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ROLE OF BIOEFFECTORS AND SOIL AMELIORATES ON COWPEA YIELD GROWN ON SALINE SOIL WITH AID OF ^{15}N ISOTOPE DILUTION TECHNIQUE

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ABSTRACT: A field experiment was conducted on salt affected soil cultivated with cowpea crop (*Vigna unguiculata*) under drip irrigation system to evaluate the impact of using mineral fertilizer, fulvic acid, and seaweeds with or without bio-inoculation to combat soil salinity stress. All soil ameliorates were applied in combination with or without *Aspergillus terreus* inoculation. Seeds of cowpea plants were inoculated with *Bradyrhizobium* spp for all fertilization and amendment treatments. ^{15}N isotope dilution technique was followed to accurately distinguish between N-derived sources gained by cowpea crop. Results indicated that shoot fresh weight of plants treated with full dose of mineral fertilizer was enhanced by fungal inoculation comparing to the un-inoculated plants. Similar trend, but to somewhat lower extent, was noticed with both root and seeds. Fresh weight of shoot, root and seeds of plants either inoculated or not were decreased, except shoot of un-inoculated one, when seaweeds were added as compared to those fertilized with mineral fertilizer. Similar trend, but to somewhat higher extent, was noticed with fulvic acid treatment. Contrary, seeds of plants were severely decreased in the presence of fulvic acid comparing to those treated with seaweeds or mineral N-fertilizer. The positive effect of fungal inoculation on enhancement of fresh weight was noticed with plants fertilized with mineral N-fertilizer. Nitrogen uptake by shoots of inoculated plants was higher in case of mineral fertilizer than those recorded with seaweeds but nearly similar to those induced by application of fulvic acid. In contrast, the un-inoculated plants didn't reflect any significant variation between the fertilization treatments. N uptake was increased with application of mineral fertilizer comparing to those resulted from application of seaweeds or fulvic acid. But the effect was not significant in fungal inoculated plants. N uptake by seeds of inoculated plants fertilized with full dose of mineral fertilizer was higher than those of plants treated with fulvic acid but nearly closed to those induced by addition of seaweeds. The highest values of nitrogen derived from fertilizer (Ndff) were recorded with application of mineral fertilizer. This holds true for all plant organs. Remarkable enhancement of Ndff was noticed in case of fungal inoculated plants comparing to the un-inoculated ones. Nitrogen derived from air (%Ndfa) ranged from 35% up to more than 60% depending on plant organ and fungal or non-fungal inoculation treatments. Ndfa were higher in un-inoculated plants than those recorded with the inoculated one. Seeds accumulate more Ndfa than roots or shoot.

Key words: Bio-effectors, cowpea, fulvic acid, ^{15}N technique, salinity, seaweed.

INTRODUCTION

Soil salinity is one of the major stresses severely harm plants especially in drylands which suffered from water scarcity. Under these conditions the grown plants may adversely be affected at physiological scale and the physico-chemical properties could be reduced causing

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improper conditions for healthy growth of grown crops (Munns and Tester, 2008). Various plant growth and development processes are adversely affected by salt stress as a major environmental stress, resulting in reduce of seed yield and crop quality. In this regard, the salt tolerance of vegetable crops is important because the cash value of vegetables is usually high compared to field crops (Gorai *et al*., 2010).

Cowpea, as one of beans crops, has a high nutritional value, and it is rich in proteins, carbohydrates, and mineral salts. Seeds contain 24-28% protein, 48-56% carbohydrate, 1.5% fat, and good amounts of vitamins C, B and minerals (Tshovhote *et al.,* 2003). Many ways were followed to compete salinity stresses, seaweeds, as organic additive, is considered one of them. It includes the macroscopic, multi cellular marine algae that commonly inhabit the coastal regions of the world's ocean where suitable substrata exist (Ugarte *et al*., 2006). Brown seaweeds are the most commonly used in agriculture (Blunden and Gordon, 1986) such as *Fucus* spp., *Laminaria* spp., *Sargassum* spp. and *Turbinaria* spp. are used as bio fertilizers in agriculture (Hong *et al*., 2007). The benefits of seaweeds as source of organic matter and fertilizer nutrients have been demonstrated earlier (Blunden and Gordon, 1986). Approximately 15 million metric tons of seaweed products are produced annually (FAO, 2006), considerable portion of which issued for nutrient supplements and as bio stimulants or bio-fertilizers to increase plant growth and yield. Biostimulants (Bio-effectors) are defined as ‘‘materials, other than fertilizers, that promote plant growth when applied in small quantities’’ and are also referred to as ‘‘metabolic enhancers’’ (Zhang and Schmidt, 2000). The beneficial effects of seaweed extract application on plants, such as early seed germination and establishment, improved crop performance and yield, elevated resistance to biotic and abiotic stresses, and enhanced post harvest shelf-life of perishable products (Norrie and Keathley, 2006). Seaweed components include macro- and micro-nutrients, amino acids, vitamins, cytokinins, auxines and abscisic acid- like growth substances affect cellular metabolism in treated plants leading to enhanced growth and crop yield. Seaweed extracts are bioactive at low concentrations diluted as 1:1000 or more (Stirk *et al.,* 2003).

In addition, humic and fulvic substances are the major organic components of lignites, soil, and peat. Humic and fulvic acids are produced by the biodegradation of organic matter resulting in a mixture of acids containing phenolate and carboxyl groups. Fulvic acid are humic acid with a higher oxygen content and lower molecular weight (Bulgari *et al*., 2015). Seaweed extract and humic acid increased leaf hydration under dry soil conditions as well as root growth, shoot growth, and antioxidant capacity (Zhang *et al.,* 2002; Zhang and Ervin, 2008). Moreover, these extracts are high in cytokinins, combined with humic acid which increased drought tolerance as well as endogenous cytokinin content (Zhang and Ervin, 2004).

Therefore, this work aimed at evaluation of bio-effector fungi, seaweeds, and fulvic acid used as soil ameliorates to compete the adverse stresses of soil salinity. ^{15}N isotope dilution technique was used to accurately distinguish between N-derived sources or gained by the tested crop.

MATERIALS AND METHODS

Field experiment was conducted in the farm of Soil and Water Research Department, Inshas, Sharquia Governorate, Egypt. Salt affected soil with sand texture, pH, 7.94; EC, 13.2 dS m^{-1}; OM%, 0.37; Available-N, 10.7 mg kg^{-1}; was transferred from Ras Suder valley, South Sinai Governorate, Egypt. Soil was spread out in grooves with 0.6 m width, 9 m long and 0.6 m depth per plot. Cowpea seeds (*Vigna unguiculata*) var. Kareem 1, were provided by Legume Department, Field Crops Research Institute, Agriculture Research Center, Giza, Egypt. Soil chemical and physical analyses were carried out according to Carter and Gregorich (2008).

Field Experiment Description

Treatments of mineral N-fertilizer, seaweeds and fulvic acid were randomly distributed in the field plots with three replicates under drip irrigation system. Seaweeds and fulvic acid were applied at 1 g l^{-1}, concentration. Some chemical composition of both fulvic (Talkha Fertilizer

Company, Egypt,) and seaweeds (HUMIN TECH GmbH Company, Germany) are listed in Table 1. Completely randomized block design was followed. Each treatment was applied with and without fungal inoculation treatments. Size of plot was 5.4 m^2 with 0.6 m width and 9 m long. Cowpea seeds were cultivated at rate of 10 seeds per hill then thinned to 5 seedlings after 15 days from planting. Cultivation took place on 5^{th} October 2015 and plants harvested on the 1^{st} March 2016. Irrigation was carried out twice a week according to water requirement of cowpea crop.

Fertilization Treatments

Recommended dose of phosphorus (25 kg P $plot^{-1}$, equal to 357 kg P ha^{-1}), and potassium (8.5 kg K $plot^{-1}$, equal to 119 kg K ha^{-1}) were added at the beginning of experimental practices during soil preparation for cultivation, in the form of super-phosphate and potassium sulfate, respectively. Urea-N fertilizer was added at rate of 25 kg $plot^{-1}$, equal to 357 kg ha^{-1} in the ^{15}N labeled urea form with 2% ^{15}N atom excess which splitted into three equal doses applied at 21, 45 and 75 days from cultivation. Labeled fertilizer was added to micro-plot with 0.6 m width and 1.5 m long equal to 0.9 m^2. One quarter of ^{15}N labeled urea was added in combination with seaweeds or fulvic acid treatments.

Biofertilization treatments

All abovementioned mineral or other fertilization treatments were also applied under fungal inoculation with *Aspergillus terreus* provided by Soil Microbiology Unit of Soil and Water Research Department, Atomic Energy Authority, this fungi was classified as halophytic bio-agent which helps in promotion and improvement of plant growth by increasing the availability of some nutrients especially from organic sources under salinity condition .Fungal inoculant was applied into the soil at rate of 500 ml suspended solution containing 5×10^8 viable spores. Seeds of cowpea were inoculated with *Bradyrhizobium* spp., which compatible with tested crop as described by Vincent (1970). Wheat crop was used as a reference crop to determine and calculate the portion and absolute figures of nitrogen derived from air using the $^{14}N/^{15}N$ isotope dilution concept described in IAEA (2001). In this regard, following standard equations were used for quantifying N derived fro fertilizer (Ndff) and N derived from air (Ndfa):

Ndff (%)=^{15}N (%) atom excess in treated plants/ ^{15}N (%) atom excess in fertilizer × 100

Ndfa (%) = 1 - ^{15}N (%) atom excess in inoculated plant/ ^{15}N% atom excess in reference plant × 100

Statistical Analysis

Data of the current study were statistically analyzed using Statistical Software Program (PC-Mstat) according to Power (1985). Means of treatments were compared with the least significant deference (LSD) at the 0.05 level according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Dry and Fresh Weight

Fresh weight of shoot was enhanced with fungal inoculation (*Aspergillus terreus*) when compared to the un-inoculated plants that fertilized with full dose as mineral fertilizer (Table 2). Relatively, the increments account for 10% over the un-inoculated treatment. Similar trend, but to somewhat lower extent, was noticed with both root and seeds fresh weight. Increments in root and seeds of inoculated plants over those un-inoculated were insignificant. Fresh weight of shoot, root and seeds of plants either inoculated or not were decreased, except shoot of un-inoculated one, when treated with seaweeds as compared to those fertilized with mineral fertilizer. Similar trend, but to somewhat higher extent, was noticed with shoot and root when plants treated with fulvic acid. Contrary, seeds were severely decreased in the presence of fulvic acid comparing to seaweeds or mineral N-fertilizer. Generally, the lowest value of cowpea fresh weight was detected with root (143 kg ha^{-1}) of inoculated plants when fertilized with seaweeds. The positive effect of fungal inoculation on enhancement of fresh weight was noticed with plants fertilized with mineral N-fertilizer. Application of seaweeds and fulvic acid reflected the superiority of un-inoculated plants over the inoculated one when fresh weight of shoot and root was considered. In case of seed's fresh weight, inoculated plants were superior over the un-inoculated plants under all fertilization treatments. Similar trends were observed with dry matter yield of plant organs.

Table 1. Fulvic acid and seaweeds gradient

Fulvic acid	Value	Seaweeds	Value
Fulvic acid	38%	Algenic acid (*W/W*)	50%
N organic	1.5%	K_2O (*W/W*)	16%
K_2O	2%	Fe	1%
Fe	0.3%	P_2O_5	4%
Zn	0.2%		
Mn	0.2%		
Mo	0.5%		
pH	4		
Density	1.28 kg l^{-1}		

Table 2. Effect of fertilization treatments and inoculation on fresh and dry weight (kg ha^{-1}) of cowpea plants grown on salt affected soil

Plant organ	Shoot			Root			Seed		
	Fungal inoculation								
Fertilization treatments	With	Without	Mean	With	Without	Mean	With	Without	Mean
	Fresh weight (kgh^{-1})								
Urea-N	1680	1524	1602.0	273.0	261	267.0	1550	1450	1500.0
Seaweeds	1084	1548	1316.0	143.0	188	165.5	1464	909	1186.5
Fulvic acid	1580	1974	1777.0	168.0	193	264.5	869	557	976.5
Mean	1448.0	1682.0	1565.0	194.7	214.0	232.3	1294.3	972.0	2663.0
LSD (0.05)	T:44.1 ; B:29.1; TB:88.8			T:1.4; B:0.9; TB:2.7			T:88.8; B:59.6; TB:179.6		
	Dry weight (kgh^{-1})								
Urea-N	1075	975	1025.0	171	167	169.0	999	914	709.0
Seaweeds	705	984	844.5	86	116	101.0	941	577	759.0
Fulvic acid	1019	1273	1146.0	103	123	113.0	534	356	445.0
Mean	933.0	1077.3	1005.2	120.0	135.3	127.7	824.7	615.7	637.7
LSD (0.05)	T:18.0; B:12.0; BT: 36.2			T:0.4; B:0.3; BT:0.8			T:37.6; B:20.6; BT:75.5		

T, fertilizers; B, inoculation, TB, interaction

Nitrogen uptake

Nitrogen uptake by shoot of inoculated plants was significantly affected by fertilization treatments where it was higher in case of mineral N--fertilizer than those recorded with seaweeds but nearly similar to those induced by application of fulvic acid (Table 3). In contrast, the un-inoculated plants didn't reflect any significant variation between the fertilization treatments. N uptake by shoot as affected by fertilization treatments was higher than those of the inoculated plants. This holds true with all treatments.

N uptake by root seems to be lower than those recorded with shoot. It increases with application of mineral fertilizer comparing to those resulted from application of seaweeds or fulvic acid, but the increase was not significantly affected by inoculation treatments. Values affected by seaweeds or fulvic acid treatments were nearly closed to each other. Root-N uptake values as affected by fertilization treatments could be rank as follows: mineral-N ≥ seaweeds > fulvic acid; mineral-N > fulvic acid > seaweeds for inoculated and un-inoculated plants, respectively.

Dealing with N uptake by seeds, results revealed that inoculated plants fertilized with full dose of mineral fertilizer has a higher N content than those treated with fulvic acid but nearly closed to those induced by addition of seaweeds. In this respect, values of N uptake affected by mineral fertilizer and seaweeds approximately doubled those of fulvic acid treatment. Similar trend of fertilization treatments, but to somewhat lower extents, was noticed with N uptake by seeds of the inoculated plants.

In conclusion, nitrogen uptake by seeds was the highest as compared to other plant parts. It is mainly positively affected by full mineral fertilizer treatment. Nitrogen uptake by shoot or root didn't respond well to inoculation whereas values of the un-inoculated plants surpassed those of the inoculated one. Reversible, but significant, trend was observed with N uptake by seeds. Generally, seaweeds were superior over fulvic acid and relatively caused 68.5% increase.

In this regard, the integration of bio- and mineral fertilizers plays most important role in improving soil fertility, yield attributing characters and in that way final yield has been reported by many workers (Kachroo and Razdan, 2006; Son *et al.*, 2007; Ragab *et al.*, 2016). In addition, Khaled (2012) found that a combined application of organic fertilizers (compost, compost tea, humic acid) or with the different mineral N fertilizer rates markedly increased number of sesame capsules plant^{-1}, seed weight plant^{-1}, seed yield kg fad.$^{-1}$, and weight of 1000 seeds (g). He explained that enhancement might be attributed to the stimulation of growth by directly improving the nutrient availability, or indirectly by promoting the cation exchange capacity of plants (Ingham, 2005). Marketable lettuce yield was significantly higher in compost amended plots than those minerals fertilized (Lahoz *et al.*, 2009). Consequently, application of humic sustances-soluble humic and fulvic acids fractions-shows inconsistent, yet globally positive, results on plant growth (Du Jardin, 2015). Most biostimulant effects of HS refer to the amelioration of root nutrition, *via* different mechanisms. One of them is the increased uptake of macro- and micronutrients. Another important contribution of HS to root nutrition is the stimulation of plasma membrane H^+-ATPases, which convert the free energy released by ATP hydrolysis into a transmembrane electrochemical potential used for the import of nitrate and other nutrients. Besides nutrients uptake, proton pumping by plasma membrane ATPases also contributes to cell wall loosening, cell enlargement and organ growth (Jindo *et al.*, 2012).

Also, fresh seaweeds used as source of organic matter and as fertilizer are ancient in agriculture, but biostimulant effects have been recorded only recently. This prompts the commercial use of seaweed extracts and of purified compounds, which include the polysaccharides laminarin, alginates and carrageenans and their breakdown products. Other constituents contributing to the plant growth promotion include micro- and macronutrients, sterols, N containing compounds like betaines, and hormones (Craigie, 2011; Khan *et al.,* 2009). In plants, nutritional effects *via* the provision and micro- and macronutrients indicate that they act as fertilizers, beside their other roles such as anti-stress effects (Calvo *et al.,* 2014).

Table 3. Effect of fertilization treatments, fungal and bacterial inoculation on nitrogen uptake (kg ha^{-1}) by shoot, root and seeds of cowpea plants grown on sand saline soil

Plant organ	Shoot			Root			Seed		
	Fungal inoculation								
Fertilization treatments	With	Without	Mean	With	Without	Mean	With	Without	Mean
Urea-N	1.133 a	1.231 a	1.182	0.142 a	0.134 a	0.138	25.77a	21.11ab	23.44
Seaweeds	0.210 b	1.271ab	0.741	0.134 a	0.109 b	0.122	24.28a	13.10c	18.69
Fulvic acid	0.964 a	1.109 b	1.037	0.097 b	0.126 ab	0.112	13.45b	8.65d	11.05
Mean	0.769	1.204	0.987	0.124	0.123	0.124	21.177	14.287	17.73
LSD (0.05)	T:0.241; B: 0.198; TB: 0.342			T: 0.039; B: 0.032; TB: 0.05			T:2.342; B:2.336;TB:2.446		

Means in the same column followed by the same letter are not significantly different at P≤ 0.05

Nitrogen derived from fertilizer-Ndff

Nitrogen derived from fertilizer (% and kg ha^{-1}) was recorded the highest values with application of solely mineral fertilizer (Table 4). This holds true for all plant organs. Remarkable enhancement of Ndff was noticed in case of fungal inoculated plants comparing to the un-inoculated ones. These values of Ndff tended to decrease with application of seaweeds and fulvic acid combined with quarter dose of mineral-N fertilizer. In case of Ndff by root, values did not reflected remarkable differences between seaweeds and fulvic acid and in the same time the fungal inoculation was not effective on Ndff gained by roots. In this connection, a slight difference was noticed in case of shoot when comparison was held between seaweeds and fulvic acid or fungal inoculated and un-inoculated plants. Remarkable differences between Ndff by seeds of inoculated and un-inoculated plants were recorded for inoculated over the un-inoculated one. Comparison between seaweeds and fulvic acid showed the superiority of seaweeds over fulvic acid when Ndff by shoot and seeds was considered and this trend was found true with or without fungal inoculation.

Nitrogen derived from air (Ndfa)

Application of mineral fertilizer solely resulted in portion of N derived from air (%Ndfa) ranged from 35% upto more than 60% depending on plant organ and fungal or non-fungal inoculation treatments (Table 5). It is worthy to observe that portions and absolute values of Ndfa were higher in fungal un-inoculated plants than those recorded with the inoculated one. This holds true with all plant organs. In this respect, considering the absolute values, seeds accumulate more nitrogen gained from air followed by those of roots then those of shoot.

Plants treated with seaweeds induced portions and absolute values of Ndfa by root, shoot and seeds nearly closed to those recorded with plants fertilized only with mineral-N. Values of Ndfa by all plant organs tended to decline when cowpea plants treated with fulvic acid. This phenomenon was more vigorous in case of Ndfa by seeds comparing to either root or shoot systems. It seems that fungal inoculation inhibited the synergistic effect of *Rhizobium* on activity of biological nitrogen fixation process. It means that some conflict may be happened between era of microorganisms and fungi in soil. These results refute those obtained by Do Rego *et al.* (2015) who found that improving of seed production was obtained by inoculation, significantly when the *Rhizobium* strain was inoculated in mixed inoculum with Arbuscular Mycorrhizal fungi and in the same time, the positive results of cowpea inoculation

Table 4. Nitrogen derived from fertilizer (% and kg ha^{-1}) by different plant organs as affected by fertilization treatments and fungal inoculation

Plant organ	Root				Shoot				Seed			
	Fungal inoculation											
Fertilization Treatments	With		Without		With		Without		With		Without	
	(%)	Kg ha^{-1}	(%)	Kg ha^{-1}	(%)	Kg ha^{-1}	(%)	Kg ha^{-1}	(%)	Kg ha^{-1}	(%)	Kg ha^{-1}
Urea-N	32.7	0.047	21.2	0.028	39.0	0.442	27.0	0.332	48.5	12.50	36.2	7.64
Seaweeds	27.5	0.037	20.9	0.023	37.9	0.080	27.3	0.347	47.3	11.48	36.1	4.73
Fulvic acid	27.3	0..026	22.4	0.028	35.5	0.342	25.3	0.281	45.9	6.17	35.7	3.09

Table 5. Nitrogen derived from air (% and kg ha^{-1}) by different plant organs as affected by fertilization treatments and fungal and rhizobium inoculation

Plant organ	Root				Shoot				Seed			
	Fungal inoculation											
Fertilization treatments	With		Without		With		Without		With		Without	
	(%)	Kg ha^{-1}	(%)	Kg ha^{-1}	(%)	Kg ha^{-1}	(%)	Kg ha^{-1}	(%)	Kg ha^{-1}	(%)	Kg ha^{-1}
Urea-N	39.4	0.446	60.7	0.747	35.4	0.050	55.3	0.074	40.0	10.31	53.7	11.34
Seaweeds	49.0	0.103	61.2	0.778	37.1	0.050	54.7	0.060	39.6	9.61	53.7	7.03
Fulvic acid	49.3	0.475	58.4	0.648	41.1	0.040	58.0	0.073	41.1	5.53	54.2	4.69

could be explained by performances of inoculated rhizobium. They added that the improvement is depending on geographical site and it is generally observed in sandy arenosols and in loamy sand vertisols. It is worthy to mention that these results were obtained under salinity stress (saline soil). In this regard, Choudhary (2012), Reinoso *et al.* (2004) and Llanes *et al.* (2005) explained that plants grown on saline soils has various physiological and biochemical mechanisms that allow optimal growth in saline conditions, and perhaps part of its adaptive success would depend at least on its ability to establish and maintain effective associations with plant growth promoting endophytic or rhizospheric bacteria.

Consequently, cowpea inoculation in the dryland areas of the semiarid conditions was shown to be a feasible practice due to the low rhizobium population present in the soils at the moment of crop sowing. Therefore, the use of inoculation, despite not improving costs significantly, may increase grain yield, contributing to an increased food supply (Martins *et al.*, 2003).

In addition to symbiotic nitrogen fixation, rhizobial bacteria may act as plant growth-promoting rhizobacteria (PGPR) by solubilization of inorganic P through liberation of organic acids (Glick, 2012 ; Marra *et al.*, 2012), uptake of Fe through siderophores production (Carson *et al.*, 2000), modulation of plant growth by indol acetic acid production (Boeiro *et al.*, 2007) and inhibition of ethylene synthesis by 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity (Glick 2014). Together, expression of all these features can improve the efficiency of the rhizobia–legume symbiosis (Naveed *et al.*, 2015).

Conclusion

The present study revealed that nitrogen uptake by seeds was more pronounced as compared to other plant parts. It is mainly positively affected by full mineral N treatment. Nitrogen uptake by shoot or root didn't respond well to inoculation whereas values of the un-inoculated plants surpassed those of the inoculated one. Reversible, but significant, trend was observed with N uptake by seeds. Generally, seaweeds were superior over fulvic acid. So the use of inoculation leads to increase mineral availability to plant so improve crop productivity and increase grain yield. Remarkable amounts of nitrogen utilized by plants were gained from air as a function of seed inoculation with *Bradyrhizobium* spp. that formed active nodules on plant roots. In this respect, application of ^{15}N isotope dilution technique gave the opportunity to accurately estimate the values of N either derived from mineral fertilizer or those derived from air. Also, it proves the necessity of inoculation with bio-effectors as well as fungi as eco-friendly agents to help in recognizing proper and low cost management strategy with special emphasis on environmental impact.

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دور المؤثرات الحيوية و محسنات التربة في انتاج اللوبيا المزروعة على أرض ملحية بمساعدة تقانة التخفيف النظائري (النيتروجين-١٥)

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أجريت تجربة حقلية على تربة ملحية مزروعة بنبات اللوبيا باستخدام نظام الري بالتنقيط لتقييم أثر استخدام السماد المعدني، حمض الفولفيك، الأعشاب البحرية لمكافحة الإجهاد الملحي حيت تم إضافة جميع محسنات التربة في وجود أو عدم وجود التلقيح بالفطر، أيضًا تم تلقيح بذور نبات اللوبيا بسلاله *Bradyrhizobium* spp وفى ظل معاملات التسميد المختلفة وقد استغلت تقانة التخفيف النظيري للنيتروجين-١٥ للتمييز بدقة بين مصادر النيتروجين المكتسب بواسطة محصول اللوبيا، أشارت النتائج إلى زيادة الوزن الطازج لسوق النباتات المعاملة بجرعة كاملة من السماد المعدني و الملقحة فطريا مقارنة بالنباتات غير الملقحة و قد لوحظ اتجاه مماثل ولكن بمدى أقل لكلا من الجذور والبذور، قل الوزن الطازج لكلا من السوق والجذور والبذور للنباتات سواء الملقحة أو غير الملقحة باستثناء سوق النباتات غير الملقحة والمعاملة بالأعشاب البحرية مقارنة مع تلك المسمدة بالسماد المعدني فقط، وقد لوحظ إتجاه مماثل ولكن إلى حد ما أعلى عند المعاملة بحمض الفولفيك، وعلى العكس من ذلك، انخفضت بشدة الأوزان الطازجة لبذور النباتات المعاملة بحمض الفولفيك مقارنة بتلك المعاملة بالأعشاب البحرية أو السماد المعدني، لوحظ التأثير الإيجابي للتلقيح الفطري على تحسين الوزن الطازج للنباتات المعاملة بالسماد المعدني، ازداد امتصاص النيتروجين بواسطة سوق النباتات الملقحة والمعاملة بالسماد المعدني عن النباتات المعاملة بالأعشاب البحرية، بينما كانت مماثله تقريبا لتلك النباتات المعاملة بحمض الفولفيك، وعلى العكس من ذلك لم تعكس النباتات غير الملقحة أية اختلافات معنوية بين المعاملات السمادية، ازداد إمتصاص النيتروجين مع إضافة السماد المعدني مقارنة بتلك المعاملة بالأعشاب البحرية أو حمض الفولفيك ولكنه لم يتأثر بالتلقيح الفطري، وقد إزداد امتصاص البذور للنيتروجين فى النباتات الملقحة والمخصبة بجرعة كاملة من السماد المعدني عن تلك المعاملة بحمض الفولفيك ولكنها قريبة من تلك المعاملة بالأعشاب البحرية، سجلت النتائج أعلى قيمة للنيتروجين المستمد من السماد المعدني في حالة التسميد المعدني منفردا، وهذا ينطبق على جميع أجزاء النبات، هناك تحسن ملحوظ في قيم النيتروجين المستمد من السماد المعدني في حالة النباتات الملقحة بالفطر مقارنة بغير الملقحة، تراوحت نسب النيتروجين المستمد من الهواء بين ٣٥ إلى٦٠% أو أكثر تبعا لأجزاء النبات والمعاملة سواء الملقحة أو غير الملقحة بالفطر، لوحظ أيضا زيادة القيم المطلقة للنيتروجين المستمد من الهواء الجوي في حالة النباتات غير الملقحة عن الملقحة بالفطر لذلك سجلت القيم المطلقة للنيتروجين المستمد من الهواء الجوى بواسطة البذور زيادة أكبر من تلك المسجلة بالجذور تليها السوق.

الكلمات الإسترشادية: المؤثرات الحيوية، اللوبيا، حمض الفولفيك، تقانة النيتروجين-١٥، الملوحة، الأعشاب البحرية.

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