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EFFECT OF USING OF TWO PLANTS POWDER SILYBUM (*SILYBUM MARIANUM*) AND LICORICE (*GLYCYRRHIZA GLABRA*) ON TWO TYPES OF GROWTH LEGUMINOUS PLANTS IN SOILS POLLUTED BY HEAVY METALS

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Abstract

The research aims at studying the effect of two plants powder Silybum *Silybum marianum* and licorice *Glycyrrhiza glabra* by concentrations (0, 3, 6) g/kg soil for each of them on growth cowpea *Vigna unguiculata* and beans *Phaseolus vulgaris* plants in soils polluted by two elements of cobalt with concentrations (0, 30, 60) and copper with concentrations (0, 70, 100) mg/kg soil. The results showed that soil treatment with cobalt at concentrations (60) mg/kg soil led to significant increase by concentrating peroxidase enzyme where it totaled (0.095) µm/mL , superoxide dismutase where it totaled (0.120) µg/mL/min for the two plants compared by the control treatment, meanwhile the results showed that the soil treatment with powder licorice at concentration (3) g/kg soil led to insignificant decrease by concentrating superoxide enzyme dismutase for the two plants compared to the control treatment , but when the soil is treated by powder licorice at concentration (6) g/kg soil it was observed that it caused an insignificant decrease in the concentration of Peroxides enzyme for both plants compared to the control treatment.

Key words : *Silybum marianum, Glycyrrhiza glabra,* enzyme dismutase

Introduction

The increase in the proportion of the world's population has led to an increased interest in environmental pollution and poisoning by chemicals ,as the acceleration of industrial and urban processes led to the accumulation of pollutants and toxins such as pesticides, oil products, acids and heavy metals in natural resources such as soil , air and water Which led to a decrease not only in the quality of the environment, but also the impact on both animal and plant life. Heavy metals, including copper, cobalt, chromium, lead, cadmium, mercury and nickel, are important environmental pollutants that lead to plant poisoning and are a serious threat to various ecosystems (Sethy and Ghosh, 2013). Pollution of the environment with heavy metals comes from various sources, including industrial and agricultural sources, which are fields of tremendous interest in all parts of the world. Although heavy metals are natural components in the soil at very few concentrations, the high environmental pollutants resulting from industry, agriculture and mining operations have contributed to undesirable poisonous accumulations of heavy metals (Chandra and Kang, 2016). Heavy metals such as cobalt, copper, iron, manganese, molybdenum, nickel and zinc enter the soil from various sources such as casting factories, mining, combustion and agricultural (Nagajyoti *et al.,* 2010). The problem of pollution of ecosystem with heavy metals may also arise from the long-term use of untreated waste in irrigation, which led to an increase in the concentration of heavy metals in the soil (Lu *et al.,* 2015). The heavy metals are considered important environmental pollutants and their toxicity is reckoned a grave problem where it arouses a huge concern for environmental, nutritional and toxicological reasons, as metals can affect a number of physiological and biochemical processes in the plants, and their toxicity varies with different types of plants, metal type, metal concentration and chemical form (Ackova , 2018). The soil is counted the fundamental part and most important in the environmental system where the soil is polluted significantly with heavy metals across world (Hakeem *et al.,* 2014). And the areas polluted with heavy metal acquires a great interest because of its possible danger on the safety and security of food and its harmful effects on the environmental system (Mombo *et al.,* 2015). Existence of heavy elements in the environment prevents plants from reaching their fullest potential in terms of growth and reproduction, because once they are deposited on the ground the plants are able to eat these elements from the soil and insert them into the food chain, which increases the risks of toxicity to metals on humans and animals (Roy and McDonald, 2015). Just as heavy metal pollution may severely hinder plant growth and productivity and it also increases the risk of harm to animal and human health through biomagnification (Sharma, 2012).

Materials and methods

Preparedness and soil treatment

The surface soil was taken from Governorate of Nineveh definitely area of Zayuna at depth of (0-30) cm and was air dried , then smoothened and passed through a sieve whose diameters of holes are (2) Mm. The treatments used in this study comprised two types of heavy elements namely cobalt and copper with two different concentrations for each element, and treating the soil with two types of powder of wild plants and they are Silybum *Silybum marianum* and licorice *Glycyrrhiza glabra* and in two concentrations each for them (the two plants' powder was obtained by taking the leaves of the plant for the Silybum and the roots of the licorice plant and drying them with air then they were ground using an electric mill to be ready for use in soil treatment, according to the type and concentration of the treatment), in addition to the control treatment and by three replications for each treatment and the elements were added ,each separately and powder of Silybum plant and powder of licorice plant, each was added separately according to the treatment to soil, as it was mixed outside the pot and then placed in the pot to ensure that it blended well and the experiment was carried out using plastic pots whose diameter is (23) cm and height is (20) cm, capacity of each pot is (5) kg soil and the treatments were as follows:

A- Adding cobalt in the form of ($Co (No_3)_2.6H_2o$) to the pots soil with two concentrations (30, 60) mg/kg respectively.

B- Adding cobber in the form of ($Cu (No_3)_2.3H_2O$) to the pots soil with two concentrations (70, 10) mg/kg soil respectively.

C- The soil treatment with powder of Silybum with concentration of (3) and (6) g/kg respectively.

D- The soil treatment with powder of licorice with concentration of (3) and (6) g/kg respectively.

E- The interventions:

1- The soil treatment with powder of Silybum with concentration (3) g/kg + the soil treatment with cobalt with concentration (30, 60) mg/kg soil respectively.

2- The soil treatment with powder of Silybum with concentration (6) g/kg + the soil treatment with cobalt with concentration (30, 60) mg/kg soil respectively.

3- The soil treatment with powder of Silybum with concentration (3) g/kg + the soil treatment with copper with concentration (70, 100) mg/kg soil respectively.

4- The soil treatment with powder of Silybum with concentration (6) g/kg + the soil treatment with cobber with concentration (70, 100) mg/kg soil respectively.

5- The soil treatment with powder of licorice with concentration (3) g/kg + the soil treatment with cobalt with concentration (30, 60) mg/kg soil respectively.

6- The soil treatment with powder of licorice with concentration (6) g/kg + the soil treatment with cobalt with concentration (30, 60) mg/kg soil respectively.

7- The soil treatment with powder of licorice with concentration (3) g/kg + the soil treatment with copper with concentration (70, 100) mg/kg soil respectively.

8- The soil treatment with powder of licorice with concentration (6) g/kg + the soil treatment with copper with concentration (70, 100) mg/kg soil respectively.

Agriculture and Irrigation

Cowpea *Vigna unguiculata* and beans *Phaseolus vulgaris* were obtained at shops for selling seeds and agricultural items in the city of Mosul in Iraq. The seeds were planted on 21/8/2019 (15) seeds/pot and it was taken into consideration that the distances should be equal between seeds, the pots were placed randomly under circumstances of plastic house and after passage of (60) days of agriculture date the cowpea and beans were harvested with (3) three replications per treatment.

The studied characteristics

Peroxidase enzyme effectiveness estimate

The peroxidase enzyme effectiveness was measured in the plant leaves and absorbance was read using the Spectrophotometer at wave length (470) nm according to method mentioned in (Badea *et al.,* 1999) the following law was applied :

$A = £ \times L \times C$

$C = A / 26.6$

A = The absorbance

C = Enzyme effectiveness (μm/mL)

£ = Completion coefficient equals (26.6)

Table 1: Effect of soil treatment with Silybum and licorice powder on peroxidase concentration(µm /mL) for cowpea and beans plants growth in soil polluted with cobalt and copper.

The plant type	Control	Silybum g/kg		Licorice g/kg		CO mg/kg		Cu mg/kg		30 mg/kg CO				60 mg/kg CO				Cu 70 mg/kg				Cu 100 mg/kg				Effect of plant type
										Silybum		Licorice		Silybum		Licorice		Silybum		Licorice		Silybum		Licorice		
		3	6	3	6	30	60	70	100	3	6	3	6	3	6	3	6	3	6	3	6	3	6	3	6	
	0.035 mn	0.035 mn	0.036 mn	0.038 lmn	0.034 n	0.084 c	0.095 A	0.086 bc	0.088 b	0.042 l	0.075 j	0.066 d-g	0.053 k	0.065 efg	0.059 ij	0.069 de	0.057 j	0.063 Gh	0.062 ghi	0.064 fg	0.070 d	0.065 efg	0.068 def	0.069 de	0.060 hij	
Cow-pea	0.020 x	0.028 w	0.028 w	0.031 w	0.018 x	0.086 cde	0.097 A	0.083 de	0.087 b-e	0.033 vw	0.038 uv	0.060 lmn	0.037 uv	0.055 nop	0.044 st	0.070 g-k	0.058 no	0.053 opq	0.059 mno	0.059 mno	0.082 e	0.055 nop	0.065 jkl	0.068 h-k	0.054 n-q	0.055 b
Beans	0.049 qrs	0.041 tu	0.044 st	0.045 rst	0.049 qrs	0.082 e	0.092 Ab	0.088 bcd	0.089 bc	0.050 pqr	0.076 f	0.071 f-j	0.068 h-k	0.074 fgh	0.073 f-i	0.067 ijk	0.056 no	0.073 f-i	0.064 klm	0.069 g-k	0.057 no	0.075 fg	0.070 g-k	0.069 g-k	0.066 kj	0.066 a

Effect of Silybum concentration	0.054 a	0.056 a			Effect of element type				
Effect of Licorice concentration			0.061 a	0.055 a	0.064 A		0.070 a		
Effect of powder type	0.055 a		0.058 a		Effect of element concentration				
Effect of powder concentration	0.054 a	0.056 a	0.061 a	0.055 a	0.060 a	0.069 A	0.069 a	0.070 a	
Effect of powder concentration × The plant type					element concentration × The plant type				Effect of element type × The plant type	
Cowpea	0.045 a	0.047 a	0.058 a	0.050 a	0.051 a	0.065 A	0.067 a	0.066 a	0.058 a	0.067 A
Beans	0.063 a	0.065 a	0.064 a	0.059 a	0.069 a	0.072 A	0.070 a	0.074 a	0.071 a	0.072 A
Effect of powder type × The plant type					The equations with similar letters don't differ significantly at the probability level (5%) according to the Duncan polynomialy test					
Cowpea	0.046 a		2.671 a							
Beans	0.054 a		0.062 a							

Table 2: Effect of soil treatment with Silybum and licorice powder on superoxide dismutase concentration (µg/mL/min) for cowpea and beans plants growth in soil polluted with cobalt and copper.

The plant type	Control	Silybum g/kg		Licorice g/kg		CO mg/kg		Cu mg/kg		30 mg/kg CO				60 mg/kg CO				Cu 70 mg/kg				Cu 100 mg/kg				Effect of plant type
										Silybum		Licorice		Silybum		Licorice		Silybum		Licorice		Silybum		Licorice		
		3	6	3	6	30	60	70	100	3	6	3	6	3	6	3	6	3	6	3	6	3	6	3	6	
	0.034 m	0.047 l	0.045 l	0.033 m	0.048 l	0.112 b	0.120 A	0.093 e	0.118 a	0.048 l	0.083 f	0.064 ij	0.074 h	0.063 j	0.100 c	0.067 i	0.095 de	0.051 k	0.078 g	0.077 Gh	0.075 gh	0.065 ij	0.078 g	0.095 d	0.096 d	
Cow-pea	0.020 x	0.0 st	0.048 tuv	0.048 tuv	0.050 stu	0.153 b	0.166 A	0.116 d	0.131 b	0.054 rs	0.112 de	0.078 l	0.093 i	0.084 k	0.129 c	0.086 k	0.129 c	0.056 r	0.110 ef	0.088 jk	0.105 g	0.067 nop	0.110 ef	0.099 h	0.106 fg	0.092 a
Beans	0.048 tuv	0.041 w	0.041 w	0.018 x	0.045 vw	0.071 mn	0.073 M	0.069 mno	0.105 g	0.041 w	0.054 rs	0.050 stu	0.054 rs	0.041 w	0.071 mn	0.048 tuv	0.061 q	0.046 uv	0.046 uv	0.065 Opq	0.045 vw	0.063 pq	0.046 uv	0.091 ij	0.086 k	0.057 b
Effect of Silybum concentration		0.055 b	0.077 a			Effect of element type																				
Effect of Licorice concentration				0.066 b	0.077 a	0.083 A		0.082 a																		
Effect of powder type		0.067 a		0.072 b		Effect of element concentration																				
Effect of powder concentration		0.055 c	0.077 a	0.066 b	0.077 a	0.076 b	0.089 A	0.075 b	0.089 a																	
Effect of powder concentration × The plant type						element concentration × The plant type				Effect of element type × The plant type																
Cowpea		0.063 c	0.102 a	0.078 b	0.097 a	0.098 b	0.119 A	0.095 b	0.101 b	0.108 a								0.098 A								
Beans		0.046 d	0.052 cd	0.054 cd	0.058 cd	0.054 d	0.059 D	0.054 d	0.078 c	0.056 a								0.066 A								
Effect of powder type × The plant type						The equations with similar letters don't differ significantly at the probability level (5%) according to the Duncan polynomialy test																				
Cowpea		0.092 a		0.087 a																						
Beans		0.049 b		0.056 b																						

L = Cell thickness equals (1)

Superoxide dismutase enzyme (SOD) effectiveness estimate

SOD enzyme effectiveness (unit/1g fresh weight) was measured in plant leaves and the absorbance was read using the Spectrophotometer at wave length (480) nm according to method (Bosco *et al.,* 2007)mentioned in (Misra and Fridovich, 1972) .

Statistical Analysis

The experiments were designed and statistically analyzed using the practical experiment according to completely randomized design (C.R.D) in the factorial experiments (Antar, 2010), the comparison was completed between the significant differences in the treatments rates using (Duncan's new multiple range test).

Results and Discussion

Peroxidase Enzyme (POD) Estimate

The table 1 states that the soil treatment with licorice *Glycyrrhiza glabra* powder at concentration (6) g/kg soil led to decrease occurrence by concentration of peroxidase enzyme in the leaves tissues of the two plants compared to the treatment of control and other treatments where the decrease totaled (0.034) µm/mL. As noted that the soil treatment with the element of cobalt at concentration (60) mg/kg led to occurrence of significant increase by concentrating peroxidase enzyme in the leaves tissues of both cowpea and beans plants where the increase totaled (0.095) µm/mL compared to the control treatment and other treatments . Also the table 1 stated that silybum *Silybum marianum* powder addition at concentration (3) g/kg soil to soils polluted with cobalt at concentration (30) mg/kg soil led to decease of concentration of peroxidase enzyme in the leaves tissues of the two plants compared to growth plants with soils polluted with heavy elements and soils polluted with heavy elements and treatment with plant powders where it totaled (0.042) µm/mL. Increase in concentration POD of cowpea and beans plants at treatment with heavy metals may be attributed to increase of levels of hydrogen peroxide in the plants stressed with heavy metal and the changes in POD activity in the plants cells are considered vital and good indicators for the vital and non-vital stresses (Doganlar and Atmaca, 2011). But the cause of decrease in POD concentration of cowpea and beans plants at treatment with licorice powder may be attributed to existence of metals nutrients in licorice extract, it has increased the plant's tolerance to heavy metals by preserving the mechanisms of photosynthesis and reducing the impact of heavy metals on the cell membrane, leading to a decrease or reduction in the concentration of antioxidant enzymes, including POD (Bargaz *et al.,* 2016).

Estimate of superoxide dismutase Enzyme (SOD)

The table 2 states that the soil treatment with licorice *Glycyrrhiza glabra* powder at concentration (3) g/kg soil led to significant decrease at concentration SOD for the two plants compared to the other treatments where it totaled (0.033) µg/mL/min with exception of insignificant decrease with the control treatment. As observed that the soil treatment with cobalt element at concentration (60) mg/kg led to significant increase at concentration SOD enzyme in the leaves tissues for both beans and cowpea where it totaled (0.120) µg/mL/min compared to the control treatment and other treatments except insignificant superiority at copper treatment at concentration (100) mg/kg soil. Also, the table 2 states that addition of Silybum *Silybum marianum* powder rat concentration (3) g/kg soil to the soils polluted with cobalt at concentration (30) mg/kg soil led to significant decrease at concentration SOD enzyme in the leaves tissues for the two plants compared to growth plants with soils polluted with heavy elements and soils polluted with heavy elements treatment with plants powders where it totaled (0.048) µg/mL/min. The reason for the increase in concentration SOD plants for cowpea and beans plants when dealing with heavy metals may be due to the high concentrations of antioxidant enzymes that have entered into the protection mechanisms adopted by plants, which is associated with increasing the mitigation against high stress (Awasthi and Sinha, 2013). For the decrease in the concentration of SOD plants for cowpea and beans when dealing licorice powder may be due to the fact that licorice extract contains soluble sugars, which have an important physiological role In supplying the plant with energy and preserving the water potential of leaves and plant water content, which reduces the cellular osmotic pressure and increases the tolerance of heavy metals, which leads to reduce the damage of stress on the cell membrane and thus decrease the concentration of some antioxidant enzymes including SOD (Babaeian *et al.,* 2011).

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