiza in combination with yeast extract application was the best for achieving maximum yield and its attributes in the two seasons. As an average of both seasons, it can be noticed that this combination treatment caused an increase in seed yield $plant^{-1}$, seed yield ha^{-1} and straw yield ha^{-1} amounted to 35.30, 47.96 and 34.98% more than uninoculated plants, respectively. From these results, it could be concluded that the application of the previous combined treatment may be recommended for promoting the plant growth characteristics which led to an encouragement the seed formation owning to increasing the plant capacity in building metabolites, and this in turn increased in the yield and its components. These results are in agreement with those obtained by Zaghloul *et al.* (2015) who indicated that yeast application in combined with inoculated pea plants with *Rhizobium* and mycorrhiza significantly surpassed untreated plants in number of pods, 100-seed weight and seed yield.

4. Chemical constituents

It can be seen from Table (6) that the application of any extracts caused significant increase in oil and NP percentages more than untreated plants in both seasons. Plants received seaweed extract produced higher oil and P% than that obtained by rest treatments. In this concern, Osman and Salem (2011) reported that seaweed application improved nutrients uptake, thus foliar application of seaweed extract could be a promising option for yield enhancement and high oil production. However, N percentage in seed and straw reached the highest content in plants treated with yeast. These results could be also due to the fact that yeast extract contains growth regulators and a relatively large proportion of free amino acids which led to more nodulation and nitrogen fixation, consequently increased accumulated nitrogen. These trends are in agreement with those obtained by Abbas (2013), Mahmoud *et al.* (2013) and Hammad and Ali (2014).

Obtained results in the same table demonstrated that mean values of oil and P percentages were significantly influenced by seed inoculation compared to uninoculated plants. It can be noticed that the mycorrhiza inoculation seemed to be the most effective treatment for increasing oil and P percentages, especially when combined with *Rhizobium*. This stimulative effect might be due to that mycorrhiza Helps plants to absorb and mobilize primarily phosphorous along with other important macro and micro elements and water. In this concern, Ewais (2006) stated that P element is a pathway to improve oil content which is a main constituent of phospholipids, phosphoproteins, nucleic acid and coenzymes. N uptake by seed and straw was significantly influenced by single inoculation of *Rhizobium* over control. Dual inoculation (*Rhizobium* and Mycorrhiza) showed significant effects over single inoculation. The lowest NP uptake was recorded in uninoculated plants. Similar results were obtained by Devi and Reddy (2001) and Rahman *et al.* (2010).

Concerning the interaction effect between biostimulants and bioinoculants, it can be noticed from Table (6) that the application of yeast extract enhanced the effect of dual inoculation in increasing the

chemical constituents of soybean seed and straw in both seasons. The increases in nitrogen of seeds and straw amounted to 23.88 and 37.01%, respectively when plants sprayed with yeast extract in presence of dual inoculation more than untreated plants. However, the increases due to seaweed application in combined with co- inoculants amounted to 12.36, 47.01 and 72.51 for oil, seed phosphorus and straw phosphorus, respectively more than untreated plants which had the lowest averages. In this concern, Zaghloul *et al.* (2015) showed that significant increases were observed in seed chemical constituents, i.e. N, P, K and total protein when plants inoculated with biofertilizers in combination with foliar application of yeast extract.

Table 6. Oil, N and P percentages in soybean plant as influenced by biostimulants extracts and bioinoculants.

Treatment	2012					2013				
	Unino.	Rhi	AM	Rhi+AM	Mean	Unino.	Rhi	AM	Rhi+AM	Mean
					Oil (%)					
Control	18.62 c	18.98 abc	19.68 abc	19.71 abc	19.25 C	18.43 c	19.87 abc	20.14 abc	20.21 abc	19.66 B
Seaweed	18.84 bc	19.29 abc	20.45 abc	20.60 a	19.80 A	18.89 bc	19.77 abc	20.61 ab	21.03 a	20.08 A
Yeast	18.65 c	19.14 abc	20.27 ab	20.48 ab	19.64 B	18.46 c	19.11 abc	20.67 ab	20.70 ab	19.74 B
Mean	18.70 C	19.14 B	20.13 A	20.26 A		18.59 C	19.58 B	20.47 A	20.65 A	
					Seed nitrogen (%)					
Control	4.86 c	5.29 bc	4.91 c	5.18 bc	5.06 C	4.94 d	5.52 bc	4.97 d	5.49 bc	5.23 C
Seaweed	5.17 bc	5.39 abc	5.37 abc	5.64 ab	5.39 B	5.25 cd	5.57 abc	5.51 bc	5.88 abc	5.55 B
Yeast	5.40 abc	5.68 ab	5.43 abc	6.04 a	5.64 A	5.79 abc	5.93 ab	5.71 abc	6.10 a	5.88 A
Mean	5.14 D	5.45 B	5.24 C	5.62 A		5.33 D	5.67 B	5.40 C	5.82 A	
					Straw nitrogen (%)					
Control	2.63 c	3.12 abc	2.78 bc	3.03 abc	2.89 C	2.72 c	2.99 bc	2.82 c	3.12 bc	2.91 C
Seaweed	2.84 abc	3.24 ab	3.17 abc	3.39 ab	3.16 B	2.92 bc	3.16 bc	2.91 bc	3.22 bc	3.05 B
Yeast	3.33 ab	3.40 a	3.38 ab	3.44 a	3.39 A	3.21 bc	3.52 ab	3.17 bc	3.89 a	3.45 A
Mean	2.93 B	3.25 A	3.11 AB	3.29 A		2.95 C	3.22 B	2.97 C	3.41 A	
					Seed phosphorus (%)					
Control	0.380 c	0.433 bc	0.430 bc	0.460 abc	0.426 C	0.413 c	0.467 bc	0.487 abc	0.501 abc	0.467 C
Seaweed	0.486abc	0.497 abc	0.586 a	0.589 a	0.540 A	0.517 ab	0.526 ab	0.564 a	0.577 a	0.546 A
Yeast	0.470abc	0.541 ab	0.506 abc	0.533 ab	0.513 B	0.504abc	0.517 abc	0.523 ab	0.540 ab	0.521 B
Mean	0.445 D	0.490 C	0.507 B	0.527 A		0.478 D	0.503 C	0.525 B	0.539 A	
					Straw phosphorus (%)					
Control	0.153 c	0.207 abc	0.240 ab	0.247 ab	0.212 C	0.178 c	0.253 ab	0.260 ab	0.261 ab	0.238 C
Seaweed	0.183 bc	0.250 ab	0.260 a	0.273 a	0.242 A	0.218 bc	0.222 abc	0.275 ab	0.298 a	0.253 A
Yeast	0.217abc	0.220 abc	0.203 abc	0.263 a	0.226 B	0.214 bc	0.223 abc	0.270 ab	0.282 a	0.247 B
Mean	0.184 C	0.226 B	0.234 B	0.261 A		0.203 D	0.233 C	0.268 B	0.280 A	

Conclusion

It can be concluded that the application of biostimulants has been effected on several metabolic processes, enhances plant growth and development via the increasing of photosynthesis, nutrients uptake and protein synthesis. It is a common practice to grow nodulated plants on poor agricultural soils to increase their fertility. The co-inoculation of *Rhizobium* and mycorrhiza was more effective than single inoculation. The synergistic effects between bioinoculants and biostimulants extracts could be important for efficient development to enhance soybean tolerance to environmental conditions in sandy soils and therefore improve the plant nutritional status, growth performance and yield.

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تأثير المستخلصات المحفزة للنمو على الصفات المورفوفسيولوجية والمحصولية لنباتات فول الصويا الملقحة بالريزوبيا والميكروهيزا

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الملخص العربي

ليس هناك شك في أن انخفاض إنتاج الزيوت النباتية في مصر أصبح وضعاً ينذر بالخطر ، حيث لا تتجاوز نسبة الاكتفاء الذاتى ١٥% . لذا أجريت تجربتان حقليتان بالمزرعة التجريبية بمدينة السادات – محافظة المنوفية بهدف دراسة تأثير الرش بالمستخلصات المحفزة للنمو (مستخلص الخميرة ١% ، مستخلص الأعشاب البحرية ٢%) و اللقاحات الحيوية (الريزوبيا ، الميكروهيزا) على تكوين العقد الجذرية والنمو الخضرى وامتصاص العناصر الغذائية وانتاجية وجودة فول الصويا (صنف جيزة ٢٢) بالأراضى الرملية خلال موسمي الزراعة ٢٠١٢ ، ٢٠١٣ ويمكن إيجاز أهم النتائج المتحصل عليها على النحو التالي :

١- أظهر رش النباتات بمستخلص الخميرة بتركيز ١% تفوقاً ملحوظاً لقيم معظم صفات تكوين العقد الجذرية (أعداد و أوزان العقد الجذرية للنبات) والنشاط الميكروبي (نشاط انزيمى النيتروجينيز والديهيدروجينيز ، عدد جراثيم الميكروهيزا بالتربة) وصفات النمو الخضرى (ارتفاع النبات ، عدد الافرع والاوراق للنبات ، الوزن الجاف للمجموع الخضري) والمحصول ومكوناته (عدد القرون على النبات ، وزن ١٠٠ بذرة ، محصول البذور للنبات ، محصول البذور والقش للهكتار) و المحتوى النيتروجينى بالبذور والقش وذلك مقارنة ببقية المعاملات الاخرى، في حين أعطت النباتات المعاملة بمستخلص الأعشاب البحرية بتركيز ٢% أعلى قيم الكلورفيل الكلى ومحتوى البذور والقش من الفوسفور ، محتوى البذرة من الزيت وذلك خلال موسمي الزراعة.

٢- تفوق التلقيح الحيوى المزدوج (الريزوبيا + الميكروهيزا) فى تحسين صفات تكوين العقد الجذرية ، النشاط الميكروبي ، النمو الخضرى ، المحصول ومكوناته ، التركيب الكيماوى مقارنة بالتلقيح الفردى بأى من الريزوبيا أو الميكروهيزا وذلك نتيجة لوجود علاقات تعاونية بين فطر الميكروهيزا وبكتريا تثبيت الآزوت الجوى. هذا وقد سَجلت النباتات غير الملقحة أقل القيم خلال موسمى الزراعة.

٣- تشير نتائج التفاعل بين عاملى الدراسة إلى أن معظم الصفات المدروسة (صفات تكوين العقد الجذرية ، النشاط الميكروبي ، النمو الخضرى ، المحصول ومكوناته ، التركيب الكيماوى) قد زادت زيادة معنوية خلال موسمي الزراعة بتلقيح النباتات بالريزوبيا + الميكروهيزا ورشها بمستخلص الخميرة. هذا وقد اعطت النباتات التي لم تعامل بأي من تلك المستخلصات أو اللقاحات الحيوية أقل القيم للصفات المدروسة خلال موسمى الزراعة.