J. Agric. Sci. Mansoura Univ., 27(11): 7385 - 7402, 2002

ALLEVIATION OF CADMIUM TOXICITY ON SOYBEAN, *Glycine max* (L.) Merr. BY INOCULATION WITH *Bradyrhizobium* AND VESICULAR-ARBUSCULAR MYCORRHIZAE OR KINETIN

Fouda, R. A. and A. A. Arafa
Dept. of Agric. Botany, Fac. of Agric., Mansoura Univ., Mansoura, Egypt.

ABSTRACT

The effects of cadmium, inoculation with either *Bradyrhizobium* or VA-mycorrhizae and kinetin application as well as their interactions with cadmium on growth, anatomical structure of both leaves and stems as well as yield and its components in soybean plants were investigated. Cadmium treatment decreased plant height, number of branches, leaf area and dry weight of shoot/plant. In addition, photosynthetic pigments contents in the leaves and carbohydrates concentrations in the shoots, pod number/plant, seed weight (g)/plant and 100 seed weight (g) were also decreased. Calcium concentration in the shoot system as well as N, protein, P and K percent in the seeds were decreased, while, Cd concentration in the shoots was increased. Inoculation with either *Bradyrhizobium* or VA-mycorrhizae and kinetin application increased all investigated parameters with the exception of Cd content in shoots in Cd-polluted soil. It is interesting to mention that these treatments alleviated the adverse effects of cadmium treatment and improved the plant growth. However, kinetin application was the most effective treatment in this respect compared with other treatments. Regarding the anatomical structure of plant, cadmium treatment decreased stem diameter and thickness of cortex, xylem, phloem and pith tissues thickness. Metaxylem vessels diameter was also decreased as well as thickness of either leaflet blade or mesophyll tissue thickness due to reducing thickness both palisade and spongy tissues thickness. The size of the midvein vascular bundle was also decreased as indicated by its dimension.

Keywords: Soybean, Cadmium, Inoculation, *Bradyrhizobium*, VA-mycorrhizae, Kinetin, Toxicity, Anatomical structure.

INTRODUCTION

Recently, there is an increasing interest to study the adverse effects of environmental stresses on plant growth and development. Plant may expose to different stresses during its life, including excess accumulation of heavy metals in cultivated soils. Toxic levels of cadmium occur in some soils as a result of environmental pollution due to mining, smelting, industrial pollution and pesticide application. Cadmium has not only a toxic metal effect but it also easily taken up by plants and translocated to different plant parts, thereby leading to impaired metabolism and reduced plant growth, hence crop production (Aschmann and Zasoski, 1987 and Sheoran *et al.*, 1990).

Consumption of agricultural food products containing high Cd levels result in increased Cd accumulation in human organs such as kidney and liver, and may impair their functions (Bernard and Lawerys, 1984). Generally, visual symptoms of Cd toxicity include reduction of plant growth, an intervinal chlorosis and necrosis with red brown discolouration of leaves (Naquib *et al.*, 1986 and El-Saied, 2001). High levels of Cd in the rhizosphere are often accompanied with morphological, physiological and structural responses (Sheoran *et al.*, 1990 and El-Saied, 2001). Most of these responses can reduce plant growth in various plant species (Abo-elatta, 2002), by affecting several physiological processes such as water absorption, photosynthesis and nutrient uptake (Sheoran *et al.*, 1990). Moreover, it alters both carbohydrate status and plant hormones of shoot system (Dixon, 1990 and Hassan and Wahdan, 1991). Photosynthesis has been found to be the most sensitive process affected by Cd toxicity (Sheoran *et al.*, 1990).

It is well established that inoculation with both *Rhizobium* and mycorrhizae may play an important role in increasing metal tolerance. Previous results of Leyval *et al.* (1991) and El-Enany and Abd-Alla (1995) revealed that microbial inoculation can not only increase the ability of plants to grow in the presence of toxic metals, but it also decrease metal accumulation in plants growing in polluted soils and, thus, protect the host against phytotoxic metal effects. In addition, several studies have been shown that kinetin application ameliorated the deleterious effects of soil pollution with cadmium on growth of many crops (Gadallah, 1995a and El-Saied, 2001).

Therefore, the present study aimed to clarify the toxic effects of Cd on soybean plant growth and its yield components in addition to the effect of Cd on certain physiological aspects and the anatomical structure of leaves and stems. Moreover, inoculation by either *Rhizobium* or *Glomus mossae* and application of kinetin were studied to assess if their application could provide useful recovery the adverse effects of Cd toxicity.

MATERIALS AND METHODS

Two pot experiments were carried out during the two successive seasons of 2000/2001 and 2001/2002 in the Experimental Station and Laboratories of Agric. Botany Dept., Fac. of Agric., Mansoura Univ., Mansoura, Egypt.

Soybean seeds:

Soybean seeds (*Glycine max* (L) Merr. cv. Giza 21 were obtained from the Agricultural Research Center (ARC) Dokki, Giza, Egypt.

Microorganisms:

Bradyrhizobium japonicum USDA 110 was obtained from Microbiol. Res. Dept., Soil, Water and Environ. Res. Instit., ARC, Giza, Egypt, in lyophlized form, multiplied in nutrient broth and centrifuged then prepared again in suspension. Inoculation was done by adding 5 ml bacteria suspension per pot at 21 days after sowing.

Mycorrhizae were obtained from Fac. of Sci., Mansoura Univ., Egypt. Roots of *Allium cepa* infected with VA micorrhizal fungus were used as inoculum. The spore suspension of VA-mycorrhizae was placed 5 cm below the soil surface at the rate of 5 ml/pot after 21 days from sowing.

Kinetin:

Kinetin (6-furfuryl amino purine) was purchased from Sigma Chem. Co. LTD England, and applied as spray treatment at 21 days old.

Plant growth:

Plastic pots 30 cm diameter were used. Each pot was filled with 5 Kg clean air-dry soil. The mechanical and chemical analysis (Piper, 1950) of the used soil are illustrated in Table (1). The pots were divided into two sets.

The first set of pots was irrigated with water, while the second was irrigated with 150 ppm cadmium chloride solution (500 ml per pot). Uniform seeds were sown on the 5th of May in both seasons. The pots were kept in the the Agric Farm of Agric. Bot. Dept. under a normal day/night and irrigated with tap water when required. The other agricultural practices were done according to the advises of the Ministry of Agriculture and Land Reclamation. After 21 days from sowing the plants were thinned to leave 3 uniform young plants per pot. Roots of soybean plants were microscopically investigated before beginning VA-mycorrhizae treatment to ensure that no exotic fungal infection was occurred. The pots were arranged in a complete randomized block design with three replications to form the following treatments.

1- Control.
2- 150 ppm Cd
3- Inoculation with *Bradyrhizobium*
4- Inoculation with VA mycorrhizae
5- Kinetin 25 ppm
6- 150 ppm Cd + *Bradyrhizobium*
7- 150 ppm Cd + VA mycorrhizae
8- Cd 150 ppm + 25 ppm kinetin

Table (1): Mechanical and chemical analysis of the used soil during the two growing seasons of 2000/2001 and 2001/2002.

Soil contents	2001	2002
1- Mechanical analysis:		
Sand %	25.8	24.5
Silt %	19.7	21.6
Clay %	54.5	53.9
2- Chemical analysis:		
EC at 25°C	0.54	0.56
PH (soil reaction)	7.70	7.80
S.P.	62.1	63.7
F.C.	43.2	45.1

At the vegetative growth stage (45 days after sowing), plant height, number of branches, dry weight of shoot system and leaf area per plant were recorded.

At harvesting (95 days from sowing), number of pods per plant, seed weight per plant (g) and 100 seed weight (g) were determined.

Chemical analysis:

Photosynthetic pigments in the second leaf were determined by the method of Mac Kinney (1941).

Total soluble carbohydrates were determined in the shoot system by anthrone method as described by Dubois *et al.* (1956).

Ca^{++} and Cd^{++} cations were determined in the shoot system by the atomic Absorption Spectrophotometry (BHF 80 B).

Seeds were dried, ground and digested (Peterburgski, 1968) to estimate N, P, K and total nitrogen (Block *et al.*, 1965), phosphorus (Jackson, 1967) and potassium (Peterburgski, 1968). Seed protein content was calculated.

Anatomical studies:

Samples were taken from the middle of the 3rd internode of the main stem and the midrib region of the terminal leaflets of the second compound leaf and fixed in FAA solution, dehydrated in alcohol series and embedded in paraffin wax (52-54°C m.p.). Cross sections at 15-20 μm thick were prepared using a rotary microtome, stained in saffranin-light green combination, cleared in clove oil and mounted in Canada balsam (Gerlach, 1977) and examined microscopically.

All the chemical analysis excepted the photosynthetic pigments contents and anatomical structure were carried out during the second season.

Statistical analysis:

Data were statistically analyzed according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Morphological characters:

Visual morphological symptoms of Cd toxicity, *i.e.*, retardation of the plant growth, an intervinal chlorosis and necrosis with a red brown discolouration of leaves were observed throughout the experimental period during the two growing seasons.

The results in Table (2) indicated that Cd^{++} adversely affected plant growth in both seasons expressed by plant height, number of branches per plant, leaf area per plant and dry weight of shoot system per plant.

The reduction of plant growth caused by heavy metals including cadmium correlated with alteration of root physiology, thereby inhibiting both root development and its elongation (Woolhouse, 1983 and Sresty and Madhava Rao, 1999). In this context, Mukherjee and Sharma (1988) noted that the depressing effect of cadmium on plant growth may be attributed to an inhibition of RNA and protein syntheses and consequently inhibiting cell division (El-Saied, 2001) added that Cd^{++} decreased water and nutrient uptake by roots resulted in an inhibition of plant growth.

Moreover, the negative effect of Cd^{++} on leaf area may be due to the slow rate of movement of nutrients and hormone transport from the root to shoot (Abo-Hamed *et al.*, 1987). Moreover, Cd^{++} reduced cell turgor potential and cell wall elasticity leading to formation of small cells and intercellular space areas (Barcelo *et al.*, 1988).

Table (2): Effects of cadmium, inoculation with either *Bradyrhizobium* or VA mycorrhizae and kinetin as well as their interactions on plant height, number of branches, leaf area (cm2) and dry weights of soybean plants during the two growing seasons of 2000/2001 and 2001/2002.

Treatments	Plant height (cm)		No of branches/plant		Leaf area (cm)/plant		Dry W of shoot per plant (g)	
	2001	2002	2001	2002	2001	2002	2001	2002
Control	40.5	41.7	2.6	2.7	116.9	118.7	2.19	2.10
Cd (150 ppm)	23.9	25.6	1.1	1.1	78.0	79.9	1.60	1.70
Bradyrhizobium (R)	46.0	52.1	3.2	3.3	136.4	137.8	2.60	2.70
VA mycorrhizae (M)	42.2	44.3	3.1	3.2	127.4	128.6	2.40	2.40
Kinetin 25 ppm (K)	58.8	63.0	3.7	4.2	153.2	155.9	3.50	3.80
Cd + R	40.4	44.6	2.9	3.2	131.7	133.8	2.50	2.60
Cd + M	42.7	45.2	2.9	3.0	122.2	123.8	2.30	2.30
Cd + K	46.5	53.3	3.5	4.0	147.2	147.9	2.90	3.40
L.S.D. at 5%	7.1	6.9	0.35	0.13	2.9	3.44	0.28	0.16

Results in the same table show that inoculation with either *Bradyrhizobium* or VA mycorrhizae as well as kinetin application and their interactions with cadmium treatment increased all the above mentioned morphological characters.

The beneficial effect of these microorganisms on plant growth may be attributed to the promoting effects of both *Bradyrhizobium* and VA mycorrhizae on nutrient uptake and the nutritional status especially nitrogen and phosphorus which are necessary for plant growth (Hauka, 2000). However, nitrogen causes an increase in the meristimatic activity of plant (Saleh *et al.*, 1982), and an increase in the levels of the endogenous GA3 and auxin contents (Helaly *et al.*, 1985), where GA3 is known to increase cell elongation. Besides, *Rhizobium* increases nitrogen fixation through an increase in nitrogenase activity, as well as Cd^{++} binding protein content which play an important role in the detoxification of excess Cd^{++} and increase resistance of *Rhizobium* to Cd^{++} toxicity (El-Enany and Abd-Alla, 1995). They added that nodules accumulated cadmium at a higher extent than roots and shoot. Moreover, nodules act as a barrier restricting the transport of Cd to shoots (Jarvis and Jones, 1978).

The increasing effect of kinetin on plant height may be attributed to its effects on increasing cell division and cell enlargement (Arteca, 1996). He added that kinetin promotes cell expansion and enlargement caused by increasing water uptake as a result of an increase in the osmotic potential of the cells. The important role of kinetin on overcoming the adverse effect of Cd^{++} may be attributed to an increase of water nutrient uptake (El-Saied, 2001). In addition, kinetin stimulates the rate of movement of nutrients and hormones from roots which accelerate the rate of leaf expansion in developing leaves (Richmond and Lang, 1975). Generally, kinetin proved to be the most effective treatment in this respect.

Photosynthetic pigments:

Data presented in Table (3) reveal that Cd^{++} treatment decreased chlorophylls (a, b) and their total as well as carotenoid contents in the leaves of soybean plants.

Inoculation with either *Rhizobium or* VA mycorrhizae as well as kinetin treatment and their interactions with Cd^{++} treatment increased significantly all photosynthetic pigments. Kinetin was the most effective treatment in this respect as compared to the other treatments.

From the previous studies, it is appeared that cadmium not only caused an inhibition in photosynthesis process but also a reduction in the photosynthetic pigments (Sheoran *et al.*, 1990). They added that chlorophyll reduction might be the major cause of decreased photosynthesis. heavy metals including cadmium can inhibit photosynthesis in various ways viz., through decreased stomatal conductance, reduction of photosynthetic pigments, inhibition of chloroplast activity.

In addition, the reduction in photosynthetic pigment concentrations of cadmium-treated plants may be attributed to its effect on chloroplast ultrastructure by delaying formation of thylakoid membranes which resulted in smaller chloroplasts (Krupka *et al.*, 1987). Moreover, the reduction in photosynthetic pigments may be attributed to the substitution of Mg by Cd^{++} causing denaturation in chlorophyll molecule (Kupper *et al.*, 1998), inhibiting production of chlorophyll by affecting the synthesis of 5-aminolaevulinic acid (Stobart *et al.*, 1985), as well as inhibition of chlorophyll biosynthesis and activation of its enzymatic degradation (Somashekaraiah *et al.*, 1992).

Table (3): Effects of cadmium, inoculation with either *Bradyrhizobium* or VA mycorrhizae and kinetin as well as their interactions on both photosynthetic pigments and carbohydrates concentrations (mg/g dry weight) in the shoot system during the two growing seasons 2001/2002.

Treatments	Chlorophyll A		Chlorophyll B		Total Chlorophyll		Carotenoids		Total soluble carbohydrate mg/g dry w	
	2000	2001	2000	2001	2000	2001	2000	2001	2000	2001
Control	1.08	1.18	1.03	1.04	2.11	2.22	0.29	0.31	11.7	12.9
Cd (150 ppm)	0.95	0.96	0.63	0.70	1.58	1.66	0.23	0.26	6.8	7.4
Bradyrhizobium (R)	1.54	1.57	1.22	1.25	2.76	2.82	0.39	0.43	13.7	15.7
VA mycorrhizae (M)	1.30	1.33	1.17	1.23	2.47	2.56	0.36	0.39	15.3	17.9
Kinetin 25 ppm (K)	1.82	1.86	1.68	1.81	3.50	2.67	0.60	0.63	17.2	20.3
Cd + R	1.49	1.54	1.19	1.23	2.68	2.77	0.35	0.42	11.4	15.4
Cd + M	1.27	1.31	1.13	1.16	2.40	2.47	0.32	0.36	12.8	13.2
Cd + K	1.80	1.82	1.50	1.59	3.30	3.41	0.56	0.58	11.8	17.1
L.S.D. at 5%	0.19	0.18	0.18	0.24	0.18	0.17	0.03	0.03	3.9	2.8

The effect of applied microorganisms on photosynthetic pigments may be attributed to an increase in N_2 fixation, this increase led to an increase in cytokinin content (Dixon, 1990). Cytokinin is known to delay senescence of plant tissue through its effect on reducing the loss of chlorophyll (Gadallah, 1995b).

The positive effect of fungal treatment with VA mycorrhizae on the photosynthetic pigments may be due to the fact that the presence of mycorrhizae can increase the uptake of heavy metals by the host plant, and this can mean better supplies of iron and trace elements, but also facilitates the entry of toxic elements such as cadmium (Larcher, 1995). He added that the mycorrhizal fungi can bind heavy metals in their cell wall xomplex and so are able to buffer the plants against toxic effects.

The role of kinetin on overcoming the depressing effect of Cd^{++} on photosynthetic pigment content may be attributed to its effect on delaying senscence (Bardford-Kent, 1983) by increasing the number of chloroplasts in the leaf, inducing chlorophyll synthesis (Brzenkova and Makronozov, 1976) and retarding the chlorophyll degradation (Inanova and Kapachina, 1984).

Carbohydrate concentration:

Data presented in Table (3) indicated generally that total carbohydrate concentrations tended to decline in Cd-treated plants. On the other hand, inoculation with microorganisms or kinetin treatment as well as their interactions with Cd^{++} treatment increased carbohydrate content. Similar results were obtained by El-Saied (2001).

The reducing effect of cadmium on carbohydrate contents may be attributed to a reduction of leaf area, photosynthetic pigment contents (Tables 2 and 3) and consequently reduction in the photosynthesis and accumulation of carbohydrates. Similarly, El-Saied (2001) noted that the decrease in leaf area and photosynthetic pigments in cadmium-treated plants was accompanied by a decrease in carbohydrate contents in sorghum leaves. He added that, the major effect of heavy metals has been reported to be on photosynthetic electron transport chain and O_2 evaluation by reducing CO_2 fixation through their effect on either light or dark reactions of CO_2 assimilatory process (Weigel, 1985).

The beneficial effects of kinetin treatment on carbohydrate contents may probably due to an increase of leaf area, production of photosynthetic pigments and consequently stimulates the photosynthetic activity (El-Saied, 2001).

Howard and Withan (1983) noted that an increase in soluble carbohydrates by kinetin in Cd^{++} treated plants may probably due to an increase in invertase activity which consequently led to a simultaneous increase in soluble activity.

3. Calcium and cadmium concentrations in shoots:

Fig. (1) shows the effects of Cd-treatment, inoculation with either *Bradyrhizobium* or VA mycorrhizae and kinetin application as well as their interactions with Cd-treatment on the average Ca^{++} and Cd^{++} concentrations in the shoots of soybean plants.

Generally, there is an antagonism between both Ca^{++} and Cd^{++} uptake and translocation, with increasing Ca^{++} uptake by plant, Cd was decreased.

It is clearly that Cd-treatment increased Cd^{++} concentration in soybean shoots, such increase was related with decreased Ca^{++} concentration. On the other hand, inoculation with either *Bradyrhizobium* or VA-mycorrhizae and kinetin application increased Ca^{++} concentrations in shoots of soybean plants while the concentration of Cd^{++} was decreased.

Abo-Kassem *et al.* (1997) noted that Cd^{++} inhibited absorption and accumulation of Ca^{++} in soybean plants.

The lower concentration of Cd^{++} with increasing Ca^{++} concentration may be attributed to the competition between Ca^{++} and Cd^{++} for negative binding sites on the cell walls of roots, permeability of membranes (Fett *et al.*, 1994) as well as increase soil pH.

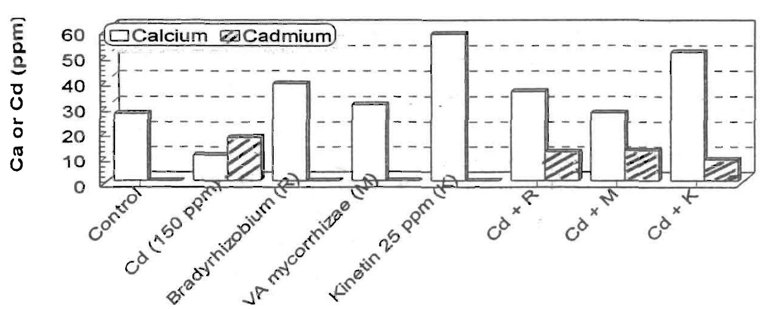

Fig. (1): Calcium and cadmium concentrations in dry shoot (ppm per plant) as affected by different treatments.

The beneficial effect of inoculation by *Bradyrhizobium* on overcoming Cd-toxicity may be attributed to an increase in nodulation and metalloprotein (Cd-binding protein) formation. Such increase in metalloprotein play an important role in the detoxification of excess cadmium and increased resistance of soybean plants to Cd-toxicity (EL-Enany and Abd-Alla, 1985).

The reduction in Cd uptake by inoculation with VA-mycorrhizae may be attributed to reducing metal uptake or translocation from root to shoot, through an accumulation of cadmium in fungal vacuoles or in the cytoplasm (Turnau, *et al.*, 1993) Moreover, the mycorrhizal fungi can bind heavy metals in their cell wall complex and so are able to buffer the plants against toxic affects (Larcher, 1995).

The reduction in Cd concentrations under kinetin treatment may be attributed to its fact on increased transpiration rate (El-Saied, 2001). Moreover, kinetin increased the stability of leaf membranes, probably due to its effects on cell membrane permeability (Gadallah, 1995b) as well as increased in Ca^{++} concentration (Fig. 1)

It seems clear that inoculation with either *Bradyrhizobium* or VA-micorrhizae and treatment with kinetin can play an important role in the decrease of metal accumulation in soybean plants growing in polluted soils by Cd and thus protect the plant against Cd-phytotoxic.

However, heavy metals uptake is not dependent on the concentration of the metals alone, but also differes from species to species of plant and fungus (Larcher, 1995). He added that the presence of mycorrhizae can increase the uptake of heavy metals by the host plant, and this can mean better supplies of iron and trace elements but also facilitates the entry of toxic elements such as cadmium.

Anatomical structure:

Leaflet structure:

The leaf blade internal structure of soybean plant is similar to other dicotyledons plants. It consists of upper and lower epidermis and mesophyll tissue, which differentiated into palisade and spongy parenchyma. Epidermis, one layer of completely arranged parenchymatous cells, which are flatted parallel to the leaf surface. Mesophyll tissue differentiated into palisade and spongy parenchyma. The palisade parenchyma cells are elongated and compactly arranged. The spongy parenchymatous cells are loosely arranged with numerous large intercellular spaces. The vascular bundle in the medvein is an upon collateral bundle having a narrow cambial zone (Fig. 2.B).

It can be concluded from Table (4) and Fig. (2B) that cadmium treatment decreased leaflet blade thickness in the midrib region due to a decreaseg in the parenchyma cells and their dimensions. The size of the midvein vascular bundle was also decreased as indicated by its dimension as well as xylem and phloem tissues thickness. Moreover, metaxylem vessel diameter was also decreased. Most anatomical features measured in the leaflet blade of soybean plants inoculated with either *Bradyrhizobium* or VA mycorrhizae and kinetin application as well as their interactions with cadmium treatment (Table 4) and (Fig. 2C, D, E and F) were greatest when compared with both control and Cd-treated plants. However, treatment with kinetin was the most effective in this respect.

The inhibiting effect on leaflet structure of Cd-treated plant and other heavy metals may be due to their inhibiting effect on both cell division and elongation (Srestly and Madhava Rao, 1999).

The stimulating effect on most anatomical features in the leaflet of soybean plants inoculated with both microorganisms may be related to an enhanced supply of nutrients particularly nitrogen and phosphorus for the host plants (Abd El-Fattah and El-Katony, 1996). Nitrogen led to an increase in the meristimatic activity (Saleh *et al.*, 1982). The increase in leaflet blade thickness due to kinetin application may be attributed to its effect on promotion of cell division and cell enlargement (Arteca, 1996), such promotion led to an increase in both palisade and spongy tissues thickness.

Table (4): Measurements of some anatomical characters (µm) in terminal leaflet of the 2nd compound leaf of soybean as affected by inoculation with either *Bradyrhizobium* or VA mycorrhizae and kinetin as well as their interactions with Cd-treatments during the second growing season.

Treatments	Counts and measurements								
	Leaflet thick-ness in the midrib	Meso-phyll tissue thick-ness	Pali-sade tissue thick-ness	Spongy tissue thick-ness	Midrib V.B dimension		Xylem tissue thick-ness	Phloem tissue thick-ness	Meta-xylem vessele dia-meter
					Length	Width			
Control	750	232	113	119	307	400	210	90	32
Cd (150 ppm)	655	2200	92	108	260	335	110	84	24
Bradyrhizobium (R)	1014	340	160	180	340	493	215	118	48
VA mycorrhizae (M)	978	295	141	154	323	487	213	110	45
Kinetin 25 ppm (K)	1320	450	216	234	400	580	259	133	56
Cd + R	844	300	147	153	309	441	205	104	43
Cd + M	783	282	135	147	290	395	197	85	39
Cd + K	972	350	156	194	367	432	243	113	50
L.S.D. at 5%	45	27.5	15.0	9.7	29.2	33.3	15.0	4.1	3.1

Stem structure:

The stem structure of soybean plants as seen in transverse section consists of the epidermis, ground tissue and the vascular system (Fig. 3B). Ground tissue differentiated into cortex and pith. The vascular collateral bundles arranged in complete cylinder. Two types of collateral bundles are present, *i.e.*, large and small bundles. The large bundles are separated with few small one. Data in Table (5) and Fig. (3B) indicated that Cd-treatment decreased stem diameter, cortex thickness, number of cortical cell layers, large vascular bundle dimension due to a decrease in both phloem and xylem tissue thickness. Pith tissue and metaxylem vessel diameters were also decreased when compared with control one. Data in the same Table and illustrated in Figs. (3 C, D, E and F) show that microorganisms inoculation and kinetin treatment as well as their interactions with Cd-treatment increased the previous anatomical parameters compared with those in both control and Cd-treated plants.

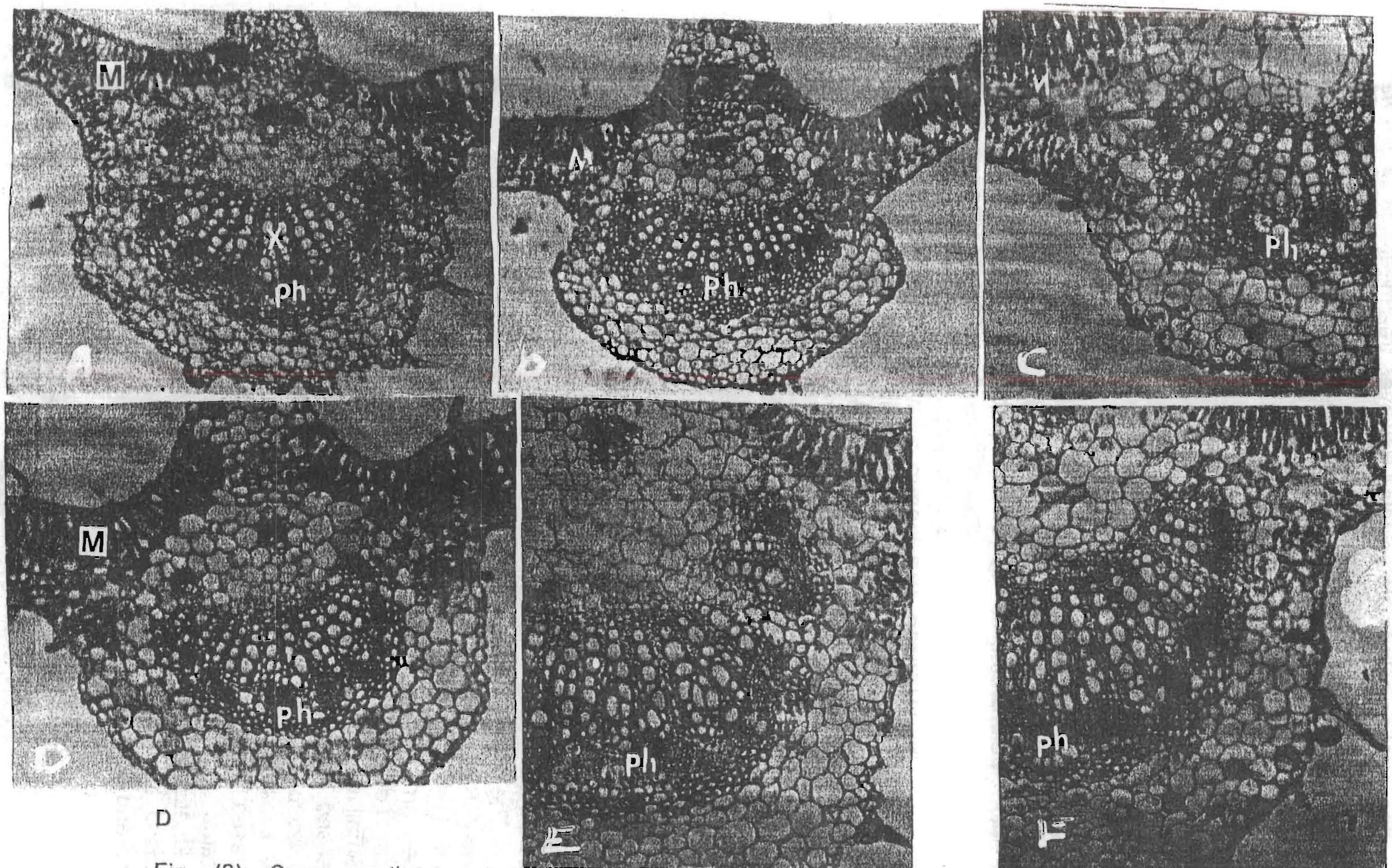

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Fig. (2): Cross sections cut through the midvein of soybean leaflets affected by cadmium, inoculation with either *Bradyrhizobium* or VA mycorrhizae and kinetin (Obj. x4. Oc. X15).

A: Control B: Cadmium 150 ppm C- *Bradyrhizobium* D: VA-mycorrhizae E: Kinetin 25 ppm
F: Cadmium + kinetin Ph: phloem X: Xylem M: Mesophyll tissue

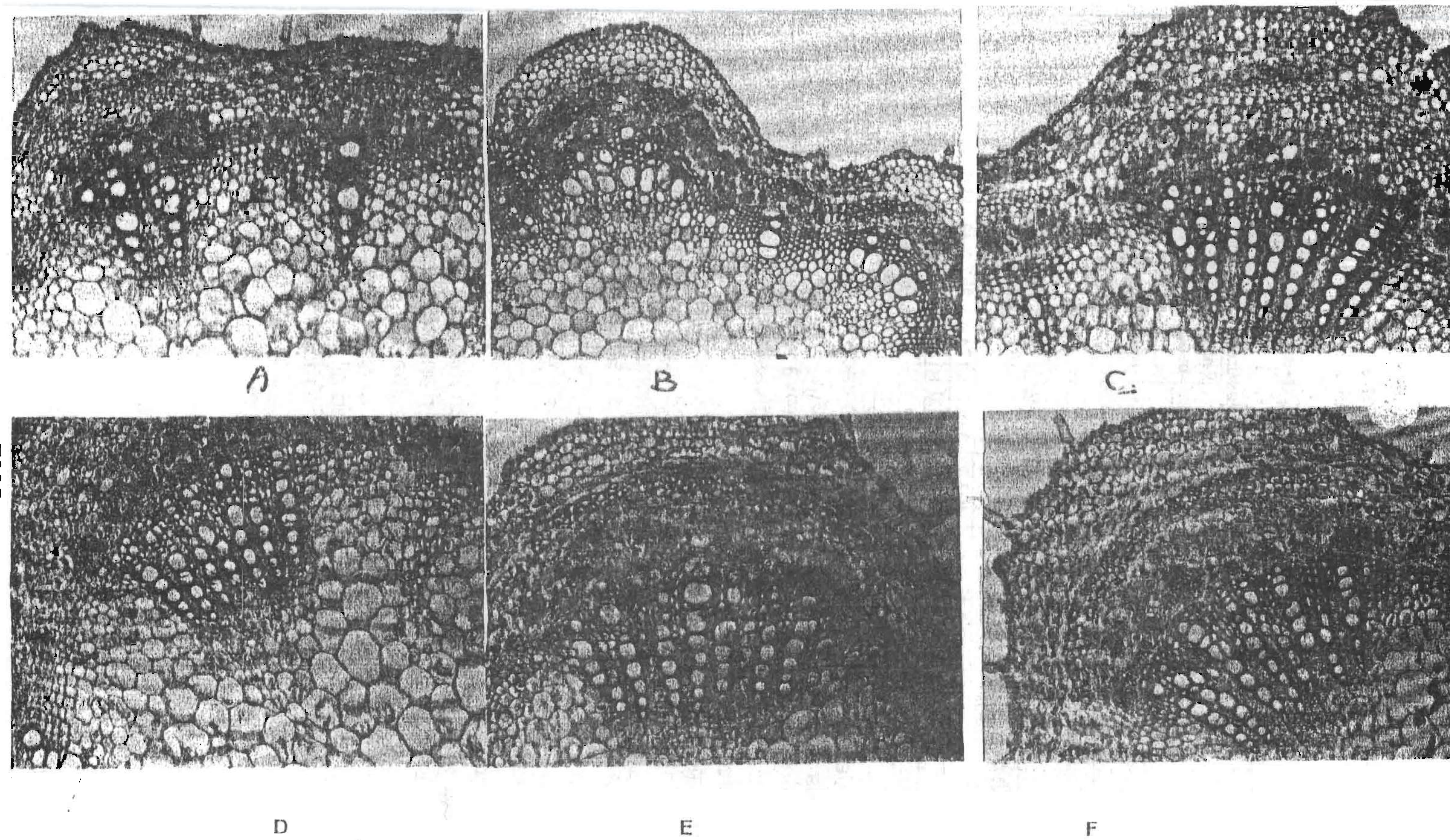

Fig. (3): Cross sections of soybean stems affected by cadmium, inoculation with either *Bradyrhizobium* or VA mycorrhizae and kinetin.as well as their interactions with cadmium (Obj. x4, Oc. X15).

A: Control B: Cadmium 150 ppm C- *Bradyrhizobium* D: VA-mycorrhizae E: Kinetin 25 ppm

F: Cadmium + kinetin Ph: phloem X: Xylem M: Mesophyll tissue

Table (5): Counts and measurements of some anatomical characters (μm) in soybean stem as affected by cadmium, inoculation with either *Bradyrhizobium* or VA mycorrhizae and kinetin as well as their interactions with Cd-treatments during the 2nd growing season 2001/2002.

Treatments	Counts and measurements								
	Stem diameter (μm)	Cortex thick-ness (μm)	No of cortical layers	L.V.B. dimension		Phloem tissue thick-ness (μm)	Xylem tissue thick-ness (μm)	Meta-xylem vessel diam-eter (μm)	Pith tissue diam-eter (μm)
				Length	Width				
Control	1142.1	105.4	7.0	316.7	240.2	130.1	195.5	40.2	720
Cd (150 ppm)	884.5	99.2	6.3	275.3	160.8	92.0	188.3	32.8	510
Bradyrhizobium (R)	1489.2	197.0	8.0	457.2	372.0	186.2	280.0	49.3	835
VA mycorrhizae (M)	1374.9	204.6	8.4	382.3	400.1	167.0	226.6	45.7	788
Kinetin 25 ppm (K)	1890.5	220.5	8.9	560.0	723.0	204.7	364.1	68.0	1110
Cd + R	1355.3	142.1	7.7	423.2	335.0	174.4	257.2	43.1	760
Cd + M	1262.3	180.3	7.9	349.0	378.0	152.0	203.0	41.5	733
Cd + K	1793.8	200.7	8.1	499.1	660.1	191.5	320.2	56.4	993
L.S.D. at 5%	105.7	4.2	0.5	22.2	35.4	13.7	6.9	6.2	0.98

The decrease in stem diameter due to cadmium treatment and other heavy metals may be attributed to the reduction of meristem size and decreasing number of mature cells (Stiborova *et al.*, 1986) as well as decrease in the cortex tissue thickness and vascular bundle dimension as shown in Table (5).

The increase in stem diameter caused by microbial inoculation or kinetin application and their interactions with Cd-treatment could be attributed to the increase recorded in the above mentioned anatomical parameters compared with those of both the control and Cd-treated plants. Fouda (1998) noted that the increase in vascular bundle dimensions by kinetin treatment may be due to a stimulation in cambial cell activity forming secondary vascular tissues.

Yield and its components:

Data in Table (6) reveal that in both seasons cadmium treatment decreased significantly number of pods per plant, seed weight (g) per plant and weight of 100 seed. It is clear in the same Table that inoculation with either *Bradyrhizobium* or VA mycorrhizae and kinetin application as well as their interactions with Cd-treatment increased all the abově mentioned yield parameters. Generally, kinetin proved to be the most effective in this respect. The reduction in yield and its components by Cd-treatment may be related to a decrease in branch number carrying pods, dry matter accumulation (Table 6), photosynthetic pigment contents (Table 3). Malan and Farrant (1998) reported that the reduction in yield of soybean plants by Cd-treatment was attributed to the decrease in photosynthetic rate, carbohydrate accumulation, seed number per pod and seed size. Moreover, El-Saied (2001) added that the reduction in yield of *Sorghum* plants by Cd-treatment may be due to a decrease in photosynthetic pigment formation resulting in inhibition of photosynthetic activity which led to reduction in assimilates translocation toward reproductive organs.

Table (6): Yield and its components of soybean plants as affected by cadmium, inoculation with either *Bradyrhizobium* or VA mycorrhizae and kinetin as well as their interactions during the two growing seasons 2000/2001 and 2001/2002.

Treatments	Pod.no/plant		Weight seeds/plant (g)		100 seeds weight (g)	
	2000	2001	2000	2001	2000	2001
Control	25.0	33.7	22.5	23.2	12.4	13.9
Cd (150 ppm)	18.0	24.0	14.3	17.6	10.0	10.6
Bradyrhizobium (R)	45.0	46.0	33.7	34.4	27.9	29.1
VA mycorrhizae (M)	41.3	43.7	30.6	32.2	23.9	24.7
Kinetin 25 ppm (K)	58.0	62.0	39.6	40.5	34.4	35.4
Cd + R	48.3	50.0	30.1	30.6	24.3	24.1
Cd + M	38.0	40.0	27.0	27.8	21.9	22.7
Cd + K	47.0	49.0	35.5	36.3	31.2	31.7
L.S.D. at 5%	5.6	5.3	2.1	1.9	1.6	1.6

The enhancing effect of inoculation with both microorganisms on yield may be attributed to their enhancing effect on plant growth, dry matter accumulation, number of branches per plant, photosynthetic pigment content as well as total carbohydrate contents (Tables 2 & 3). These results led to an increase in assimilates translocation toward reproductive organs.

Overcoming the adverse effects of Cd-treatment on seed yield by kinetin application may be related to enhancing the expansion of leaf area, photosynthetic pigment contents and photosynthetic activity which, in turn, increased carbohydrate accumulation and consequently increased the yield capacity of Sorghum-treated plants with cadmium (El-Saied, 2001). Moreover, Jiang *et al.* (1998) noted that kinetin promoted formation and differentiation of flower primordia and improved flower bud formation in *Cucumis* plants.

Seed quality:

The effects of cadmium treatment, inoculation with either *Bradyrhizobium* or VA mycorrhizae as well as kinetin application and their interactions with Cd-treatment on percentage of nitrogen, protein, P and K in soybean seeds during the second season are presented in Table (7). It is clear that Cd-treatment decreased significantly N, protein, P and K percentages in the seeds. While, interaction with both microorganisms and kinetin treatment increased these parameters in the seeds. Treatment with kinetin was the most effective in this respect.

Generally, the reduction in mineral percentages due to Cd-treatment may be attributed to the inhibition of nutrient uptake and transport. This effect may be due to an inhibiting effect of heavy metals on root growth (Gupta, 1997). Moreover, the reduction in nitrogen percent in the seeds by Cd-treatment may be attributed to its effects on inhibition of the nodulation, nitrogenase activity and consequently nitrogen fixation (El-Enany and Abd-Alla, 1995) and through, alterations of the root function (Barceleo *et al.*, 1988) or interference with the translocation of fixed nitrogen from roots to shoots (Jarvis *et al.*, 1976). Such decreases were related with the decrease in protein content.In addition, the reduction in protein content by Cd-treatment may attributed not only to inhibition in protein synthesis (Vassilev *et al.*, 1997) but also the metabolism of primary amino acids (Orzechowski *et al.*, 1997).

The enhancement of nutrient uptake by mycorrhizal infection may be attributed to direct hyphal uptake and/or indirect effects brought about by morphological and physiological changes in the host roots (Abd-El-Fattah and El-Katony, 1996). They added that the beneficial effect of mycorrhizae on nutrient uptake seems to be related to an enhanced supply of nutrients particularly phosphorus and nitrogen for the host plant. Such enhancing effects of both microorganisms resulted in increasing the absorptive area of the root system (Abd-El-Ati *et al.*, 2000). Diaz *et al.* (1996) concluded that VA-mycorrhizae can play an important role in the restoration of contaminated soils, by protecting the plants from high levels of heavy metals and this effect can be partially due to the improvement of the P status for the plant.

Table (7): Effect of cadmium, inoculation with either *Bradyrhizobium* or VA mycorrhizae and kinetin as well as their interactions on percentages of N, protein, K and P of soybean seeds during the second season 2001/2002.

	N%	Protein %	P%	K%
Control	4.8	30.0	0.22	3.6
Cd (150 ppm)	3.1	19.6	0.13	1.8
Bradyrhizobium (R)	6.1	38.7	0.35	4.4
VA mycorrhizae (M)	4.9	30.7	0.31	4.2
Kinetin 25 ppm (K)	6.4	40.0	0.46	4.9
Cd + R	5.7	35.5	0.31	4.1
Cd + M	4.6	28.5	0.29	3.9
Cd + K	6.2	39.0	0.39	4.7
L.S.D. at 5%	0.78	4.9	0.04	0.08

The stimulating effects of kinetin on mineral uptake and protein percentage noticed in the present investigation may be attributed to an increase in the stability of cell membrane permeability (Gadallah, 1995b), an increase in mineral uptake and transport through an increase in transpiration rate (El-Saied, 2001). He added that the increase in protein percentage due to kinetin treatment may be attributed to its effect on increasing cytokinin content. Cytokinins increase the activity of cytoplasm ribosomes leading to stimulation RNA synthesis followed by an increase in protein synthesis (Wareing and Phillips, 1970). Hussein *et al.* (1983) noted that the increase in protein content may be due to an increase in the amino acid content and an increase in the activities of the enzymes related to soluble protein synthesis. It could be concluded that inoculation with either *Bradyrhizobium* or VA-mycorrhizae and kinetin plays an important role in the protection of soybean plants against Cd-toxicity. However, kinetin proved to be the most effective treatment in this respect as compared to all other treatments and treated plants. Generally, with regard to heavy metals, uptake is not dependent on the concentration of metals alone, but also differs from species to species of plant and fungus. The presence of mycorrhizae can increase the uptake of heavy metals by the host plant and this can mean better supplies of iron and trace elements, but also facilitates the entry of toxic elements such as cadmium. On the other hand, the mycorrhizal fungi can bind heavy metals in their cell wall complex and so are able to buffer the plants against toxic effects. In addition, the species spectrum of the mycorrhizae-forming fungi may change in polutted soil.

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تخفيف التأثير السام للكادميوم على فول الصويا بواسطه التلقيح ببكتريا البراديرايزوبيوم والميكروبزا أو المعامله بالكينتين

رمضان عبد المنعم فودة و عرفه أحمد عرفه

قسم النبات الزراعى، كليه الزراعة، جامعه المنصورة

نظرا لأهميه فول الصويا كغذاء للإنسان والحيوان فقد أستهدف البحث دراسه إمكانيه التغلب على التأثير الضار للكادميوم بإستخدام التلقيح ببكتيريا البرادايرايزوبيم وفطريا الميكوريزا والمعاملة بالكينتين وذلك بسبب زيادة تراكم الكادميوم فى بعض الأراضى نتيجه تلوث البيئه وزيادة استخدام المبيدات الى جانب سرعة انتقاله داخل النبات ومن ثم تأثيرة على السلسله الغذائيه. وقد أدت المعامله بالكادميوم الى نقص طول النبات وعدد الأفرع ومساحه الورقه والوزن الجاف للمجموع الخضرى للنبات بالإضافه الى نقص محتوى الأوراق من صبغات البناء الضوئى ومحتوى الكربوهيدرات فى المجموع الخضرى للنبات، وعدد ووزن البذور/ نبات ووزن المائه بذرة كما أدى الى نقص محتوى المجموع الخضرى من الكالسيوم وكذلك محتوى البذور من النيتروجين والبروتين والفوسفور والبوتاسيوم الا انه أدى الى زيادة محتوى المجموع الخضرى من الكادميوم.

على الجانب الآخر أدى التلقيح بالبرادايرايزوبيوم او الميكوريزا وكذا المعامله بالكينتين الى زيادة كل الصفات السابقة فى التربه الملوثه بالكادميوم فيما عدا نقص محتوى المجموع الخضرى من الكادميوم. أدت كل المعاملات السابقة الى التغلب على الآثار الضارة للكادميوم وتحسين كل الصفات السابقه وكانت المعامله بالكينتين أكثر المعاملات تأثيرا فى هذا المجال مقارنه بالمعاملات الأخرى. تشريحيا أدت المعامله بالكادميوم الى نقص قطر الساق وسمك أنسجه القشرة ، الخشب، اللحاء والنخاع وكذلك قطر أوعيه الخشب التالى بالإضافة الى نقص سمك الوريقة والنسيج المتوسط فى الوريق وذلك لنقص كم من النسيج العمادى والأسفنجى. كما نقص حجم الحزمه الوعائيه للعرق الوسطى معبرا عنه بنقص ابعادها الا أن التلقيح بالبرادايرايزوبيوم او الميكوريزا وكذا المعامله بالكينتين الى زيادة كل الصفات السابقة فى التربه الملوثة بالكادميوم.