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REDUCTION OF Mn TOXICITY TO SOYBEAN BY *Bradyrhizobium japonicum*

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ABSTRACT

Two preliminary experiments were conducted to investigate the effect of Mn concentrations on the performance of soybean in two different soil types induced activation and those which are harmful to soybean plants and *Bradyrhizobium japonicum* cells in the first experiment sequential concentrations of Mn (from zero to 500 ppm) to clay soil planted with soybean in small pots. The second one carried out by addition of some selected Mn concentrations resulted from the first experiment (zero, 100, 500, 1000, 2500, 3000 and 5000 ppm) to flasks containing yeast mannitol medium, then inoculated with *Bradyrhizobium japonicum* (St 110) cells. In the main pot experiment clay and calcareous soils were used. A sequential concentrations of Mn were added to both soils (zero, 300, 500, 1000 and 2000 ppm) in presence or absence of rhizobial inoculation.

The results indicated that all Mn concentrations had no obvious significant effects on germination % and natality %. Embryonic internod and shoot length were significantly increased till concentrations 400 and 1500 ppm for each respectively. Viable counts of rhizobial cells significantly increased due to low levels of Mn (100 and 500 ppm), but heavily significant declines were recorded when concentrations above 500 ppm were applied. Rhizobial inoculation significantly increased seed yield and most of other growth parameters. Soil types neither affects crop yield nor P and K contents of seeds. Plants which were grown in clay soil exhibited an increase in N-content than in calcareous soil. Nodules had no consistent effects due to increasing Mn levels. While N-content of plants significantly declined. K-content exhibited nearly similar trend. However, P-content generally did not affected. In the clay soil, all Mn concentrations till 1000 ppm did not significantly affect seed yield of inoculated plants but a significant increase was observed at 2000 ppm. In calcareous soil, the addition of manganese caused non-significant decrease in seed yield. Inoculation with rhizobia obviously decreased Mn uptake by plant in both soil types.

INTRODUCTION

Lead has been used as antiknock additives to gasoline as tetra-ethyl and tetra-methyl lead (Zimdahl and Hassett, 1979). Lead emissions into environment exerts many complications on crops grown around high ways (Impens, 1987). Many efforts were exerted to eliminate lead from the environment (Vesilind *et al.*, 1990).

An alternative antiknock additive must be used as Organic manganese compound which woud be used as suitable alternative to lead compounds (Vesilind *et al.*, 1990). The authors also stated that 2-methyl cyclopentadienyl manganese tricarbonyl has antiknock properties and is currently under consideration as a replacement for lead alkyls. Low levels of Mn is essential but high levels is considered neurotoxic. Pulmonary toxicity also occurs as high levels of exposure and developmental toxicity to fetuses, Howard and Gina (1997) and David *et al.* (2000).

Manganese is an important microelement for plant nutrition, Hegazy et al. (1992) found that the foliar application of faba bean with Mn resulted in a significant increase in yield. Also, William (1999) reported a significant increase in seed yields of lupinus due to foliar application with Mn.

Importance of manganese in plant nutrition is concentrated in activation of certain enzymes. In this connection, Mengel and Kirkby (1987) stated that Mn bridge ATP with the enzyme complex (phosphokinases and phosphotransferases). Decarboxylases and dehydrogenases of TCA cycle and chloroplasts RNA polymerase are also activated by Mn^{2+}. They added that the most well documented role of Mn in green plants is that the water splitting and O_2-evolution system in photosynthesis, the so called Hell reaction. It has now confirmed that Mn is required for both higher and lower plants.

Manganese deficiency negatively affects plant growth, the deficiency in plant is in the range of 10-20 µg Mn/g dry matter. Mn deficiency affect chloroplasts, as they are the most sensitive of all other organelles. In whole plants, tissue which are suffering from Mn deficiency have small cell volume, cell wall and the interepidermal tissue is shrunken. Intervienal chlorosis occur in the leaves (Mengel and Kirkby, 1987).

Mn toxicity, unlike deficiency is not restricted to a narrow critical concentration range. Ranging from 200 ppm in maize to 3500 in sunflower associated with 10% reduction in dry matter. Toxicity symptoms are brown spots in older leaves surrounded by chlorotic areas. Sometimes excess of Mn can induce a deficiency of other nutrients such as Fe, Mg and Ca (Alloway, 1995).

Some studies pointed to the importance of plant inoculation with some microorganisms in order to bioremediation of heavy metals. In this respect, Nour El-Dein (1997) found that inoculation of soybean plants with rhizobia decreased Pb concentrations in plants as well as decreasing the detrimental effects of Pb on plant growth. Gutnikc (2000) documented that bacteria have an important role in adsorption of heavy metals. He mentioned that many bacterial polysaccharides have been shown to bind heavy metals with varying degrees of specificity and affinity.

The effects of increasing ambient Mn concentrations on environment, vegetation and health have received very little attention. Thus the present study aimed to evaluate the effects of different Mn concentrations, added to media of rhizobia, on viable counts of *B. japonicum* cells. The effects of gradual levels of Mn on nodulation, NPK uptake, yield and yield components of inoculated and un-inoculated soybean plants was also evaluated. The detoxification effect of soybean-rhizobial inoculation and mitigation of hazardous effects of high levels of Mn on plants growth were also assessed.

MATERIALS AND METHODS

Three experiments were conducted two preliminary & pot experiments; the first preliminary was carried out to evaluate the effect of different sequential manganese levels added to the soil on soybean growth parameters (germination %, embryonic internode length, shoot and root length). These concentrations were; zero, 50, 100, 200, 300, 400, 500, 600, 750, 1000. 1250, 1750, 2000, 2250, 2500, 2750, 3000, 3500, 3750, 4000 and 5000 µg/g soil. Mn concentrations in soil were made by addition of certain volume of manganese from stock solution (50,000 µg/ml) as $MnSO_4$ to 200 gm soil to attain the desired concentration per gram of soil, they were left to dry, well mixed and put in small pots (7.5 cm diameter and 12 cm height). Three soybean seeds (Clark variety) were sown in each pot, and irrigated with tap water as they need. Concentrations which attained clear variations in dry *weight of plant, as a main character*, were chosen for the second preliminary experiment, to be added to rhizobial liquid cultures. These concentrations were; zero, 100, 500, 1000, 2500, 3000 and 5000 µg/L.

The second preliminary experiment was undertaken by additing of the desired Mn concentrations from stock solution containing 50000 µg/ml, in flasks each contain 250 ml yeast mannitol medium, then autoclaved at 1.5 lb for 15 minutes. 10 ml stock rhizobial culture containing 1.3 x 10^9 cells/ml were used for inoculation of Mn amended media (250 ml for each) in flasks with 500 ml capacity. Inoculated flasks were incubated at 30 $\pm$ 1°C for seven days. They tested for purity, then number of rhizobial cells in cultures of each Mn concentration were determined by using dropping method (Somasegran and Hoben, 1985) (four replicates were used for each concentration).

In the third experiment (a pot trial), four Mn concentrations plus control (zero level) were chosen as two low concentrations (300 and 500 ppm) which did not give significant variations on plant dry weight compared to the control, and other two high concentrations (1000 and 2000 ppm) which gave significant variations compared to the control. The pot experiment was conducted at winter season of 2002 in wire proof greenhouse at Sakha Agricultural Research Station, Kafr El-Sheikh, Sakha. Two soil types were used, clay and calcareous soil. Pots had diameter of 15 cm and 25 cm height. The chemicaand mechanical composition of these soils are shown in Table (1).

Soils were amended with Mn by preparing a stock of Mn (50, 000 µg/ml) as $MnSO_4$. Certain volume of this stock was added to the soil in order to obtain the desired Mn levels. After drying, each Mn level-soil was mixed thoroughly and added to the pots as 10 kg soil/pot. All pots were fertilized with 2 grams superphosphate, 1.5 gm urea for un-inoculated control and 0.4 gm for inoculated one as well as 0.5 gm KSO_4 per pot. Soybean seeds were inoculated with rhizobia (*B. japonicum*, St 110), which kindly obtained from Lab. of Bacteriology, Sakha Agricultural Research Station, Sakha. The seeds of un-inoculated treatment (control) were left without inoculation.

Split split plot design with six replications was used. The main of manganese levels (Mn) were as following zero, 300, 500, 1000 and 2000 ppm. The sub-plots were soil types (S) i.e. clay or calcareous and sub-sub plots were inoculation with rhizobia (I_1) or un-inoculation (I_0).

Seeds were sown as five seeds/pot. After germination, plants were thinned to three/pot. Six replicates for each treatment were made. All plants were irrigated on regular intervals

Table (1):Chemical and mechanical analysis of the soil.

Soil type	Chemical composition										
	Total CO_3 content	pH	EC	Soluble anions meq/L				Soluble cations meq/L			
				CO_3^{-2}	HCO_3^{-1}	Cl^{-1}	So_4^{-2}	Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}
Clay	4.3	7.64	1.69	0.0	5.5	3.3	9.3	7.0	2.0	8.3	0.8
Calcareous	21.19	7.75	4.3	0.0	4.32	25.90	20.78	8.1	8.8	32.25	1.94

Soil type	Mechanical composition			Texture
	Sand %	Silt %	Clay %	
Clay	18.87	32.73	48.4	Clay
Calcar.	47.61	27.26	22.40	Sandy clay loam

Plant samples were collected after 70 days of planting for determining the number and dry weight of nodules. At the harvesting time plants were collected for determining plant dry weight (gm/plant), seed yield (gm/plant) and seed index (g). Seeds were washed thoroughly with tap water and then with distilled waters, oven dried, then grinded in a steal mill and keep in paper pages for chemical analysis. N was analysed using microkjeldhal methods as reported by (A.O.A.C., 1975), P was measured colourimetrically according to Olsen *et al.* (1954), and K was measured by flame-photometer as described by Richard (1945). Available manganese of plant material were determined according to (Chapman and Pratt, 1961).

All data were subjected to the statistical analysis as usual technique of analysis of variance (ANOVA), mentioned by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Response of germination %, natality %, length of embryonic stem, shoot length and plant dry weight of soybean to Mn:

In a preliminary experiment a gradual concentrations of Mn (from zero to 5000 ppm in soil) were made to get the suitable Mn concentrations which might be used for the main experiment.

Table (2) demonstrated that increasing of soil Mn concentrations up to 5000 ppm did not attain significant variations in germination percentage of soybean seeds. These results are in agreement with those of Mengel and Kirkby (1987) and Unni *et al.* (1995).

Results of plants natality % after 45 days of sowing did not exhibit consistent significant trend with the increasing of Mn concentrations. Natality % have inconsistent decreases due to addition of high levels of Mn. Death of some plants may be as a result of toxicity of high levels of Mn that initiated brown spots in older leaves and induced deficiency of other nutrients such as Fe, Mg and Ca leading to dryness of these leaves (Mengel and Kirkby, 1987). Results of embryonic internode length and shoot length gave similar trends. The increase of Mn levels till 1500 µg/g soil resulted in significant increments than control treatment (zero Mn). The high values recorded at the range from 50 to 1250 µg/g soil, then inconsistent decrease took place till (5000 µg/g.).

Elongation of embryonic internode may be due to chlorophyll degradation (Mengel and Kirkby, 1987) which may lead to elongation of internods and shoot system to collect high amounts of light.

Plant dry weight was gradually decreased with increasing Mn concentrations of soil. The significant decreases, generally, started from 1000 mg/g soil, to give 40% reduction. The highest Mn concentration (5000 µg/g soil) reduced the dry weight up to 54.0%. High levels of Mn induced brown spots surrounded by chlorotic areas. This observation was noted also by Mengel and Kirkby (1987). They added that a deficiency of other nutrients such as Fe, Mg and Ca may also occur. Thus the toxicity symptoms may be the main factor causing decrease in plant dry weight.

Table (2):Influence of different concentrations of Mn supplemented to the soil on germination %, natality %, length of embryonic internode, shoot length and dry weight of plants/pot.

Mn concentration ppm	Germination %	Natality % after 45 days of sowing	Embryonic internode length (cm)	Shoot length (cm)	Dry weight of plants (gm/pot)
Zero	75.00 a	66.67 b	4.23 abc	13.14 abc	1.103 h
50	50.00 a	41.69 a	6.48 f	15.71 b-c	1.028 gh
100	41.67 a	50.00 ab	6.23 ef	16.26 b-e	0.780 a-g
200	66.87 a	41.67 a	6.48 f	18.51 e	0.935 d-h
300	63.34 a	66.67 b	6.94 f	18.32 de	0.950 e-h
400	63.34 a	58.34 ab	5.40 de	14.40 a-d	0.877 b-h
500	75.00 a	66.67 b	6.23 ef	14.09 abc	0.828 b-h
600	66.67 a	41.67 a	6.33 f	13.99 abc	0.633 a-d
750	75.00 a	66.67 b	6.48 f	15.76 b-e	0.968 fgh
1000	58.33 a	58.63 ab	6.96 f	16.18 b-e	0.663 a-f
1250	75.00 a	66.67 b	6.63 f	16.70 cde	0.890 c-h
1500	66.67 a	58.34 ab	5.39 de	15.31 a-c	0.745 a-g
1750	83.34 a	66.67 b	4.89 bde	16.34 b-e	0.785 a-g
2000	83.34 a	58.35 ab	4.66 a-d	14.98 a-e	0.708 a-f
2250	86.34 a	41.67 a	4.13 ab	12.39 ab	0.568 ab
2500	66.67 a	66.87 b	5.13 cd	14.55 a-d	0.673 a-f
2750	86.67 a	41.67 a	3.89 a	15.90 b-e	0.495 a
3000	75.00 a	58.34 ab	4.56 a-d	15.55 b-e	0.645 a-e
3500	83.34 a	66.67 b	4.53 a-d	15.55 b-e	0.725 a-g
3750	66.67 a	66.67	4.74 a-d	15.96 b-e	0.615 abc
4000	75.00 a	50.00 ab	4.68 a-d	16.99 cde	0.600 abc
5000	66.67 a	50.00 ab	4.03 ab	11.66 a	0.508 a

Means with different symbols are significantly different

Effect of Mn levels on viability of rhizobia:

Our results in Table (3) indicated that addition of low levels (50 and 500 ppm) of Mn in the media of rhizobia (*B. japonicum*) resulted in highly significant increase in viable counts of rhizobia (i.e. 5.5 and 7.65 x 10^8 per ml), respectively, compared to 2 x 10^8 for control. In contrast, addition of high Mn concentrations to rhizobial cultures (from 1000 to 5000 ppm) exhibited highly significant decreases in viable counts of rhizobia (2 x 10^6 cells/ml) in culture supplemented with 5000 ppm Mn.

Table (3):Effect of different Mn concentrations (ppm) on *B. japonicum* rhizobial cells/ml of culture.

Mn concentration (ppm)	*Number of cells/ml*
Zero	2.00×10^{8} C
100	5.50×10^{8} d
500	7.65×10^{8} e
1000	6.0×10^{7} b
2500	4.6×10^{7} ab
3000	2.0×10^{7} ab
5000	2.0×10^{6} a

Means with different symbols are significantly different

In accordance with our results, Alloway (1995) state that Raulin as long ago (1863) recognised that Mn is essential element, as he demonstrated that it was needed for the growth of the fungus *Rhizopus nigricans*. Mn activates enzymes and involve in the synthesis of glycoprotein and present in metaloenzymes as arginase and pyruvate carboxylase. On the other hand, Mn may be toxic for living organisms when present in high concentrations up to 200 ppm (Mengel and Kirkby, 1987). Mn is normally added to the soil in the form of $MnSO_4$, MnO, or as an addition to macronutrient fertilizers. Quantities applied may be from < 10 to > 100 kg Mn/ha (Alloway, 1995) for the synthesis of glycoprotein and present in metaloenzymes as arginase and pyruvate carboxylase. Fritze *et al.* (1989) stated that soil respiration and fungal hyphal length were reduced due to high concentrations of Mn in soil. Tyler *et al.* (1989) reviewed that the toxic action of heavy metal ions are essentially exerted on enzymes, and the inhibition of enzymes may be due to (1) the masking of catalytically active groups (2) protein denaturation (3) effects on enzyme configuration (4) competition with activating metal ions for enzyme and substrate sites involved in the enzyme-substrate complexes, as well as (5) the activities enzymes that depend on amino and sulphyahdrile groups are easily inhibited.

Response of number and dry weight of nodules, dry weight of shoot and root, and seed yield to Mn addition:

Table (4) showed the influence of Mn concentrations, soil types and inoculation with *B. japonicum* on soybean number and dry weight of nodules, dry weight of shoot, dry weight of root and seed yield. Data indicated that Mn from (0.0 to 2000 ppm) levels did not significantly affect number of nodules, dry weight of nodules or shoot dry weight, but there were significant variations in case of root dry weight and seed yield. There were increases in root dry weight at 1000 and 2000 ppm Mn and significant increase in seed yield using 2000 ppm Mn (10.425 gm/plant compared to 9.515 gm/plant for control). Mengel and Kirkby (1987) stated that nodules are tolerant to high levels of Mn whereas they normally contain Fe and Mn in their structure. Larry *et al.* (2000) indicated that Mn is required for ureide degradation in leaves and he added that it was hypothesized that increased leaf Mn would elevate ureide accumulation. Although, the data of Table (4) show the effect of Mn levels on plants grown in clay and calcareous soils, this led to obscuring of the difference between Mn levels effects, whereas each soil type have a contradicting trend to other for number and dry weight of nodules as well as shoot dry weight.

Regarding effect of soil types on nodule numbers in calcareous soil, it attained highly significant increase than clay soil did (59 and 22 nodule respectively). Dry weight of nodules have the same trend with significant difference (0.784 and 0.686 g/plant). Root dry weight at clay soil was significantly higher than those of calcareous soil (9.002 and 6.475 g/pot). But there were insignificant variations for shoot dry weight or seed yield. Calcareous soil was more efficient than clay soil under circumstances of higher concentration of Mn. In this respect, Mengel and Kirkby (1987) mentioned that increasing Ca in soil decreased activity of Mn. Moraghan (2002) stated that liming decreased seed-manganese concentration. Thus calcareous soil may be more suitable than clay soil when using high manganese levels.

Table (4):Effect of manganese concentrations (ppm) and soil types in the presence or absence of inoculation with *B. japonicum* on number and dry weight of nodule, dry weight of shoot and root and seed yield of soybean.

Treatment	No. of nodules/ plant	Dry weight of nodules gm/plant	Shoot dry weight (gm)/plant	Root dry weight (gm)/plant	Seed yield (gm)/plant
Concentration (C) (ppm)					
Zero	45	0.471	21.321	7.238	9.315
300	45	0.775	22.229	6.088	8.060
500	39	0.754	20.537	6.909	7.790
1000	34	0.668	22.760	9.532	8.425
2000	40	0.738	22.353	8.927	10.425
F-test	N.S	N.S	N.S	*	*
L.S.D. 0.05	-	-	-	3.197	2.28
0.01	-	-	-	4.00	-
Soil types (S)					
Clay	22.0	0.686	22.442	9.002	8.522
Calcareous	59.0	0.784	21.238	6.475	9.084
F-test	**	*	N.S	**	N.S
Inoculation (I)					
Inoculated I_1	81.00	1.47	22.811	7.103	11.83
Un-inoculated I_0	0.00	0.00	20.869	8.373	5.78
F-test	**	**	**	**	**
Interaction					
C x S	*	*	**	**	Ns
C x I	*	N.S	**	**	**
S x I	**	*	*	N.S	**
C x S x I	**	*	**	**	**

* = Significance at 0.05 N.S. Not significant

** = Significance at 0.01

Inoculation of soybean plant with *B. japonicum* rhizobia significantly increased all the above mentioned parameters except for root dry weight. These results are in accordance with many authors (Nour El-Dein, 1997; Drevon, 1999; Gohar *et al.*, 1999 and Monib *et al.*, 1999).

Data in Table (5) illustrated the effect of interaction between Mn levels, soil types and inoculation on the same criteria mentioned in Table (4). Nodule number exhibited a decrease due to Mn concentration for both soil types and reached to the lowest levels at concentrations 500 and 1000 ppm for calcareous soil (102 and 92, respectively, compared to 135 for control). However, un-inoculated plants had no nodules.

Dry weight of nodules showed a significant consistent decrease than control due to Mn levels for clay soil. The reverse was true for calcareous soil. This difference between clay and calcareous soils may be due to increasing amounts of Ca in calcareous soil which may alleviate the toxicity of Mn on nodules mass (Morghan, 2002). The toxicity of Mn on nodulation process may be due to influence of Mn on carbohydrate biosynthesis, where high levels of Mn caused necrotic and chlorosis in older leaves (Mengel and Kirkby, 1987), leading to decrease in carbohydrate biosynthesis. They also mentioned that high levels of Mn can induce deficiency of other nutrients as Fe, Mg and Ca which harmfully affects plant growth and nodules formation and function. In calcareous soil, Mn levels till 2000 ppm caused increase in nodules dry weight. This may be due to the presence of high level of Ca in calcareous soil. Nguyen *et al.* (2001) stated that a combination of gypsum and lime was more effective in correcting Mn toxicity, and they added that soybean growth was better correlated with leaf Ca/Mn ratio than with leaf Mn.

Table (5):Effect of interaction between Mn concentrations (ppm), soil types and rhizobial inoculation on the number and dry weight of nodules, dry eight of shoot and root and seed yield of soybean.

Conc-entration ppm	No. of nodules/plant		Dry weight of nodules (gm)/plant		Shoot dry weight (gm)/plant		Root dry weight (gm)(plant)		Seed yield (gm)/plant	
	I_1	I_0	I_1	I_0	I_1	I_0	I_1	I_0	I_1	I_0
Clay soil										
Zero	45.7 ab	0.0 a	1.64 a	0.0 a	20.28 b	17.96 c	4.66 b	8.18 bc	9.80 b	8.60 a
300	44.70 b	0.0 a	1 37 ab	0.0 a	20.39 b	20.57 bc	6.82 b	5.45 c	9.67 b	5.43 b
500	53.0 a	0.0 a	1.32 b	0.0 a	24.62 b	22.75 ab	6.53 b	8.77 bc	9.07 b	5.50 b
1000	42.0 ab	0.0 a	1.28 b	0.0 a	27.79 a	24 81 a	15.77a	10.16 b	9.10 b	6.07 b
2000	34.7 b	0.0 a	1.26 b	0.0 a	30.298 a	24.96 a	6.99 b	16.69 a	15.50 a	6.50 ab
Calcareous soil										
Zero	135.0 a	0.0 a	1.33 b	0.0 a	28.32 a	18.73 b	5.67 a	10.94 a	12.63 ab	6.23 a
300	134.0 a	0.0 a	1.73 a	0.0 a	29.68 a	18.28 b	4.40 a	7.66 ab	10.97 b	6.17 a
500	102.0 b	0.0 a	1.7 a	0.0 a	26.24 a	16.53 b	7.24 a	5.09 b	12.50 ab	4.10 a
1000	92.0 b	0.0 a	1.39 b	0.0 a	20.35 b	18.10 b	7.50 a	4.70 b	14.27 a	4.27 a
2000	125.0 a	0.0 a	1.70 a	0.0 a	20.16 b	18.0 b	5.45 a	6.57 b	14.77 a	4.93 a

Means within each raw of each soil type (are with different symbols are significantly different.

In respect to shoot dry weight, there was an increase with the increase of Mn levels, for clay soil. In case of calcareous soil a significantly decrease than control was observed at 1000 and 2000 ppm for inoculated plants by 28.1 and 28.8%, respectively. No significant variation is recorded in respect to uninoculated treatment.

Dry weight of root (gm/plant) of clay soil exhibited a general significant increase with the increase of Mn concentrations for both inoculated and un-inoculated treatments. This may be attributed to the response of oats to escape from polluted soil zone. Nour El-Dein (1997) found similar observations due to a pollution of soil with lead. In case of calcareous soil, Mn additions decreased the dry weight than control (zero Mn) for un-inoculated plants. There were no significant variations than control for inoculated plants. This confirm the role of inoculation with rhizobia in detoxification of Mn to the plant.

The treatment with the concentration 2000 ppm Mn, inoculated plants showed significant increase in seed yield than control, (15.5 and 9.8 gm/plant, respectively) for clay soil, and insignificant increase for calcareous soil. In contrast, uninoculated plants induced decreases in seed yield, for both soil types, with the increasing of Mn concentrations, the effect was significant in case of clay soil and insignificant for calcareous soil. These results confirm our concept that rhizobial inoculation of soybean plants not only increase plant growth and saving a lot amounts of N-fertilizer (about 7%), but also detoxify soil Mn through its contribution in declining detrimental effects of Mn on plant growth and yield (Table 4). In the present study, it is noticed that final seed yield was decreased in case of both clay and calcareous soils for un-inoculated plants, this indicate that Mn concentration from 500 ppm and above were harmful for seed yield of soybean in un-inoculated plants. The toxicity may be due to inducing auxin deficiency caused high IAA oxidase activity or induce a loss of apical dominance and proliferation of auxiliary shoots (Mengel and Kirkby, 1987). In case of plants inoculated with *B. japonicum* rhizobia, they exhibited marked increases in seed yield. This confirm that rhizobial inoculation helps the plant to overcome the toxic effect of Mn, in addition to its main role in N_2-fixation. This finding was recorded before by Nour El-Dein (1997). He found that inoculation of soybean plants with *B. japonicum* decreased toxicity of lead on the plant. Lieu *et al.* (2002) also found that nodules naturally contained Mn and Fe oxides and the heavy metals in nodules are associated with Fe and Mn. Mechanism of microbial bioremediation and detoxification of metals reported by many investigators and could be due to one or more of the following reasons:

1. Adsorption of microorganisms to metals on their surfaces (Zimdahl and Koeppe, 1979; Beveridge and Koval, 1981 and Bustard *et al.*, 1997).
2. Bind metals on their polysaccharides layer (Gutnike,, 2000).
3. Mn oxidizing microorganisms influence Mn oxidation turning it to less available form (Mengel and Kirkby, 1987).
4. Collection of heavy metals in nodules and partially prevent their translocation to the shoot (Nour El-Dein, 1997).

Effects of addition of different Mn concentration on N, P & K uptake and Mn contents in seeds of soybean:

Table (6) illustrated the effects of Mn concentrations, soils types and rhizobial inoculations on the contents of soybean seeds from N, P, K and Mn. A continuous decrease in N-content was observed till 1000 µg/g soil Mn and the concentrations 300 and 500 µg/g soil attained significant differences than control (zero Mn).

This effect was nearly due to the drastic effect of Mn on seed yield which heavily affected especially by higher concentrations of Mn. On the other hand El-Hawary *et al.* (1994) found that high levels of Mn included in the product fulaz (0.75 kg/feddan) induced significant decrease in N%.

Table (6): Effect of Mn concentrations (ppm) and soil types in the presence or absence of rhizobial inoculation on soil Mn concentration as well as soybean contents of N, P, K and Mn.

Treatment	N-content mg/kg	P-content mg/kg	K-content mg/kg	Mn-content mg/kg
Concentration (C) (ppm)				
Zero	66.60	3.072	18.575	86.91
300	59.11	2.750	16.858	93.06
500	52.59	2.990	14.759	92.16
1000	56.51	3.434	16133	95.69
2000	68.73	3.432	15.892	100.34
F-test	**	N.S	N.S	**
L.S.D. 0.05	8.19	1.194	5.020	-
0.01	11.60	1.668	7.029	3.449
Soil types (S)				
Clay	65.38	3.138	15.333	86.28
Calcareous	56.04	3.133	17.553	98.49
F-test	**	N.S	N.S	*
Inoculation (I)				
Inoculated I_1	81.12	4.165	21.330	88.24
Un-inoculated I_o	40.30	2.106	11.557	99.03
F-test	**	**	**	*
Interaction				
C x S	**	N.S	*	**
C x I	**	*	**	**
S x I	**	*	**	N.S
C x S x I	**	N.S	**	**

* = Significance at 0.05 N.S. = Not significant
** = Significance at 0.01

Plants grown in clay soil had highly significant value than those in calcareous soil (65.38 and 56.04). Inoculation with rhizobia increased N-content of soybean seeds by highly significant level than un-inoculated ones (18.12 and 40.30 mg/kg seeds). These results agreed with the observations of (Somasegaran and Hoben, 1985).

Mn application did not significantly affect soybean seeds P-content (Table 6). In this concern, there was no difference between the two used soils. Contrarely, inoculation with *B. japonicum* rhizobia sharply increased P-content of soybean seeds than did un-inoculated plants (4.165 and 2.106 mg/kg seed. Soybean seeds K-content had the same trend of P-content. The values in case of inoculated and un-inoculated seeds were 21.330 and 11.557 mg/kg seeds). This increase may be due to the effect of inoculation which gave high seed productivity.

Mn content of soybean seeds as affected by increasing Mn levels of soil, soil type and inoculation with rhizobia (Table 6). It is noted that seed-Mn content significantly increased with the increasing of Mn levels. On the other hand, Mn content of seeds of clay soil was much lower than ($P < 0.05$) those of calcareous soil (86.29 and 98.49 mg/kg, respectively). Inoculation with rhizobia had a potential role in decreasing Mn content of seeds ($P < 0.05$); 38.4 in comparison to 99.03 mg/kg seeds for inoculated and uninoculated plants respectively in this regard, Nour El-Dein (1997) found a similar finding in case of lead added to the soil.

There was an obvious declining of Mn concentration, in seeds of inoculated plants than those of un-inoculated, these results are in accordance with results of Nour El-Dein (1997) who found that inoculation of soybean plants with *B. japonicum* decreased plant lead concentration than those of un-inoculated plants. These findings confirm our viewpoint that inoculation with rhizobia could be used as heavy metal detoxificant, in addition to its main role as N_2-fixer. Therefore, we recommend inoculation with rhizobia to leguminous plants especially in case of soil polluted with heavy metals.

Table (7) showed the effect of interaction between Mn concentrations, soil types and rhizobial inoculation on N, P, K and Mn contents of soybean seeds. In general, N-content of soybean seeds decreased with the addition of Mn to the soil, in both clay and calcareous soil types. In case of inoculated plants, some Mn concentrations attained significant decreases than control (zero Mn), then were 1000 ppm Mn and 300, 500 and 2000 ppm Mn for plants grown in clay and calcareous soil types respectively. While, un-inoculated plants showed consistent significant decreases in N-content than control, for both soils concentrations 500, 1000 and 2000 ppm Mn. Inoculated plants induced a moderate tolerance against high levels of Mn toxicity in both clay and calcareous soil types.

Regarding P-content inoculated plants of clay soil attained insignificant increases than control while un-inoculated plants showed insignificant decreases. In calcareous soil, inoculated plants exhibited increases in P-content being significant at 1000 and 2000 ppm Mn, while uninoculated plants showed insignificant decreases. El-Hawary (1994) recorded significant decreases in N, P and K% of un-inoculated soybean plants as influenced by Fulaz-containing Mn with 0.75 kg/feddan. The increase recorded in the present study is mainly due to the effect of rhizobial inoculation which mitigate the detrimental effect of high levels of Mn on P-contents. These results support the concept of detoxification of inoculation to Mn hazardous effect.

Table (7):Effect of interaction between Mn (ppm) concentrations (ppm), soil types, and rhizobial *inoculation* on soybean *N, P, K and Mn* contents (mg/kg) of soybean seeds.

Concent-ration ppm	N-content µg/gm of seeds		P-content µg/gm of seeds		K-content µg/gm of seeds		Mn-cotnent µg/gm of seeds	
	I_1	I_0	I_1	I_0	I_1	I_0	I_1	I_0
Clay soil								
Zero	73.00 bc	65.73 a	4.32 ab	2.23 a	22.4 a	19.8 a	88.88 a	77.75 a
300	79.80 b	96.17 c	3.52 b	2.18 a	22 4 a	10.5 b	87.00 a	84.00 b
500	69.67 c	45.10 b	4.57 ab	1.62 a	14.6 b	13.0 b	90.00 a	92.37 c
1000	58.27 d	47.60 b	4.93 a	1.79 a	15 5 b	9.2 b	91.38 a	96.00 c
2000	*128.67 a*	*49.77 b*	*4.55 ab*	*1.67 a*	*13 7 b*	12.4 b	82.88 a	97.50 d
Calcareous soil								
Zero	89.80 a	37.87 b	3.04 b	2.69 a	19.3 c	12.8 a	90.38 a	90 63 a
300	74.27 b	46.20 a	3.14 b	2.16 a	23.7 bc	10.9 ab	86.25 a	115.00 a
500	71.690 b	23.70 c	3.60 b	2.18 a	24.4 abc	7.1 ab	*82.63 a*	*103.63 a*
1000	96.50 a	23.67 c	5.07 a	1.95 a	29.3 a	10.6 ab	86.88 a	108 50 a
2000	69.30 b	27.20 c	4.91 a	2.60 a	28.1 ab	9.4 ab	96.13 a	124.88 a

***Means within each raw of each soil type (are with* different symbols are significantly different.**

The influence of Mn concentrations on K contents of seeds differs between inoculated and *un-inoculated plants grown in clay soil (Table 7). In* inoculated plants, showed significant decreases at 500, 1000, 2000 ppm Mn (14.6, 15.5 and 13.7 mg/kg), respectively, compared to (22.4 mg/kg for control) concentrations in un-inoculated plants caused significant decreases than control. In calcareous soil, Mn concentrations attained increases in K-*content of inoculated plants and* reached to significant levels for concentrations 1000 and 2000 ppm. Un-inoculated plants exhibited insignificant decreases in K-contents with the addition of Mn. Inoculation with rhizobia was able to eliminate the toxic effect of Mn on K-*uptake by seeds* (Table 7). Moreover, it helped Mn treated plants to be more healthy than *those of control* (without Mn), as they became able to uptake more K than those of control, without any harmful effect on plant growth or yield (as shown in Table 5).

The above *interpretation may be supported by the calculated* data from the present work, where it is noted that at 1000 ppm Mn, inoculated treatments inclay soil attained 20.0% decrease, 7.5%, 8.0% increase and 30 0% decrease, than control, in comparison to 27.5%, 19.7 and 53.5% decreases in N, P, K of un-inoculated plants, respectively. For calcareous soil, *there were 7.5%, 66.8% and* 51.8 increases, in comparison to 37.4, 27.5% and 17.2% decreases in N, P, K of un-inoculated plants, respectively. These calculations indicate that the inoculation with rhizobia undubitably contribute in mitigating and detoxifying the *harmful effects of high Mn concentrations on* the physiological systems of the plant, as mentioned by Rizzo *et al.* (1992) and Richard *et al.* (1993) that the presence of tolerant bacteria to heavy metals in soil remarkably decreased the harmful effect of heavy metals upon the grown crops.

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اختزال سمية المنجنيز لنبات فول الصويا باستخدام ريزوبيـــا الـبريدى ريزوبيـــم جابونيكوم

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تم إجراء تجربتين مبدئيتين ، استخدم فى الاولى تركيزات متتالية من المنجنيز (من صفر إلى ٥٠٠٠ جزء فى المليون) وذلك بإضافتها إلى تربة طينيه زرعت بنبات فـــول الصويـــا فـــى اصص صغيرة. واجريت التجربة الثانية عن طريق اضافة بعض تركيزات من المنجنيز والمختاره من التجربة الاولى وهى (صفر ، ١٠٠ ، ٥٠٠ ، ١٠٠٠ ، ٢٥٠٠ ، ٣٠٠٠ ، ٥٠٠٠ جزء فـــى المليون) إلى دوارق مخروطيه بها بيئه مانيتول الخميره ، لقحت بخلايا ريزوبيا فول الصويا سلالة (١١٠). أما في التجربة الاساسية وهى تجربه الأصص فقد استخدم فيها كلا من تربه طينيه وكذلك الجيرية. اضيفت تركيزات متتابعة من المنجنيز للتربة (صفـــر ، ٣٠٠ ، ٥٠٠ ، ١٠٠٠ ، ٢٠٠٠ جزء فى المليون) في وجود او عدم وجود التلقيح بالريزوبيا.

أوضحت النتائج ان تركيزات المنجنيز لم يكن لها تأثيرات معنوية على نســـبة الانبـــات ونسبة النباتات الحية. زاد كل من طول السلامية الجنينيه وطول المجموع الخضرى زيادة معنويـــه حتي تركيز ٤٠٠ ، ١٥٠٠ لكل منهما على التوالى. ادت الاعداد الحية من خلايا الريزوبيا بدرجـــة معنوية عند اضافة التركيزات المنخفضة من المنجنيز (١٠٠ و ٥٠٠ جزء فى المليون) ولكن سجل انخفاض معنوى شديد بسبب التركيزات الاعلى من ٥٠٠ جزء فى المليون. سبب التلقيح بالريزوبيا زيادة معنوية فى إنتاجيه البذور وكذلك معظم خصائص النمو الاخرى. لم تؤثر نوعيه التربة علـــى انتاجيه المحصول ولا على محتوى البذور من الفوسفور والبوتاسيوم. أظهرت النباتات التى نميـــت فى التربة الطينية زيادة فى محتوى البذور من النتروجين مقارنة بتلك التى نميت فى تربة جيريـــة. لم تتأثر عملية تكوين العقد بدرجة معنوية بسبب زيادة مستويات المنجنيز ، ولكن انخفض محتـــوى البذور من النتروجين معنويا. اظهر محتوى البذور من البوتاسيوم اتجاها مماثلا تقريبا ، بينما لـــم يتأثر المحتوى من الفوسفور بصفة عامة. لم تقل الانتاجيه من البذور للنباتات الملقحة والمنزرعـــة فى تربة طينيه حتى تركيز ١٠٠٠ جزء فى المليون من المنجنيز ولكن لوحظ زيادة معنوية عنـــد تركزي ٢٠٠٠ جزء فى المليون. فى التربة الجيرية سبب اضافة المنجنيز نقص غير معنوى فـــى انتاج البذور. خفض التلقيح بالريزوبيا من محتوى المنجنيز ببذور النباتات المنزرعة فى كلا مـــن نوعى التربة المستخدمين.