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Impact of cobalt application to soil and mycorrhizal fungi inoculation on growth and some nutrients content of barley and Egyptian clover plants grown in calcareous soil

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Abstract Two pot experiments were carried out at the green-house of Faculty of agriculture (Saba Bash), Alexandria University to study the effect of addition of cobalt to the soil in form of cobalt sulphate by preparing six different concentrations, (0.0, 10, 20, 40, 80, and 160 mg of cobalt/ kg soil) in relation to two species of mycorrhiza inoculation each alone (*G. intraradiaces* and *G. macrocarpium*) on growth and quality of barely and clover as forage crops under calcareous soil conditions during 2012-2013 and 2013-2014 growing seasons, respectively. Experimental design was randomized complete block design (R.C.B.D) with one hundred and eight experimental units and three replicates. The study was carried out, by planting the seeds in plastic pots in glass houses. After the appearance of three leaves, the pots were irrigated once only by each particular concentration, of cobalt. The obtained results showed that addition of cobalt to the soil of barley and clover (forage crops) and mycorrhizal inoculation had significant effects on all growth parameters and the tested nutrients content in the plants of the two crops. The Co significantly increased the contents of N, P, K, and Co in shoot and root of the tested plants. It was found that *G. intraradiaces* was more effective than *G. macrocarpium* for the studied traits. Also, the recommended cobalt concentration for barley crops was lower 20 mg/l) than that for E.Clover (40mg/l). However, Co concentration above 20 and 40 mg/kg soil for barley and E. Clove, respectively, reduced growth of plants.

Key wards: Cobalt application, cobalt, Mycorrhiza, Barley, E. Clover, growth parameters, macronutrients.

تأثير إضافة الكوبالت للتربة والتلقيح بفطر الميكورايزا على نمو ومحتوى بعض العناصر الغذائية لنباتي الشعير والبرسيم المصري النامية في أرض جيرية

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الملخص اجريت هذه الدراسة في الصوبة الزجاجية بكلية الزراعة ساباباشا جامعة الاسكندرية لدراسة تاثير اضافة الكوبالت مباشرة للتربة وعلاقة ذلك بتلقيحها بالميكورايزا وتاثيره على نمو ومحتوى العناصر الغذائية في نباتي الشعير والبرسيم المصري كمحصولي علف تحت ظروف التربة الجيرية خلال موسمي النمو 2012–2013 و 2013–2014 على التوالي. حيث تم ري النباتات بعد ظهور ثلاث أوراق حقيقية رية واحدة بمحلول الكوبالت (على صورة كبريتات الكوبالت) في عدة تركيزات مختلفة من الكوبالت (0.0، 10، 20، 40، 80، و 160ملجم/ كيلو جرام تربة) كما تم استخدام نوعين من الميكورايزا (*G. intraradiaces* and *G. macrocarpium*)) والمعاملات وزعت عشوائيا على 108 وحدة تجريبية (أصيص) مع ثلاث مكررات، ونتج عن ري نباتات الشعير والبرسيم المصري (كعلف حيواني) ب محلول الكوبالت عند تركيزاته المختلفه وتلقيحها بالميكورايزا الى زيادة معنوية في كل صفات النمو المختلفة مثل الوزن الخضري والجذري الطري والجاف وارتفاع النباتات وعدد الأوراق بالإضافة إلى حساب طول الجذر وسمكه ومساحته السطحية ونصف قطره إضافة إلى نصف المسافة بين الجذور ومحتوى العناصر الغذائية المختبرة في المجموع الخضري والجذري. أدت اضافة الكوبالت الى زيادة محتوى N ،P ، K والكوبالت في النباتات المختبرة . وكانت الميكورايزا (*G. intraradiaces*) اكثر فاعلية من النوع (*G. macrocarpium*) وكان التركيز الامثل للكوبالت هو التركيز (20 ملجم/ كيلو جرام تربة) لنبات الشعير، و التركيز (40 ملجم/ كيلو جرام تربة) لنبات البرسيم المصري. وكان محتوى الكوبالت في نباتات الشعير والبرسيم يتماشى مع كميات الكوبالت المتاحة في التربة والتي تزداد بازدياد تركيزات الكوبالت المختلفة المضافة للتربة.

الكلمات المفتاحية: اضافة الكوبالت، الكوبالت، الميكورايزا، الشعير، وصف النمو، العناصر الكبرى.

Introduction

Barley (*Hordeum vugare*_L.) is the major cereal crops in many dry areas of the middle east, north Africa and west Asia [11. Its distribution is worldwide and is of considerable economic importance for animal feed and human consumption. Throughout the world around, 80% of the grown barley is used to feed animals [5].

Berseem or Egyptian Clover (*Trifolium alexandrinum* L.) is an annual legume that is a vine with climbing growth habit, great productivity due to its high growth rate and good fodder recovery after cutting, and high levels of crude protein. It is well adapted to a range of environments and is usually grown in the Mediterranean, central European, and Southeast Asian Countries for forage production [14, 15].

Cobalt is required by Rhizobia for nitrogen fixation and indirectly by leguminous and other plants [41]**.** On the other hand, low concentrations of cobalt can have a favorable effect on plant growth of non-leguminous crops [45]**.** Cobalt affects metabolism and plant growth and is an essential component of several enzymes and co-enzymes [39]. Its beneficial effects include retardation of leaf senescence, inhibition of ethylene biosynthesis, and stimulation of alkaloid biosynthesis [39]**.**

There are three main methods of adding micronutrients to crops: soil fertilization، foliar sprays and seed treatment.

The cobalt applied to the soil at seedling stage (At the third truly leaf) through irrigated once with concentrations of cobalt. [37] reported that the effect of cobalt application on the growth of barely plants was significant on the clay soil and insignificant on the clay loam and sandy loam soils .The higher dry matter yields were obtained with the application of cobalt to the soils at the rate of 20 mg Co/Kg soil. Also [19] demonstrated that, all cobalt treatments (2.5, 5.0, 7.5, 10.0 and 12.5 ppm) significantly increased the growth, yield of root and minerals composition (except Fe content). Cobalt at 7.5 ppm caused the maximum growth, root yield, minerals composition and sugar yield of sugar beet compared to control and other cobalt rates. In the same line [6] concluded the different concentration of cobalt applied to soil after one month from transplanted the seedlings (0.0, 7.5, 15.0 22.5, 30.0 ppm) on herb yield, essential oil content and its composition of *Ocimum basilicum*. Cobalt at 15.0 ppm gave the greatest values of fresh and dry herb yield (66.20 and 13.19 ton/ ha). Increasing Co from 0.0 to 7.5, 15.0 and 22.5 ppm significantly increased the essential oil yield from 38.39 to 94.67, 266.78 and 181.49 L/ha. While the highest level of Co (30ppm) recorded 91.15 L/ ha.

[20] reported that the highest significant figures of N, P, K, Ca, Mg, Mn, Zn, and Cu were obtained by using cobalt at 20.0 ppm for growing maize plant.

[35showed that the effect of applying cobalt as foliar application at the rate of 0.24 and 0.48 g/L in broad bean on clay loam soil (7kg/pot). The results clearly indicate that the percentage of N, P and K in response to the different rates recorded significant increase in comparison with untreated plants.

Cobalt accumulation can occur in Anisopappus chinensis (Asteraceae), as foliar Co concentration increases in relation to Co concentration in the nutrient solution without a decrease in the biomass or toxicity symptoms [32].

[13] concluded that application of Co at 20 mg kg-1 level increased maximum growth and yield of fodder maize and detrimental effect were seen at higher Co level. Whereas, highest reduction in plant height, green forage yield dry matter yield of leaves, stem and root of maize was observed at Co at 80 mg kg^{-1} level.

Arbuscular mycorrhizal fungi (AMF) plays an important role in vegetation restoration because of symbiosis with plant root; they can facilitate mineral absorption by host plant, stability and improve soil structure, affect the population structure and preserve species diversity [10].

[22] studied the effect of cobalt and mycorrhizae (*Gigaspora gigantean*) on growth and yield of corn as monocots and soybean as dicots. The seedling (at the third truly leaf) were irrigated with cobalt sulphate once, with 0, 4, 8, 10, 12, 16 and 20 ppm cobalt. They found that the cobalt with mycorrhizae inoculation under low phosphorus level enhanced the growth, yield quantity and quality in both corn and soybean, but this positive impact was more significant in the dicot plants compared to monocot.

The aim of this research was to study the effect of cobalt application to the soil directly (soil method) and mycorrhizal fungi inoculation on growth and nutrients content of barley (non-legumes) and Egyptian Clover (legumes) grown on calcareous soil.

Materials and Methods

Soil

Surface calcareous soil sample (0-15cm) was collected from Al-Hamam region at the northern western coast of Egypt. The soil sample was air dried ground to pass 2 mm sieve and thoroughly mixed before using. The characteristics of this soil are presented in Table (1). Practical size distribution was determined by the hydrometer method according to Black *et al*, [9]. Field capacity was measured by saturated the soil samples through capillary rise then pull the gravitational water from the samples and drying at 105°C for 24 hours as reported by [9]**.** Soil organic matter was determined by Walkley and Black method according to [27]. The water soluble Ca^{2+}, Mg^{2+}, HCO^{-3}, Cl^{-}, pH and EC in the soil were measured according to Jackson [26]. The calcimeter method was used to determine the total carbonates volumetrically [9]. The amounts of available nitrogen and potassium were determined according to Jackson [26], that of phosphorus was determined as described by Murphy and Riley [36], and that of cobalt was determined using the methods of [33].

Experimental procedures

Two pot experiments were carried out during the growing season 2012/2013 and 2013/2014 under the greenhouse conditions of the Agriculture Faculty (Saba Bash), Alexandria University to study effect of cobalt applied to soil in relation to Mycorrhiza inoculation on growth and quality of barely and clover, as forage crops under calcareous soil conditions. Plastic pots (12.5 cm in diameter and 11.5 cm deep) were washed, labeled, and a filter paper placed on the bottom of each pot to prevent soil leakage then a weight of 1 kg calcareous soil was assigned for each pot leaving 5 cm upper without soil. The barley and E. Clover were fertilized by recommended dose of super phosphate (15% P_2O_5), which was added and mixed with soil in each pot during the preparation of the experimental soil at the rate of (90 and 200kg P_2O_5/fed respectively, while N fertilizer was added in the form of (NH_4NO_3,33%) at the rate of (100 and 50kg N/fed) in three equal dose, and K fertilizer was added in the form of K_2SO_4 ($K_2O_5$0%), at the rate of (50 and 100 kg/fed) for barley and clover, respectively. A spoon of mycorrhiza inoculate was added to the soil after filling 2/3 of whole quantity of soil in pots, furthermore a tea spoon of mycorrhiza inoculate was applied in every hole under the seeds before planting. Ten seeds of barely or clover were sown in each pot and the plants were thinned to six plants per pot after three weeks from sowing.

All pots were watered to 80% of field capacity daily. The seeds of Egyptian clover (*Balady* 1) were inoculated prior to sowing with the specific strain of rhizobium trifolii. Normal practises were followed as usual in both plants. The Cobalt and arbuscular-mycorrhizal (AV) species treatments for each experiment were distributed in completely randomized block design with three replicates. The cobalt was applied as follows:

Table 1. The main physical and chemical properties of the experimental soil

Soil property	Particle size distribution				Soil moisture content			
Physical	Sand %	Silt%	Clay%	Texture	F.C % (w:v)			
	73	14	13	Silty loam	20			
Chemical	pH (1:1)		EC(dSm^{-1}) (1:1)		Tot $CaCO_3$,%		O.M (%)	
	8.0		2.14		19.95		1.06	
	Water soluble cation (meq/l)				Water soluble anion (meq/l)			
	Ca^{2+}	Mg^{2+}	K^+	Na^+	HCO_3^-	CO_3^-	SO_4^{2-}	Cl^-
	7.0	2.5	0.87	13.1	4.00	-	5.08	14.00
	Available nutrients (mg/kg soil)							
	N	P	K		DTPA-Co			
	60	7	185		0.196			

Cobalt application to the soil

When plant seedlings started to form their first three true leaves, cobalt sulphate as a source of cobalt was added to soil pots at the rate of 0, 10, 20, 40, 80 and 160 mg Co Kg^{-1} soil.

Morphological root parameters

Plant roots were removed from each pot and separated from soil by washing under a jet of tap water on a 0.5 mm sieve. Excess moisture was blotted from the cleaned roots by wrapping up the roots in layers of paper towel for 3 min [42]. For each pot three samples of 0.3 g fresh weight were used for the determination of root length by the line intersect method of [44].

- **Root length** [44]

$$RL = \frac{11}{14} \times N \times G$$

Where:
RL= root length, N = sum of horizontal and vertical crossing,
G= length of the grid unit (2cm or 1cm).

- **Root Surface Area** [8]

Surface area of a 1cm root cylinder (SAC) was calculated as follows:

$$SAC = 2\pi \times r_0$$

Where SAC = surface area of the root cylinder and r_0 = root radius

- **Root radius**

Estimation of root radius (r_0) (cm) was based on the assumption that the specific weight of root is almost equal to that of water,1 g cm^{-1} [8].

$$r_0 = \sqrt{\frac{RFW}{\pi RL}}$$

Where RFW = root fresh weight (g) and RL = root length (cm) and r_0 = root radius

- **Mean half distance between roots (*r_1*)**

Mean half distance between neighbouring roots (r1) was calculated according to [42]:

$$r_1 = \sqrt{\frac{V}{\pi RL}}$$

Where V = volume of the soil in the pot (cm^3) and RL= root length per pot

Plant analyses

Samples of shoots were measured from each pot and weighed, washed with running tap water and then with distilled water .The samples were air dried for few hours, and weight was measured, then oven dried at 65°C for 48 hours and grounded after recording the oven-dry weight of shoots. After dryness, the plant samples were ground by mill well and 0.5g of oven-dried plant materials were digested with H_2SO_4-H_2O_2 digest [34] and the digested solutions, were analysed for total nitrogen, phosphorus, potassium and cobalt. Total N was determined colorimetrically by *Nessler* method [12]. The vanado molydate colorimetric method was used to measure P in the digested plant samples [26] using spectrophotometer at 480 nm wave length. Cobalt concentration was determined in the digested solution [26] using the atomic absorption spectrophotometer (Model SpectrAA-200).

The obtained data were statistically analyzed according to the technique of analysis of variance (ANOVA) and the least significant difference (L.S.D) method was calculated to test the difference between the treatment means at α =0.05 level, as described by Gomez and Gomez [25], by using CoStat computer software package (Co Stat, Ver. 6.311., 2005.

Results and Discussions

1. Barley and E. Clover growth parameters

A. Shoot growth:

Obtained results in table (2) demonstrated that inoculated both barley seeds and Egyptian clover seeds with *G. intraradiaces* mycorrhizal species produced the highest shoots; highest number of leaves/plant; heaviest shoot fresh weight and dry weight. Conversely, uninoculated seeds showed the lowest values for the respective traits of barley and Egyptian clover crops.

Considering cobalt concentrations effect, results presented in table (2) pointed out that applied 20 mg cobalt/kg soil gave the highest number of barley leaves /plant (9.49), heaviest shoot fresh and dry weights and highest shoot. While, applied 40 mg cobalt /kg soil for E.clover showed the highest shoot (60.27cm), shoot fresh and dry weights (2.97 and 0.76g) and highest number of leaves/plant. That means the leguminous plants required more cobalt than other plants.

On the contrast, the lowest values of the studied traits of both barley and E. clover resulted from 160 mg cobalt/kg soil application.

That's due to bacteria on root nodules of legumes (Beans – alfalfa and clover) required cobalt to synthesize vitamin B_{12} and Fix nitrogen from air [46]**.** The results showed that the importance of cobalt to the growth and development of leguminasea plants was mainly due to the effect of cobalt on the activity and population of both atmospheric nitrogen micro-organisms of *Azotobacter* and *Nitrobacter* [40]. These results are in the same line with [17,29].

B. Root growth parameters

The studies root characteristics of barley and E.clover plants were significantly affected by cobalt application, mycorrhizal inoculation and their interactions as shown in table (3).

Results presented in table (3) demonstrated that cobalt soil application at 10 and 20 mg /Kg showed the heaviest fresh weight (3.12 and 3.16 g), tallest roots length (1456.03 and 1496.02 cm) and highest root surface area (238.86 and 243.12 cm^2) of barley plants, while applied 160 mg cobalt /Kg soil produced the highest root radius (0.02930 cm) and highest mean half distance between roots (0.473 cm). However, the heaviest root dry weight (0.74 g) resulted from 20mg cobalt /Kg soil application. These results are agreement with [16, 18]. They reported that application of cobalt at 20 mg /kg soil was more effective in increasing all parameter growth, yield quality and quantity.

Considering mycorrhizal effect, inoculated seeds of barley plants with *G. interadiaces* showed the highest valued (3.49 g, 0.72 g, 1659 cm, 0.0298 cm, 0.519 cm and 269.39 cm^2) for root fresh and dry weights, root length, radius, mean half distance between roots and root surface. In the contrary, the lowest values for respective traits resulted from sowing uninoculated seeds. Increased growth of plants inoculated with mycorrhizal fungi (AMF) is usually attributed to mycorrhizal colonization through increased capacity of plant roots to take up water and nutrients [2].

Root fresh and dry weights, root length, root radius, distance between roots and root surface area of E. Clover were significantly affected by cobalt soil application and mycorrhizal inoculation besides their interaction. Results in table (3) indicated that the heaviest root fresh weight (1.45 g), tallest roots (2412.8 cm) and highest root surface area (209.61cm^2) resulted from 40mg cobalt/ kg soil application, while soil applied with 20 or 40 mg cobalt/ kg soil produced the heaviest root dry weights (0.33 and 0.33 g).

On the contrast, soil treated with 80 or 160 mg cobalt / kg soil gave the lightest root fresh weights (0.60 and 0.59 g) shortest roots (1073.07 and 981.25 cm) and lowest root surface (89.81 and 85.45 cm^2) of E.clover plants, respectively, as shown in table (3). These results agreement with **[**29] they found that cobalt up 50 mg/kg soil decreased plant growth parameters such as root and shoot length, total leaf area, shoots and roots dry weights.

On the other side, E. Clover seeds inoculated with both mycorrhizal species showed heavy root weight, length and surface area compared with uninoculated seeds. Inoculation seeds with *G. intraradiaces* showed the heaviest fresh (1.22 g) and dry (0.32g) roots; tallest roots (2006.46 cm), as shown in table (3). In the same line, [2] reported that the root length of carrot plant inoculated with mycorrhizal fungi was significantly longer than that of the other plants without mycorrhizal fungi. Also, these results were agreement with the finding of [7].

With respect to mycorrhizal inoculation effects, obtained results table (3) revealed that inoculated barely seeds and E. clover seeds with *G. intraradiaces* produced the heaviest root fresh weight, dry weight, highest root length and highest root surface (269.39, 175.31 cm^2) for the two successive plants. On the contrast, uninoculated seeds showed the lowest values for the respective characters in barely and E. clover plants, receptively. These results are in agreement with those obtained by [22] in some higher plants. Indigenous mycorrhiza inoculum was successful in colonizing plant roots and resulted in better plant growth and yield [38].

However, uninoculated seeds combined with 80 mg cobalt/kg soil produced the highest mean half distance between roots (0.5742 cm) and root radius (0.03157 cm) paralled with (0.03136 and 0.03138 cm) that resulted from zero and 160 mg cobalt/ kg soil application.

On the other hand inoculated E.clover seeds with *G intraradiaces* combined with 40 mg cobalt /kg soil produced the highest root fresh weight (1.678g) and root radius (0.0150 cm) while 20 mg cobalt /kg soil application gave the heaviest root dry weight (0.5191 g) and root length (2858.33 cm). In the same line, [2] reported that the root length of carrot plant inoculated with mycorrhizal fungi was significantly longer than that of the other plants without mycorrhizal fungi. Also, these results were agreement with the finding of [7]**.** They studied the effect of *G. intraradiaces and G. macrocarpium* on growth by maize grown in a calcareous soil. They found that the plants treated with *G. intraradiaces* had higher mycorrhizal colonization rates than *G. macrocarpium* -treated plants. Two mycorrhizal species increased shoot and root DW and ***G.*** *intraradiaces* was more effective.

The highest mean half distance between roots (0.5563 cm) resulted from 160 mg cobalt /kg soil application and uninoculated E. clover seeds.
Conversely, high Cobalt concentrations, especially 160 mg /kg soil application combined with uninoculated barely and E. clover seeds, generally showed the lowest values for the studied root characteristics.

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2. Barley and E.clover macronutrients content:

With regards to interaction effects, obtained results in table (4) showed that applied 20 mg cobalt/ kg soil and inoculated barely seeds with *G. intraradiaces* species produced the highest NPK contents and uptake, respectively, by barely plants. On the other hand, inoculated E. clover seeds with the same mycorrhizal specie combined with 40 mg cobalt /kg soil produced the highest NPK contents and uptake by plants. That could be due to high requirements of cobalt for some leguminous plants such soybean plants [28].

In the contrary, applied 160 mg cobalt / kg soil to uninoculated seeds with mycorrhizae, generally showed the lowest NPK content and uptake with barely and E. clover plants. So leguminous plants required little more cobalt than non-leguminous. Also [23] reported that cobalt has

colonizing plant roots and resulted in better plant growth and yield [38].
However, uninoculated seeds combined with 80 mg cobalt/kg soil produced the highest mean half distance between roots (0.5742 cm) and root radius (0.03157 cm) paralled with (0.03136 and 0.03138 cm) that resulted from zero and 160 mg cobalt/ kg soil application.

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They found that the plants treated with *G. intraradiaces* had higher mycorrhizal colonization rates than *G. macrocarpium* -treated plants. Two mycorrhizal species increased shoot and root DW and ***G.*** *intraradiaces*
significant promotive effect on yield, fruits oil, fruits macro and micro nutrient, fruit quality and endogens hormone comparing to control, but increase cobalt concentration has negative effect on plant growth [47,20] who reported that the highest significant figures of N, P, K, Ca, Mg, Mn, Zn, and Cu were obtained by using cobalt at 20.0 ppm for growing maize plant.

With respect to mycorrhizal inoculation effect, results presented in table (4) revealed that the highest NPK content in barely and E. clover plants and, resulted from inoculation with *G. intraradiaces*. However uninoculated seeds with mycorrhizae gave the lowest NPK content and uptake in barely and E. clover plants. In the same line, [1] reported that the root growth of tagetes inoculated with mycorrhizal increased significantly than tagetes without mycorrhizae. Also this result is in agreement with those reported by [3]**.** This increase coincided with an increase in free indole -3-butyric acid (IBA) [30]. Finer roots are more efficient in nutrient absorption from the soil [42].

Table 2. The main effects of Mycorrhiza inoculation and cobalt concentration on shoot growth parameters (SGP) of barley and E. Clover plants

Cobalt application	Shoot height (cm)		No of leaves/ plant		Shoot fresh weight (g/plant)		Shoot dry weight (g/plant)	
	Barley	E.Clover	Barley	E.Clover	Barley	E.Clover	Barley	E.Clover
Mycorrhiza inoculation								
Control	44.20c	53.04c	8.38c	47.29	1.61c	2.14c	0.61c	0.53c
G.M	47.52b	54.64b	9.21b	52.59b	1.95b	2.26b	0.69b	0.57b
G.I	49.52a	57.16a	9.92a	55.39a	2.16a	2.51a	0.76a	0.58a
$LSD_{.05}$	0.63	1.01	0.18	2.33	0.025	0.04	0.008	0.007
Cobalt concentration (mg/l)								
Control	46.45cd	56.78c	9.08b	48.54c	1.73e	2.48c	0.67de	0.60c
10	48.11a	57.91bc	9.32ab	53.0b	2.05b	2.55bc	0.70b	0.61bc
20	48.22a	58.84ab	9.49a	59.25a	2.15a	2.56b	0.73a	0.62b
40	47.5ab	60.27a	9.24ab	61.5a	2.01c	2.97a	0.69c	0.76a
80	46.96bc	51.96d	9.08b	47.3c	1.81d	1.86d	0.67d	0.46d
160	46.04d	43.93e	8.81c	40.95d	1.70e	1.40e	0.66e	0.35e
$LSD_{.05}$	0.89	1.43	0.26	3.3	0.035	0.06	0.01	0.01

G.I = *G. intraradiaces*, G.M= *G. macrocarpium,* and Cont=control (without mycorrhizae inoculation

The values in each column followed by the same letter are not significant at the 0.05 probability level

Table 3. The main effect of Mycorrhiza inoculation and cobalt application concentration on root growth parameter of barley and E.Clover plants

Cobalt application	Root fresh weight (g/plant)		Root dry weight(g/plant)		Root length (cm/plant)		Root radius (cm)		Mean half distance between roots (cm)		Root surface (cm^2)	
	Barley	E.Clover	Barley	E.Clover	Barley	E.Clover	Barley	E.Clover	Barley	E.Clover	Barley	E.Clover
Mycorrhizae inoculation												
Cont	2.37c	0.81c	0.61c	0.20c	860.34c	1306.1c	0.026c	0.01293b	0.375c	0.35c	160.14c	115.29c
G.M	3.04b	0.89b	0.66b	0.23b	1318.71b	1730.96b	0.272b	0.01398a	0.419b	0.37b	224.03b	138.51b
G.I	3.49a	1.22a	0.72a	0.32a	1659a	2006.46a	0.0298a	0.01417a	0.519a	0.43a	269.39a	175.31a
$LSD_{.05}$	0.06	0.04	0.023	0.014	58.18	105.67	0.00029	4.40	0.010	0.01	7.39	5.07
Cobalt Concentration (mg/kg)												
Control	2.85c	0.94d	0.64c	0.23	1157.57c	1794.2c	0.0285b	0.01323b	0.46ab	0.37c	203.08c	144.28d
10	3.12a	1.09c	0.71ab	0.25b	1456.03a	1865.98bc	0.0264d	0.01374ab	0.403d	0.36cd	238.86a	160.13c
20	3.16a	1.16b	0.74a	0.33a	1496.02a	1959.77b	0.0264d	0.01395a	0.402d	0.35d	243.12a	168.93b
40	2.99b	1.45a	0.69b	0.33a	1294.12b	2412.8a	0.02725c	0.01388a	0.430c	0.30e	220.72b	209.61a
80	2.94bc	0.60e	0.64c	0.20d	1223.02bc	1073.07d	0.02876b	0.01341ab	0.458b	0.46b	212.08bc	89.81e
160	2.76d	0.59e	0.55d	0.15e	1041.81d	981.25d	0.02930a	0.01395a	0.473a	0.48a	189.27d	85.45e
$LSD_{.05}$	0.09	0.05	0.033	0.019	82.28	149.45	0.0003	6.22	0.01	0.01	10.46	7.18

G.I = *G. intraradiaces*, G.M= *G. macrocarpium,* and Cont=control (without mycorrhizae inoculation

The values in each column followed by the same letter are not significant at the 0.05 probability level

Table 4. The main effect of Mycorrhiza inoculation and cobalt application concentration on N, P and K content and uptake of barley and E. Clover plants

Cobalt application	Plant N content (g/kg d.m)		Plant P content (mg/g d.m)		Plant K content (mg/g d.m)		Plant N uptake (mg/plant)		Plant P uptake (mg/plant)		Plant K uptake (mg/plant)	
	Barley	E.Clover	Barley	E.Clover	Barley	E.Clover	Barley	E.Clover	Barley	E.Clover	Barley	E.Clover
Mycorrhiza inoculation												
Control	11.64c	18.63c	3.24c	4.17c	27.94c	23.33c	6.93c	7.48c	2.01c	1.81c	17.22c	10.93c
G.M	16.97b	22.61b	5.66b	5.22b	30.14b	27.4b	12.06b	9.79b	3.89b	2.52b	20.89b	13.63b
G.I	21.46a	25.92a	13.40a	6.75a	34.39a	30.13a	16.07a	12.77a	10.18a	3.66a	26.18a	15.71a
$LSD_{.05}$	0.42	1.78	0.26	0.31	0.39	0.78	0.33	0.95	0.24	0.21	0.57	0.65
Cobalt Concentration (mg/kg)												
Control	15.62d	19.68cd	4.71e	4.72d	29.15c	26.58cd	10.67e	8.57c	3.16e	2.32d	19.55d	13.81c
10	17.96b	21.84c	10.13b	5.25c	31.79b	27.38bc	13.11b	9.87c	7.65b	2.70c	22.81b	14.45bc
20	19.41a	24.96b	12.12a	5.8b	35.03a	27.83b	14.45a	12.29b	9.38a	3.11b	26.05a	15.36b
40	17.49b	33.36a	7.64c	7.8a	31.52b	30.66a	12.21c	18.79a	5.44c	4.85a	21.89c	19.49a
80	16.88c	18.2de	5.45d	4.47de	29.7c	25.9d	11.32d	6.32d	3.67d	1.73e	20.08d	10.27d
160	12.77e	16.1e	4.53e	4.23e	27.76d	23.3e	8.36f	4.25e	2.85e	1.25f	18.19e	7.16e
$LSD_{.05}$	0.59	2.51	0.37	0.44	0.56	1.11	0.47	1.34	0.34	0.29	0.81	0.91

G.I **=** *G. intraradiaces* , G.M= *G. macrocarpium* , and Cont=control (without mycorrhizae inoculation

The values in each column followed by the same letter are not significant at the 0.05 probability level

3. Cobalt availability and Cobalt content in barley and Clover plant content:

Regarding mycorrhizal inoculation effects, results in table (5) indicated that inoculated barely seeds and E. clover produced the highest cobalt availability (9.48, 12.31 mg/kg d.m), shoot cobalt content (0.862, 0.680 mg/kg d.m), root cobalt content (6.94, 11.01 mg/kg d.m) and plant cobalt content (7.61 and 11.70 mg/kg d.m) in two respective crops.

Conversely uninoculated seeds of the two studies crops showed the lowest availability and cobalt contents.

Concerning cobalt soil application effect, results presented in that table revealed that increasing cobalt application to soil significantly and gradually increased cobalt availability and content in shoot, root and plant of both barely and Egyptian clover.

Cobalt application at 160 mg /kg soil showed the highest cobalt availability (15.65, 18.51 mg/kg d.m), shoot content (0.99, 1.29 ppm), root content (9.84, 21.14 ppm) and content (10.47, 22.44 mg/kg d.m) in barely and E. clover crops respectively. Conversely, sowing both crops without cobalt application showed the lowest cobalt available, shoot, root and barely plant (0.217, 0.49 , 1.16 and 1.65 mg/kg d.m) and (1.0, 0.25, 1.52 and 1.78 mg/kg d.m) respectively for E. clover , besides 10mg cobalt / kg soil application (2.52 mg/kg d.m) for E.clvoer root cobalt content. [21] showed that cobalt content in fenugreek grains significantly increased when cobalt addition increasing in plant growing media. These results are agree with those obtained by [24] who found that increasing cobalt concentration in plant media significantly increased cobalt content in faba bean plants compared with control. So, as the cobalt considered an essential element for legume plants, therefore the cobalt content of E. clover is higher compared to cobalt content of barley. These are agreement with those obtained by [32]. They found that cobalt accumulation can occur in Anisopappus chinensis (Asteraceae), as foliar Co concentration increases in relation to Co concentration in the nutrient solution without a decrease in the biomass or toxicity symptoms.

From the previous results, it could be concluded that inoculated both barely and Egyptian seeds with both mycorrhizal species studied showed higher values of all the studied traits compared with uninoculated treatment. Inoculated seeds with *G. intraradiaces* species showed the highest values. . These results agreement with [31] who found that AM colonization increased the biomass, growth of plants and availability of essential nutrients to wheat plants comparing to control.

That could be due to the extra-radical mycelium (ERM) are tiny hyphal threads which reach far out into the soil and effectively serves as an extension of the plant's root system by increasing the surface area available for nutrient acquisition, including nitrogen, which can be taken up in the form of ammonium, nitrate or from organic sources [4].

Cobalt application at 20 and 40 mg/kg soil to barely and E.clvoer respectively showed the highest values of the studied traits, except for Fe content, where sowing both crops without cobalt application to uninoculated seeds showed the highest Fe content.

Table 5. The main effect of Mycorrhiza inoculation and cobalt application concentration on the content of available Co^{2+}, Shoot Co^{2+}, root Co^{2+} and Plant Co^{2+} of barley and E. clover root of barley and E. Clover plants

Cobalt application	Available Co^{2+} (mg/kg soil)		Shoot Co^{2+} (mg/kg d.m)		Root Co^{2+} (mg/kg d.m)		Plant Co^{+2} (mg/kg d.m)	
	Barley	E.clover	Barley	E.clover	Barley	E.clover	Barley	E.clover
Mycorrhiza inoculation								
Cont	6.04c	7.16c	0.511c	0.50c	3.95c	5.22c	4.46c	5.73c
G.M	8.37b	10.33b	0.638b	0.59b	5.99b	8.89b	6.63b	9.49b
G.I	9.48a	12.31a	0.862a	0.68a	6.94a	11.01a	7.61a	11.70a
$LSD_{.05}$	0.422	0.77	0.013	0.07	0.13	0.95	0.13	0.97
Cobalt Concentration (mg/kg)								
Control	0.217f	1.0f	0.49f	0.25e	1.16e	1.52e	1.65f	1.78e
10	2.66e	5.49e	0.51e	0.36de	2.94d	2.52e	3.46e	2.89e
20	5.90d	7.98d	0.63d	0.41cd	3.36c	4.31d	3.99d	4.72d
40	10.33c	11.45c	0.66c	0.47c	7.03b	8.06c	7.69e	8.54c
80	13.03b	15.18b	0.72b	0.76b	9.39a	12.70b	10.12b	13.46b
160	15.65a	18.51a	0.99a	1.29a	9.84a	21.14a	10.47a	22.44a
$LSD_{.05}$	0.59	1.10	0.019	0.10	0.18	1.35	0.19	1.38

G.I = *G. intraradiaces*, G.M= *G. macrocarpium* , and Cont=control (without mycorrhizae inoculation

The values in each column followed by the same letter are not significant at the 0.05 probability level

[23] reported that cobalt has significant promotive effect on yield, fruits oil, fruits macro and micro nutrient, fruit quality and endogens hormone comparing to control, but increase cobalt concentration has negative effect on plant growth [20, 47].

Regarding cobalt availability, root, shoot and plant content increasing cobalt soil application up to 160 mg/kg soil showed the highest values of the studied traits, especially it's interactions with *G. intraradiaces* inoculation. [7] reported that the effect of *G. intraradiaces* on growth by maize grown in a calcareous soil.

They found that the plants treated with *G. intraradiaces* had higher mycorrhizal colonization rates than *G. macrocarpium* -treated plants. Also [43] reported that inoculation of the host plants with mycorrhizae, significantly increased the dry weight, shoot length, total N, P and K as well as chlorophyll concentration in the plants.

4. Conclusion:

It could be concluded for the soil application methods *G. intraradiaces* was more effective than *G. macrocarpium* for the studied traits. Also, the recommended cobalt concentration for barley crops was lower (20 mg/kg soil) than that in E. clover (40 mg/kg soil) as legume plants needs Cobalt supply. Cobalt supply enhances nitrogen fixation in all rhizobium species and hence promotes legume growth. These conclusions get under the same conditions of this study.

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