SIGNIFICANCE OF N-BIOFIXATION AND FOLIAR SPRAY WITH Zn AND Co ELEMENTS FOR IMPROVING FABA BEAN PRODUCTIVITY UNDER FIELD CONDITIONS OF MIDDLE NILE DELTA AREA, EGYPT

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ABSTRACT:

A field experiment was conducted on a clayey soil during two successive winter seasons of 2007-2008 and 2008-2009 at Damas village, Mit Ghamer district, El Dakahlia Governorate, Egypt to evaluate the effectiveness of seed inoculation with rhizobium and foliar spray with zinc and cobalt on faba bean (*Vicia faba* L.) yield and its components as well as seed protein and nutrient contents. Zinc and cobalt sulphates were applied at three rates of 0, 250 and 500 mg L^{-1} for Zn and 0, 15 and 25 mg L^{-1} for Co. Yield and its components (*i.e.*, number of tillers $plant^{-1}$), number & dry weight of nodules and nitrogenase activity of root nodules $plant^{-1}$ at two investigated periods of 45 and 70 days from sowing as well as number of pods $plant^{-1}$, grain contents of protein and elements (*i.e.*, N, P, K, Fe, Zn, Cu & Co). Soil content of available nitrogen forms (NH_4^+ and NO_3^-) after 70 days from sowing and at harvest were taken into consideration in this study.

The obtained data show that seed inoculation with rhizobium inoculation and foliar spray with Zn and Co led to a significantly increased in each of tiller $plant^{-1}$, number of pods $plant^{-1}$, grain protein, grain and straw yields in kg fed^{-1}. A parallel trend was occurred for the positive effect of the applied treatments on faba bean grain contents of N, K, Fe, Zn, Cu and Co, however, the greatest values were achieved at the highest rates of 500 and 25 mg L^{-1} of Zn and Co, respectively. On the other hand, no changes were observed for grain content of P by increasing the applied Zn and Co rates, may be due to the antagonism between Zn and P.

As for the beneficial effects of the applied treatments on the available nitrogen content in the experimental soil, data show that seed rhizobium inoculation as well as Zn and Co as foliar application resulted in a noticeable increase for available content of nitrogen forms (NH_4^+ and NO_3^-) at both investigated periods of 70 days after sowing and at harvest as compared to the control treatment. At the same times, it is noticed that the available N-forms gave the greatest values at 70 days after sowing as well as in case of Zn foliarly as compared to at harvest and case of Co. This was true, since such increase was more closely to the nitrogenase activity. It is noteworthy to mention that seed inoculation and Zn or Co foliar application showed a parallel trend for the pronounced increases of all tested plant and soil parameters as compared to the control treatment under both studied growing season conditions. So, it could be said that the residual effect of N-bio-fixation in the soil after harvest leading to rationalize use of N-mineral fertilizers for the next crop, which is surplus point for sustainable agriculture system.

Key words: Faba bean, N-biofixation, Zn or Co foliar spray, available nutrients in the soil and seed nutrient contents.

INTRODUCTION:

The symbiotic relation between higher plants and soil microorganisms represents one of the most striking biological phenomena. The use of symbiotic is more economical and much better than the use of chemical fertilizers, which had raised serious objection and real concern about the environmental pollution. The use of a symbiotic bacterium (rhizobium) produces enough nitrogen to support the building up of the whole protein requirements of the legumes **(Bedrous *et al*., 1990).**

Faba bean (*Vicia faba* L.) is one of the principle food legume crop in Egypt as a source of vegetable protein. It serves as an important source of protein in the human diet, especially for those with low income. In addition, faba bean plants improve the fertility of the soil via providing a substantial input of N2 through fixation. **Abdel-Maksoud *et al*. (1985)** showed that faba bean was highly responsive to inoculation with selective rhizobia strains and this was reflected on increments of seed yield and nitrogen content.

Cobalt is an important element to the plant growth, however, bacteria on root nodules of legumes (beans, alfalfa and clover) require cobalt and other trace elements to synthesize B12 and fix nitrogen from air **(Smith. 1991).** Although it is needed by rhizobia in root nodules of leguminous plants, the mechanism by which Co affects plants is not yet clearly known. Soybeans grown without cobalt are severely retorted in growth and exhibit severe nitrogen deficiency, leading to death in about one of four plants. Adding only a few ounces of cobalt per acre can resolve deficiency symptoms in ten to 21 days **(Hala Kandil, 2007)**.

In plants, zinc plays an important role as a structural constituents or regulatory co-factor in a wide range of enzymes in many important biochemical pathways (**Moore and Patrick, 1989**). There are mainly involved in: carbohydrate metabolism (photosynthesis and conversion of sugars to starch), protein metabolism (deficient plants often have reduced protein contents and elevated concentrations of certain free amino acids), auxin (growth regulator) metabolism (resulting stunning and/or resetting) and pollen formation (reduced production of pollen leads to a proportion of empty grains in the ears of self-pollinating cereals), **Moore and Patrick (1989) and Romheld and Marchner (1991)**.

MATERAL AND METHODS:

A field experiment was conducted on a clayey soil during two successive seasons of 2007-2008 and 2008-2009 at Damas village, Mit Ghamer district, El Dakahlia Governorate, Egypt. This study aimed to evaluate the effect of rhizobium leguminosarum bv. viciae (nitrogen fixing bacteria) and foliar spray with zinc and cobalt on soil content of available nitrogen forms (*i.e.*, NH_4^+ and NO_3) at both periods of 70 days after sowing and harvest. Also, faba bean yield and its components (*i.e.*, number of tillers plant^{-1}) and nodulation characters (*i.e.*, number & dry weight of nodules and nitrogenase activity of root nodules plant^{-1}) at two investigated periods of 45 and 70 days from sowing were determined. Number of pods plant^{-1}, grain contents of protein and elements (*i.e.*, N, P, K, Fe, Zn, Cu & Co).were taken into consideration in this study.

The experiment was designed in split-split design and comprised 18 treatments in three replicates. The main plots are represented by seed inoculated with rhizobium and the sub-plots with Zn and Co as foliar spraying. The plot area is 10.5 m^2 (3x3.5 m, *i.e.*, 1/400 fed). Faba bean seeds were sown on rows (50 cm in width), with hills containing two seeds each with 20 cm apart at 9^{th} November in both agricultural growing winter seasons.

Some physical and chemical properties of the experimental soil were determined by using the standard methods as described by **Black *et al.* (1965)**, **Hesse (1971) and Jackson (1976)**, and the obtained data are present in Table (1).

Table (1): Some characteristics of the studied experimental soil at initial state.

Soil character	Value	Soil character	Value
Particle size distribution %:		pH (1: 2.5 ,soil suspension)	7.50
Sand	15.70	*Chemical analysis of soil paste extract:*	
Silt	39.30	ECe (dS m^{-1})	2.73
Clay	45.00	*Soluble cations (m molc $^{L-}$1):*	
Textural class	Clay	Ca^{++}	17.20
$CaCO_3$ content %	2.80	Mg^{++}	3.71
Organic matter content %	1.02	Na^{+}	7.03
CEC (c molc kg^{-1} soil)	41.90	K^{+}	0.55
Available N (mg kg^{-1})	33.50	*Soluble cations (m molc $^{L-}$1):*	
Available P (mg kg^{-1})	9.64	CO_3^{--}	0.00
Available K (mg kg^{-1})	379.40	HCO_3^{-}	3.43
Available Zn (mg L^{-1})	1.95	Cl^{-}	11.32
Available Co (mg L^{-1})	0.075	SO_4^{--}	12.74

Plant samples:

Three plants were randomly taken from each plot at 45 and 70 days after sowing to determine number and dry weight (mg) of nodules $plant^{-1}$ and nitrogenase activity of nodules **(**n mole C_2H_4 g^{-1} dry nodules h^{-1}) as described by **Hardy *et al.* (1976).** At harvest plants were cut just above the soil surface to determine faba bean yield and its components as well as grain contents of nutrients (*i.e.*, N, P, K, Fe, Zn, Cu & Co) **(Jackson, 1976)** and grain protein content **(A.O.A.C., 1980)**.

Soil samples:

Soil samples were collected at 70 days after sowing and at harvest to determine the soil available nitrogen forms of NH_4^+ and NO_3^- by using Technicon Auto Analyzer according to **Markus *et al.* (1982)**.

Inoculants of bacteria used:

Rhizobium leguminosarum bv.viciae was kindly supplied by Department of Microbiology, Soils, water and Environment Research Institute. Agric. Res. Center, Giza, Egypt

Faba bean seeds inoculation:

Faba bean seeds were divided into two portions, the first was sown without inoculation and the second one was inoculated before sowing with rhizobial inoculants using Arabic gum as an adhesive agent.

Mineral fertilizers application:

Nitrogen fertilizer as urea (46 % N) was applied at a rate of 20 kg N fed^{-1} into two equal split doses at 35 and 60 days after sowing, P and K fertilizers as super phosphate (15.5 % P_2O_5) and potassium sulphate (48 % K_2O) were added at the rates of 100 and 50 kg fed^{-1}, respectively. Zinc sulphate ($ZnSO_4.7H_2O$) and cobalt sulphate were applied to the plants as foliar application at three rates, *i.e.*, 0, 250 and 500 mg L^{-1} for Zn and 0, 15, 25 mg L^{-1} for Co.

At the end of the experiments, the plants were harvested and the faba bean yield was recorded. The grains were ground and kept in tight bags for chemical analysis. The dried materials of seeds were digested according to **Peterburgski (1968)**. Nitrogen was determined using microkjeldahl, phosphorus by stannous chloride method as described by **A.O.A.C (1980)** and K was determined by using Flame Photometer **(Yamagnchi and Minges 1956)**. Zn, Fe, Cu and Co were determined using Atomic Absorption Spectrophotometer Perkin Elmer, according to **Cottenie *et al.* (1982)**.

Statistical analysis:

All data obtained of both the two studied seasons were averaged in a mean value for each of the tested plant and soil parameters, and then statistically analyzed by using L.S.D. at 0.05 as described by **Snedecor and Cochran (1980)**.

RESULTS AND DISCUSSION:

I. Faba bean yield and its components:

Results in Table (2) reveal that seed rhizobium inoculation significantly increased number of tillers $plant^{-1}$, number of pods $plant^{-1}$, grain protein %, grain and straw yields (kg fed^{-1}). These increases in faba bean yield and its components are more attributed to the increase of nitrogen biofixation in faba bean plants due to increasing nitrogenase activity (Table 3). These results are in agreement with those stated by **Wang and Patill(1995) and Vessey (2004)** who showed that rhizobium inoculation enhanced seed yield and its components of pea and other legumes.

Table (2): Effect of seed inoculation and different applied rates of Zn and Co as foliar application on faba bean yield and its components.

Treatment			No. of tillers $plant^{-1}$	No. of pods $plant^{-1}$	Grain yield (kg fed^{-1})	Grain protein %*	Straw yield (kg fed^{-1})
Seed inoculation	Applied rates of foliar spray (mg L^{-1})						
	Zn	Co					
Un-inoculation	0	0	3.35	13.80	1246.35	22.75	972.80
	250	25	3.65	15.35	1383.85	25.70	1082.90
	500	50	3.75	16.35	1537.15	27.30	1180.65
Mean			3.60	15.15	1388.95	25.25	1078.80
Inoculation	0	0	4.40	17.75	1527.65	24.60	1184.80
	250	25	5.20	19.95	1737.60	28.10	1343.90
	500	50	7.85	22.50	1972.70	31.45	1504.15
Mean			5.82	20.10	1745.98	28.05	1344.28
L.S.D. at 0.05							
Seed inoculation			0.44	1.74	155	1.80	122
Zn and Co rates			0.54	2.13	190	2.33	149

Grain N-content % x 6.25 **(Deyoe and Shellenberger, 1965)**

Data also showed that foliar application of Zn and Co led to significantly increases in faba bean yield and its components at both the first and second seasons. The greatest values of these parameters were obtained at the highest rates of both Zn and Co (*i.e.*, 500, 25 mg L^{-1}, respectively), with a more effectiveness for Zn than Co. The noticeable increases in faba bean yield and its components as a result of Zn-foliar application could be attributed to the increase in the number of tillers and pods per plant. Similar results were reported by **El-Mansi *et al*. (1990)** on pea. As for Co-foliar spraying, **Ibrahim *et al.* (1989) and El-Gizawy and Mehasen (2009)** showed that the positive effect of Co-foliar spray on faba bean plants, as compared to the control, may be due to its effective role on many developmental processes such as stem and coleoptiles' elongation, leaf disc expansion and bud development. Also, the proper doses of cobalt may help in better nodulation, and consequently a better growth and yield **(Jana *et al*., 1994)**.

II. Nodule numbers, their dry weights and nitrogenase activity:

Biological N_2-fixation by legume rhizobia symbioses is vitally important as N input in agro-ecosystem. Table (3) show that seed inoculation with rhizobium significantly increased number of nodules, their dry weights and nitrogenase activity after 45 and 70 days of sowing. These results are in line with those reported by **Abdel-Ghaffar *et al.* (1994) and Massoud *et al*. (2008)** on pea plants and other legumes. While, the presence of active nitrogenase enzyme in nodules for un-inoculated plants is owing to the indigenous rizobia in soil **(Mowad and Abd-El Rahim, 2002)**.

Table (3): Effect of seed inoculation and different applied rates of Zn and Co as foliar application on nodulation of faba bean roots.

Treatment			Nodule Nos. plant^{-1}		Nodule dry weights (mg) plant^{-1}		N2-activity (C_2H_4 g^{-1} plant^{-1})	
Seed inoculation	**Applied rates of foliar spray (mg L^{-1})**		**Days from sowing**					
	Zn	Co	45	70	45	70	45	70
Un-inoculation	0	0	15.05	10.25	31.65	21.85	151.65	109.20
	250	25	17.15	12.45	36.15	25.45	170.40	125.65
	500	50	18.50	13.50	38.40	26.95	179.85	133.00
Mean			16.90	12.05	35.40	24.70	167.30	122.60
Inoculation	0	0	29.20	22.60	76.70	53.70	315.65	220.30
	250	25	35.60	26.85	91.15	62.95	364.15	259.40
	500	50	39.65	30.40	105.20	72.65	396.00	292.70
Mean			34.82	26.62	91.02	63.10	358.60	257.47
L.S.D. at 0.05								
Seed inoculation			2.60	3.40	10.90	7.36	25.22	27.33
Zn and Co rates			3.20	4.11	13.40	9.00	30.88	33.47

Data in Table (3) show that number and dry weights of nodules and their dry weights after 45 and 70 days of sowing exhibited significantly increases as a result of foliar spray with the applied rates of Zn and Co. Such mentioned investigated scope tended to gradual increases with increment of Zn and Co rates up to the highest ones of 500 and 25 mg L^{-1}, respectively. These results are in agreement with those obtained by **Vessey (2004)**.

The positive effect of zinc on nodulation and nitrogenase activity may be due to its role which acts as metal component for some enzyme or as a functional or regulatory co-factor for the others, hence zinc increase root nodulation and nitrogenase activity by indirect way. The same results were obtained by **Abadi *et al.* (1995)** who attributed the effect of zinc on nodulation and nitrogenase activity to the improved nutritional status of rhizobium. While, the positive effect of Co on nodulation and nitrogenase activity may be attributed to the role of Co as an essential component of nitrate reductase and nitrogenase enzyme, which controls the reduction of nitrogen (Fixing N_2 to NH_3) and promotes the nodules formation of legumes. Similar results are reported by **Jana *et al*. (1994) and Abd El-Moez and Nadia Gad (2002)** who found that cobalt addition increased the nodules formation of root and atmospheric nitrogen fixation by microorganisms, which increased the nitrogen content in faba bean plants.

It is worthy to mention that a parallel trend for increased both number of nodules and their dry weights took place with the nitrogenase activity after either 45 or 70 days from sowing, which are more affected by seed rhizobia inoculation and applied foliar spray with both Zn and Co elements. These results showed a close relationship between the nodulation phenomenon and the activity of symbiotic bacteria, especially in the rhizosphere zone

III. Macro and micronutrients status in faba bean grains:

Grain element contents of N, P, K, Fe, Zn, Cu and Co are presented in Table (4), and showed that faba bean seed rhizobium resulted in a significantly increase for each of all tested elements, except P, as compared to the un-inoculated one either in the first or second seasons.

Table (4): Effect of seed inoculation and different applied rates of Zn and Co as foliar application on element contents of faba bean grains.

Treatment			Macronutrients %			Microelements (mg kg^{-1} soil)			
Seed inoculation	Applied rates of foliar spray (mg L^{-1})		N	P	K	Fe	Zn	Cu	Co
	Zn	Co							
Un-inoculation	0	0	3.35	0.22	1.35	105.00	26.00	2.85	2.85
	250	25	3.80	0.19	1.45	109.35	28.90	4.60	4.60
	500	50	4.05	0.17	1.55	112.70	32.00	7.10	7.10
Mean			3.75	0.19	1.45	109.00	29.00	4.15	4.15
Inoculation	0	0	3.95	0.25	1.60	122.60	30.60	3.35	3.30
	250	25	4.50	0.20	1.75	133.20	35.95	5.35	5.40
	500	50	4.75	0.19	1.95	144.15	39.90	8.55	6.55
Mean			4.35	0.21	1.76	133.32	35.48	5.75	5.75
L.S.D. at 0.05									
Seed inoculation			0.16	0.07	0.10	8.48	3.82	0.63	0.61
Zn and Co rates			0.19	0.02	0.12	10.40	4.68	0.77	0.74

These positive effects are more attributed to the increase in bio-fixed nitrogen that increased by nitrogenase activity of rhizobium in root nodules, and consequently increased N uptake by plants. Such favourable condition encourages the vegetative growth of faba bean plants, hence uptake of the other elements increased from the soil, besides Zn and co whose are added as foliar application, and accumulated in the different plant organs, particularly faba bean grains. These results are in line with those stated by **Daterao *et al.* (1994) and Massoud *et al.* (2005)** who reported that rhizobium inoculation increased grain nitrogen in pea and other legumes. Similar results were obtained by **Massoud (2001)** who found that rhizobium inoculation increased nutrient contents in grains of pea. With respect to faba bean grain content P, which showed no noticeable changes as a result of the applied treatments, it may be explained by the antagonism between the applied Zn as foliar application and the uptake of P from the soil.

Regarding the effect of foliar application of Zn and Co on N, P, K, Fe, Zn, Cu and Co in faba bean grains, it is observed gradual increases in the uptake of these elements with increasing the applied rates of Zn and Co as foliar application, which reached the greatest contents at the highest rates of 500 and 25 mg L^{-1}, respectively. On the other hand, faba bean grain content of P exhibited no changes by increasing of Zn and Co rates, may be due to the antagonism phenomenon as mentioned before. These results are in harmony with those obtained by **Massoud *et al.* (2005)** who found that Zn application as a seed coating or foliar had a positive effect on N, K, and Mo contents of pea. Also, **Dahdoh and Mossa (2000)** observed that addition of Zn or Co increased plant N and K contents vs decreased P and Cu in broad bean and peanut plants. Moreover, **Hala Kandil (2007)** found that Co addition increased faba bean grain contents of macro and micronutrients as well as protein percentage. In this concern, **Zhoa *et al.* (1998)** found that, up to the Zn rate of 20 g kg^{-1}, phosphorous content of plant tissues remained at a constant level, and then tends to decrease abruptly when Zn concentration went above 20 g kg^{-1}. This is mainly attributed to Zn rates stimulate phosphorous content in faba bean grains, due to the antagonism phenomenon between two elements,

IV. Available nitrogen content in the soil as affected by nitrogenase activity:

Data in Table (5) showed the effect seed rhizobium inoculation and foliar application of Zn and Co on available content of nitrogen forms (nitrate and ammonium) in the soil after either 70 days from sowing of faba bean or harvesting. Results revealed that the rhizobium inoculation increased Both NH_4^+-N and NO^+_3-N contents in the experimental soil as compared with un-treaded one. Similar results were obtained by **Massoud *et al.* (2005)**. Data also showed that increasing Zn and Co as a foliar application increased the contents of available nitrogen forms in the soil, with gradual increments reached the greatest values at the applied highest rates of 500 and 25 mg L^{-1}, respectively. However, Zn foliar application was more effective for increasing soil content of NH_4^+ and NO^-_3 than that of Co. It is noteworthy to mention that soil available N-forms, *i.e.,* NH_4^+ and NO_3^- recorded relative increase percentages reached about 92 and 84% at 70 days after sowing and at harvest, respectively, as compared to the control treatment. This was true, since such increases were more closely attributed to the nitrogenase activity.

Table (5): Effect of seed inoculation and different applied rates of Zn and Co as foliar application on soil contents of available nitrogen forms at both 70 days after sowing and harvest.

Treatment			**Soil content of available forms**			
Seed inoculation	**Applied rates of foliar spray (mg L^{-1})**		**70 days from sowing**		**At harvest**	
	Zn	Co	**NH_4^+**	**NO_3^-**	**NH_4^+**	**NO_3^-**
Un-inoculation	0	0	12.25	42.65	9.40	30.50
	250	25	17.20	45.90	13.25	34.60
	500	50	19.75	48.75	15.20	37.10
Mean			16.41	44.77	12.65	34.07
Inoculation	0	0	21.20	73.40	15.30	49.50
	250	25	24.45	81.75	17.60	54.30
	500	50	27.60	90.60	19.90	63.90
Mean			24.08	81.92	17.60	55.90
L.S.D. at 0.05						
Seed inoculation			1.68	3.80	1.30	2.9
Zn and Co rates			2.07	4.80	1.60	3.7

The increase of nitrogen forms in soil as a result of foliar application by Zn or Co may be due to high activation of rhizosphere zone as a constituent of nitrogenase enzyme, hence it increased the bio-fixed N_2 in the soil. Similar results were obtained by **Vieira *et al.* (1998) and Massod *et al.* (2005)** who found that seed coated with Mo or Zn increased the available nitrate and ammonium in the soil after pea harvesting.

CONCLUSION:

The present study showed that, the combined effect of seed rhizobium inoculation with Zn and Co as foliar spray significantly increased faba bean yield and its components. As for the achieved increases in soil available nitrogen forms after either 70 days from sowing or at harvest, it could be said that the residual effect of seed inoculation leading to rationalize use of mineral nitrogen fertilizers for the next crop, which is surplus point for sustainable agriculture system. These data need more investigations on many species of other crops and different rates of these elements, especially Co to justify its effect on plant growth, yield and elemental composition of seeds whether it adds foliarly or soil application under field conditions.

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أهمية التثبيت الحيوى للنتروجين والرش بعنصرى الزنك و والكوبلت فى تحسين إنتاجية الفول البلدى تحت ظروف الحقل فى منطقة وسط دلتا نهر النيل – مصر

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أجريت تجربة حقليه على أرض طينية خلال موسمين شتويين متتاليين ٢٠٠٧-٢٠٠٨، ٢٠٠٨-٢٠٠٩ فى قريه دماص- مركز ميت غمر - محافظه الدقهليه – مصر، لتقييم كفاءة تلقيح البذور بالريزوبيوم ورش النباتات بعنصرى الزنك والكوبلت فى صورة كبريتات على محصول الفول البلدى (*Vicia faba* L.) ومكوناته، وكذا محتوى الحبوب من البروتين والمغذيات. ولقد تمت الإضافة لكلا العنصرين بمعدلات ثلاث هى ٠، ٢٥٠، ٥٠٠ ملجم/لتر للزنك، ٠، ١٥، ٢٥ ملجم/لتر للكوبلت. وتم تقدير كميه المحصول ومكوناته ممثلة فى عدد التفريعات/نبات، عدد العقد الجذريه وزنها الجاف، نشاط إنزيم النتروجينيز بالعقد الجذريه/نبات عند فترتى ٤٥، ٧٠ يوم من الزراعة، بالإضافة إلى عدد القرون/نبات، محتوى الحبوب من البروتين وبعض العناصر (N, P, K, Fe, Zn, Cu and Co)، وكذلك محتوى التربة من صور النتروجين الميسر (NH_4^+ and NO_3^-) بعد ٧٠ يوم من الزراعة وعند الحصاد.

وتشير النتائج المتحصل عليها إلى أن تلقيح البذور بالريزوبيوم ورش النباتات بالزنك والكوبلت قد أدى إلى زياده معنويه فى عدد التفريعات/نبات، عدد القرون/نبات، محتوى الحبوب من البروتين، محصولى الحبوب والقش (كجم/فدان). وهناك إتجاه موازى للتأثير الإيجابى للمعاملات المطبقة على محتوى الحبوب من عناصر N, K, Fe, Zn, Cu and Co، حيث تحققت أعلى القيم عند أعلى معدلين ٥٠٠، ٢٥ ملجم/لتر لكل من الزنك والكوبلت على الترتيب. وعلى الجانب الآخر، لاتوجد تغيرات ملحوظة فى محتوى الحبوب من الفوسفور بزيادة معدلات إضافة كل من الزنك والكوبلت، وذلك قد يرجع إلى حدوث ظاهرة التضاد بين عنصرى الزنك والفوسفور.

وبالنسبة للتأثيرات المفيدة للمعاملات المطبقة على محتوى تربة التجربة من النتروجين الميسر، فان النتائج توضح أن تلقيح البذور بالريزوبيوم ورش النباتات بالزنك والكوبلت قد تسبب فى زيادة ملحوظة فى المحتوى الميسر من صورتى النتروجين (NH_4^+ and NO_3^-) عند فترتى ٧٠ يوم من الزراعة وكذلك عند الحصاد مقارنة بمعاملة الكنترول. وفى نفس الوقت لوحظ أن صور النتروجين الميسر قد أعطت أعلى القيم عند فترة ٧٠ يوم من الزراعة وكذا عند الرش بالزنك مقارنة بفترة الحصاد والرش بالكوبلت. وهذا حقيقى، حيث أن هذه الزيادة كانت أكثر إرتباطا بنشاط إنزيم النتروجينيز. ومن الجدير بالذكر الإشارة إلى أن تلقيح البذور بالريزوبيوم ورش النباتات بالزنك والكوبلت قد أظهر إتجاه موازى للزيادات الملحوظة فى كل المقاييس تحت الدراسة والتى تختص بالنبات أو التربة مقارنة بمعاملة الكنترول تحت ظروف كلا موسمى الزراعة. ولذا، يمكن القول بأن التأثير المتبقى للنتروجين المثبت حيويا فى التربة بعد الحصاد يؤدى إلى ترشيد إستخدام أسمدة النتروجين المعدنى للمحصول التالى، مما يدعم فكرة نظام الزراعة المستدامة.