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Minimizing the Harmful Effects of Cadmium on Vegetative Growth, Leaf Anatomy, Yield and Physiological Characteristics of Soybean Plant [*Glycine max* (L.) Merrill] by Foliar Spray with Active Yeast Extract or with Garlic Cloves Extract

[1]Fatma A. Abdo, [2]Dalia M.A. Nassar, [2]Elham F. Gomaa and [2]Rania M.A. Nassar

[1]Department of Crops Physiology Research, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.
[2]Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza, Egypt.

ABSTRACT

Pot experiments were carried out during the two successive summer seasons of 2010 and 2011 in the greenhouse of Crops Physiology Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt to investigate the effect of pollution with cadmium at concentrations of 50, 100 and 200 ppm on vegetative growth, leaf anatomy, yield and certain physiological aspects of soybean 'Giza 35'. In addition, the use of natural extracts from active yeast (60ml/L.) and garlic cloves (30 ml/L.) for minimizing the harmful effects of environmental pollution caused by cadmium on vegetative and reproductive growth as well as on leaf anatomy and physiological behaviour of soybean was also investigated. The obtained results revealed that all assigned concentrations of cadmium induced significant decrease in all investigated morphological characters of vegetative growth (plant height, number of branches and leaves/plant, total leaf area /plant and shoot dry weight/plant) and in all studied yield characters (number of pods and seeds/plant, specific seed weight and seed yield/plant) of soybean 'Giza 35'. Moreover, the significant decrease in morphological and yield characters got higher as the concentration of cadmium increased in irrigation water. Worthy to note that, soybean plants grown under stress of pollution with different levels of cadmium and sprayed with either yeast or garlic extract had better growth behavior and yield than those of unsprayed with natural extracts. Likewise, the concentrations of photosynthetic pigments (Chl. a, Chl. b and carotenoids), total sugars and phytohormones from IAA and GA_3 in leaves of cadmium polluted soybean plants were decidedly lower than those of control plants. In this respect, increasing cadmium concentration in irrigation water decreased gradually photosynthetic pigments, total sugars, IAA and GA_3 . It was found that yeast as well as garlic extracts were able to minimize the harmful effect of cadmium and improve the concentrations of photosynthetic pigments, total sugars, IAA and GA_3 in leaves of cadmium polluted soybean plants. Also, the percentage of crude protein and total lipids in seeds of cadmium polluted soybean plants were decidedly lower than those in seeds of control plants and increasing cadmium concentration in irrigation water decreased gradually the percentage of crude protein and of total lipids. It is clear that foliar application with yeast or garlic extract was sufficient for reducing the harmful effect of cadmium and improve the percentage of crude protein and of total lipids in seeds of cadmium polluted soybean plants. At the same time, the concentration of phytohormone ABA in leaves and of cadmium in leaves and seeds of soybean plants was increased due to cadmium treatments, and the application of yeast or garlic extract on soybean plants grown under stress of pollution with cadmium minimized the harmful effect of such heavy metal on concentration of ABA in leaves and on cadmium accumulation in leaves and seeds of cadmium polluted soybean plants. Concerning the anatomical structure of leaflet blades, treatment with 100 ppm cadmium decreased the thickness of both midvein and lamina due to the decrease induced in thickness of both palisade and spongy tissues as well as in the dimensions and components of main midvein bundle. The mean values of number of xylem rows and vessels/midvein bundle as well as of vessel diameter in leaflets of stressed soybean plants were decidedly lower than those of control. The application of yeast or garlic extract caused enhancement in leaflets structure of polluted plants and the best results were obtained from treatment with 60 ml. active yeast extract/L. which caused recovery of the reduction occurred in most of the histological characters where their mean values reached or surpassed those of control.

Key words: Soybean, Pollution, Cadmium, Natural extracts, Vegetative growth, Leaf anatomy, Yield, Physiological aspects.

Introduction

Soybean [*Glycine max* (L.)Merrill], the subject of the present investigation, is one of the most important annual pulse crops in the world. The cultivated form is used in human food and livestock feeds because it is a

Corresponding Author: Dalia M.A. Nassar, Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza, Egypt.
E-mail: dalia_nassar2004@yahoo.com

source of oil and high-protein meal (Harry and Kwon, 1987 and Abdo, 2008). Soybean seeds contain approximately 40% protein and 21% edible oil which is used in making margarine (Noureldin, 1998).

Pollution of the environment with toxic metals such as lead, cadmium, chromium and arsenic has increased dramatically since the onset of the industrial revolution (Nriagu, 1979). The main sources of this pollution are fossil fuel burning, mining and smelting of metalliferous ores, municipal wastes, landfill leachates, mineral fertilizers, pesticides and sewage (Forstner, 1995). Toxic metal contamination of soil, streams, and ground water pose a major environmental and human health risk. The threat that heavy metals pose to human and animal health is aggravated by their long-term persistence in the environment (Shaw, 1990). Emission from automobile exhaust is the greatest source of pollution with lead and cadmium. In addition, many areas in different agricultural places show increased levels of different heavy metals as a result of application of large amounts of cattle manures, mineral fertilizers, composts, contaminated harbor, sludge or sewage sludge (Bockhold, 1992).

Accumulation of heavy metals in agricultural soils has become a major concern for food crop production. Cadmium (Cd) is recognized as one of the most hazardous elements which is not essential for plant growth (Kabata-Pendias and Pendias, 1992). Elemental analysis of many different plants growing in laboratories and in various parts of the world near mines and industrial areas have revealed that plants do not exclude toxic heavy metals, especially cadmium, during the uptake of essential minerals (Turner, 1969; John, 1972; Williams and David, 1973 and Chaney *et al*., 1977).

Stocks and Bagchi (1995) as well as Taiz and Zeiger (1998) related the toxicity of heavy metals to their ability to cause oxidative damage to plant cells. Such damage includes enhanced lipid peroxidation, DNA damage and / or the oxidation of protein sulfhydryl groups. Likewise, Marrs (1996) reported that the oxidative stress caused by heavy metals pollution is established through a series of redox reactions initiated by hydrogen peroxide and other free radicals.

It is well known that Cd concentration in plant tissues is usually directly related to the concentration of plant available Cd in soil (Braumemer *et al.,* 1986). Huang *et al.* (1974) found that Cd, in the nutrient solution, inhibited pod fresh weight accumulation, nitrogenase activities and photosynthetic rates in soybeans. Likewise, addition of Cd to growth media depressed the production of dry matter in several plant species (Leita *et al.,* 1993). In this respect, Jalil *et al*. (1994) reported that Cd inhibited shoot and root biomass, root length and leaf area of wheat plants. Likewise, El-Ghinbihi (2000) stated that increasing cadmium level (0.0, 0.1, 0.5 and 1.0 mM) in growth media of common bean plants significantly decreased plant height, root length, fresh and dry weights of roots and stems, number as well as fresh and dry weights of leaves, leaf area, concentrations of Chl. a, Chl. b and carotenoids as well as total carbohydrates in leaves of common bean. Accumulation of cadmium in roots and leaves was significantly increased. Increasing cadmium level led to a significant decrease in total protein concentration in leaves and seeds. The yield as represented by number and weight of pods/plant as well as number and weight of seeds/pod was significantly decreased by cadmium treatments.

Also, El-Nabarawy (2002) found that the uptake of Cd in different plant organs of spinach was parallel with Cd increments in root soil medium, and that associated with continuous reduction of spinach plant organs growth, in terms of leaf number, whole plant leaf area, fresh and dry weights of roots and shoots. Likewise, Fouda and Arafa (2002) showed that cadmium treatment decreased height of soybean plant, number of branches, leaf area and dry weight of shoot/plant. In addition, photosynthetic pigments content in the leaves and carbohydrates concentration in the shoot, pod number/plant, seed weight (g)/plant and weight of 100 seeds (g) were also decreased. Whereas, Cd concentration in the shoot was increased and protein percent in the seeds was decreased. Regarding anatomical structure of soybean plant, cadmium treatment decreased stem diameter due to the decrements induced in the thickness of cortex, phloem, xylem and pith diameter as well as diameter of metaxylem vessels. Thickness of either leaflet blade or mesophyll tissues was decreased due to reducing induced in both palisade and spongy tissues thickness. The size of midvein bundle was also decreased as a result of cadmium treatment. Also, El-Gamal and Hammad (2003) stated that cadmium negatively affected growth of tomato plant represented by plant height, leaf number, dry weight of shoots and roots. Water relations, photosynthetic pigments, carbohydrates and soluble sugars as well as fruit yield and quality were also negatively affected.

It was found that the application of natural extracts protects plants against heavy metals pollution. Among these extracts, yeast and garlic were found to exert positive effect and overcome the harmful effect of some environmental stresses on plant growth. In this respect, Hanafy *et al.* (1994) observed that garlic was antagonized to lead toxicity. They found that lead concentrations were reduced in muscle and liver tissues of chickens given both lead and garlic simultaneously or garlic as a supplement after lead treatment. Garlic contains chelating compounds capable of enhancing elimination of lead and feeding can minimize lead concentration in meat food animals from a lead polluted environment. In this concern, El-Gamal and Hammad (2003) reported that garlic and yeast extracts are useful in counteracting the harmful effects exerted by cadmium on tomato plants.

Recently, Atawia and El-Desouky (1997) stated that foliar application with natural yeast extract at the full bloom stage of orange 'Washington navel' trees improves the fruit yield per tree as well as fruit quality. In this connection, El-Desouky *et al.* (1998) and Wanas *et al.* (1998) found that the natural extract of garlic cloves and of active yeast cells improve the growth, sex expression and fruit yield and quality of squash plant. Likewise, Amer (2004) and Nassar *et al.* (2011) used yeast as a natural source of phytohormones especially cytokinins and stated that yeast extract has stimulatory effects on growth and yield of bean plants. Since, these extracts contain many growth materials and essential requirements at vegetative and reproductive growth. They are rich in phytohormones, vitamins, amino acids, energy, etc. (Watt and Merrill, 1963 on garlic as well as Devlin and Witham, 1983 on yeast).

Therefore, the present work was designed to disclose the effect of pollution with cadmium on the growth, leaf anatomy, physiological behavior and yield of soybean. Moreover, the use of natural extracts from garlic or yeast in counteracting or at least reducing the deleterious effects of the environmental pollution caused by cadmium on vegetative and reproductive growth of soybean was also investigated.

Materials And Methods

Pot experiments were carried out during the two successive summer seasons of 2010 and 2011 in the greenhouse of Crops Physiology Research Department, Field Crops Research Institute, Agricultural Research Center, Giza.

Clay loam soil was collected from an ordinary agricultural field at Giza and analyzed for cadmium (Cd) by Atomic Absorption Spectrophotometer in ammonium acetate extracts (Jackson, 1973). Cadmium concentration and physical properties of the collected soil were as follows:

Cd (ppm)	Coarse sand %	Fine sand %	Silt %	Clay %	pH	Soil texture
0.16	1.9	32.6	38.4	24.7	7.4	Clay loam

Earthenware pots 35 cm inner diameter were used in this investigation. Each pot was filled with 10 kg clean air-dried clay loam soil. Seeds of soybean 'Giza 35', maturity group III, were obtained from Agricultural Research Center, Giza, Egypt and sown on 26th April in the first season of 2010 and on 2nd May in the second season of 2011 in previously prepared earthenware pots. Six seeds were sown in each pot and after 15 days from sowing plants were thinned to leave 2 uniform seedlings per pot. Calcium superphosphate (15.5% P_2 O_5) was mixed with the soil prior to sowing at the rate of 3.5 g/pot. While, ammonium nitrate (33% N) and potassium sulphate (48% K_2O) were added with water irrigation after thinning at the rate of 1.5 g/pot from each fertilizer. Other agricultural practices were done according to the recommended practices.

One month after sowing, cadmium sulphate (Cd) at concentrations of 0.0, 50, 100 and 200 ppm were introduced in the irrigation water. Yeast extract at the rate of 60 ml/L or garlic extract at the rate of 30 ml/L were used as foliar application three times in two weeks intervals starting 30 days from sowing date.

Preparation of yeast extract (YE):

The pure dry yeast powder was activated by using sources of carbon and nitrogen at the rate of 6 : 1 (Barnett *et al.,* 1990). Such ratio was suitable to obtain the highest vegetative production of yeast, each ml of activated yeast contained about 12000 yeast cells. Such technique allowed yeast cells to be grown and multiplied efficiently during conductive aerobic and nutritional conditions. To produce *de novo* beneficial bioconstituents; *i.e*., phytohormones, carbohydrates, proteins, amino acids, fatty acids, vitamins, enzymes, minerals …etc, hence allowed such constituents to release out of yeast tissues in readily form. Such technique for yeast preparation based on: 1- Nutritional media of glucose and casein as favourable sources of C, N and other essential elements (P, K, Ca, Mg, Fe, Mn, Cu, B and Mo as well as Na and Cl) in suitable balance (Barnett *et al.,* 1990). 2- Air pumping and adjusting incubation temperature. The media then subjected to two cycles of freezing and thawing for disruption of yeast tissues and releasing their bioconstituents directly before usage. Tween-20 was added as a spreading agent for tested treatments.

Preparation of garlic extract (GE):

One kg fresh mature garlic cloves were blended in one liter distilled water, frozen and thawed two times then filtered (El-Desouky *et al.,* 1998).

The experiment was made in a complete randomized design with five replicates, the replicate contains 40 pots, each four pots were assigned for one treatment. The treatments were:

1- Control (tap water).
2- 50 ppm cadmium.
3- 100 ppm cadmium.
4- 200 ppm cadmium.

5- 50 ppm cadmium + yeast extract.
6- 100 ppm cadmium + yeast extract.
7- 200 ppm cadmium + yeast extract.
8- 50 ppm cadmium + garlic extract.
9- 100 ppm cadmium + garlic extract.
10- 200 ppm cadmium + garlic extract.

Recording of data:

The current investigation involved studies pertaining to vegetative growth, leaf anatomy, physiological and yield attributes of soybean plants grown under stress of pollution with different levels of cadmium and their response to treatment with yeast or garlic extract.

For all determined characters, five pots (representing five replicates) from each treatment were used for this purpose. Data were recorded on individual plants, and then the mean plant character of recorded plants per pot was calculated as a mean of a replicate. The procedure of recording the various data was carried out in the following manner:

1- Vegetative growth characters:

The following measurements were recorded after two weeks from the last application with natural extracts; *i.e.*, at the age of 75 days in each growing season: plant height (cm), number of branches per plant, number of leaves per plant, total leaf area (cm^2) per plant and dry weight of shoot (g) per plant. For determination of dry matter, plant shoot was dried at 70^oC in an electric oven for at least 48 hours and weighed accurately.

2- Anatomical studies:

A comparative microscopical examination was performed on plant material for treatments which showed remarkable response. Tested material included the lamina of the terminal leaflet of the compound leaf corresponding to the median internode of the main stem; *i.e.,* the eighth developed leaf. Specimens were taken throughout the second season of 2011 at the age of 75 days. Specimens were killed and fixed for at least 48 hrs. in FA.A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). The selected materials were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56^oC, sectioned to a thickness of 20 micron, double stained with crystal violet-erythrosin, cleared in xylene and mounted in Canada balsam (Nassar and El-Sahhar, 1998). Sections were read to detect histological manifestations of noticeable responses resulted from assigned treatments and photomicrographed.

3- Yield characters:

Data on yield characters were recorded on individual plants at harvest time of each growing season; *i.e.,* 120 days from sowing date. The following yield and yield components per individual plant were estimated: number of pods per plant, number of seeds per plant, weight of 100 seeds (g) and seed yield (g) per plant.

4- Physiological characters:

All chemical determinations of physiological characters were carried out in the second season of 2011 on fresh green leaves, at the age of 75 days, and on mature dried seeds, at harvest time (120 days from sowing), of treated and untreated plants of soybean 'Giza 35'.

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were extracted by using dimethyl formamide and determined according to Nornai (1982) as mg/g fresh weight of soybean leaves. Total sugars were determined by using phenol-sulphoric method according to Dubois *et al.* (1956) as mg/g fresh weight of soybean leaves. Phytohormones (IAA, GA_3 and ABA) were measured in methanol extract of soybean leaves according to the method described by Vogel (1975). Phytohormones were determined as μ g/100 g fresh weight of soybean leaves.

Samples from mature dried seeds resembling various treatments were finely ground and subjected to chemical analysis for crude protein % (Pregl, 1945 and Anon., 1990) and total lipids % (Anon., 1990).

The concentration of cadmium in plant samples (leaves and dried seeds) which were digested in $HClO_4$ and H_2SO_4 acids were determined using atomic absorption spectrophotometer (Model Phillips Pu 9100).

5- Statistical analysis:

Data on morphological and yield characters of the two seasons were subjected to conventional methods of analysis of variance according to Snedecor and Cochran (1982). The combined data of both experimental seasons were used for results presentation. The least significant difference (L.S.D.) at 0.05 level of probability was calculated for each determined character under different assigned treatments.

Results And Discussion

I- Vegetative growth characters:

The combined data of two successive summer seasons (2010 and 2011) for vegetative growth characters of soybean 'Giza 35' grown under stress of pollution with different levels of cadmium and treated with either yeast or garlic extract were followed up. The investigated characters included: Plant height, number of branches/plant, number of leaves/plant, total leaf area/plant and shoot dry weight/plant at the age of 75 days. Data on vegetative characters are shown in Table (1).

It is clear that values of all vegetative growth characters in cadmium polluted soybean plants were significantly lower than those of control plants. Moreover, increasing cadmium concentration in irrigation water retarded significantly all vegetative growth characters under investigation. The maximum significant decrease below the control was recorded at 200 ppm Cd, being 38.5, 50.0, 44.4, 45.4 and 43.7% less than those of control plants for plant height, number of branches/plant, number of leaves/plant, total leaf area/plant and shoot dry weight/plant; respectively.

These results are in agreement with those reported by Fouda and Arafa (2002) on soybean. They found that cadmium treatment decreased plant height, number of branches, leaf area and dry weight of shoot/plant.

Some investigators conformed these findings on other plant species, for instance, Gadallah (1995) on sunflower stated that heavy-metal toxicity appears in the reduction of plant height and dry mass accumulation. Likewise, Ghonem *et al.* (1997) on maize as well as Singh *et al.* (1999) on cowpeas and mungbeans found that dry matter decreased with increasing cadmium concentration. Also, El-Shafie and El-Shikha (2002) on pea reported that increase soil cadmium application caused reduction in shoot and root dry weight. In this concern, El-Gamal and Hammad (2003) stated that cadmium negatively affected growth of tomato plant represented by plant height, leaf number and dry weight of shoots and roots. All, being in accordance with the present findings.

Table 1: Effect of yeast or garlic extract on some vegetative growth characters of soybean 'Giza 35' grown under stress of pollution with different levels of cadmium (Average of two seasons, 2010 and 2011 combined)

Treatments	Plant height (cm)	No. of branches / plant	No. of leaves / plant	Total leaf area $(cm)^2$/plant	Shoot dry weight (g)/plant
Control (Tap water)	69.3 B	3.2 AB	47.3 AB	3689 ABC	87.5 AB
50 ppm Cd	61.3 CD	2.9 BCD	42.2 CD	3202 DEF	78.9 CD
100 ppm Cd	54.5 E	2.3 E	35.6 E	2704 G	65.5 F
200 ppm Cd	42.7 F	1.6 F	26.3 F	2014 H	49.3 G
50 ppm Cd + Yeast extract	76.7 A	3.4 A	51.2 A	3977 A	92.7 A
100 ppm Cd + Yeast extract	68.6 B	3.1 ABC	46.3 BC	3531 BCD	85.2 ABC
200 ppm Cd + Yeast extract	57.2 DE	2.7 D	39.4 DE	3006 EFG	73.2 DE
50 ppm Cd + Garlic extract	75.8 A	3.1 ABC	48.8 AB	3785 AB	87.1 AB
100 ppm Cd +Garlic extract	64.7 BC	2.8 CD	42.7 CD	3313 CD	79.9 BCD
200 ppm Cd + Garlic extract	54.5 E	2.3 E	35.5 E	2896 FG	68.2 EF
L.S.D. (0.05)	5.9 cm.	0.38 branch	4.46 leaves	412 cm^2	7.7 g

Means having the same letter(s) are not significantly different at 0.05 level.

The reduction in vegetative growth characters of soybean plants caused by pollution with cadmium could be attributed to the deleterious effect of such metal on the activity of enzymes that involve in the regulation of growth and physiological behaviour. In this respect, Greger (1989) attributed the inhibitory effect of Cd on vegetative growth of plant species to different reasons: a- inhibiting water transport to the shoot, b- decreasing the uptake of essential elements by the roots, and c- reducing the stomatal aperture and CO_2 uptake, consequently decreasing the photosynthesis. In this respect, Vassilev *et al.* (1997) reported that heavy metals inhibited photosynthetic pigments which affected photosynthesis and caused inhibition in the growth and development of many plant species.

Data presented in Table (1) clearly show that soybean plants grown under stress of pollution with different levels of cadmium and sprayed with either yeast or garlic extract had better growth behavior than those unsprayed with natural extracts. Generally, the best results in this respect were obtained from yeast extract followed by garlic extract without significant difference between them. Thus, it could be stated that yeast as well as garlic extract had beneficial effects on all vegetative growth characters of soybean plants grown under stress of pollution with cadmium and they overcome the harmful effect of such heavy metal on vegetative growth characters of soybean plants under investigation. In this respect, some natural extracts have been applied to some economic plants as new trends for enhancing plant growth and increasing the final yield as well as diminishing the amount of fertilizers application (Atawia and El-Desouky, 1997 and Mahmoud, 2001). Yeast treatments were suggested to participate in a beneficial role during vegetative and reproductive growth through improving the content of auxins and cytokinins (Barnett *et al.*, 1990). Hanafy *et al.*, (1994) reported that garlic extract was more effective in antagonized the toxicity of heavy metals. The role of garlic in overcoming the harmful effect of some heavy metals was explained as garlic contains chelating compounds capable of enhancing the elimination of lead.

El-Gamal and Hammad (2003) found that garlic and yeast extracts are useful in counteracting the harmful effects exerted by cadmium on growth of tomato plants, being in harmony with the present findings.

II- Leaf anatomy:

Microscopical counts and measurements of certain histological characters in transverse sections through the blade of the terminal leaflet of the eighth compound leaf developed on the main stem of control plants of soybean 'Giza 35' and of those grown under pollution stress with 100 ppm cadmium as well as of polluted plants which affected by foliar spray with 60 ml/L. active yeast extract or with 30 ml/L. garlic cloves extract are presented in Table (2). Likewise, microphotographs illustrating these treatments are shown in Figure (1).

Table 2: Counts and measurements in microns of certain histological characters in transverse sections through the terminal leaflet blade of the eighth compound leaf developed on the main stem of soybean 'Giza 35' grown under pollution stress with 100 ppm cadmium and affected by foliar spray with yeast or garlic extract (Means of three sections from three specimens)

Histological characters	Treatments					
	Control	100 ppm Cd	± % to control	100 ppm Cd + yeast extract	± % to control	100 ppm Cd + garlic extract	± % to control
Thickness of midvein	1212	753	-37.9	1158	-4.5	1039	-14.3
Thickness of lamina	198	181	-8.6	207	+4.6	192	-3.2
Thickness of palisade tissue	85	79	-7.1	91	+7.1	83	-2.4
Thickness of spongy tissue	93	82	-11.8	96	+3.2	89	-4.3
Length of midvein bundle	350	236	-32.6	332	-5.1	291	-16.9
Width of midvein bundle	448	279	-37.7	528	+17.9	362	-19.2
No. of xylem rows/midvein bundle	11	8	-27.3	13	+18.2	9	-18.2
No. of vessels/midvein bundle	49	34	-30.6	55	+12.2	41	-16.3
Vessel diameter	42	30	-28.6	34	-19.1	39	-7.1

It is obvious from Table (2) and Figure (1) that cadmium treatment decreased the thickness of both midvein and lamina of examined leaflet by 37.9 and 8.6% less than those of the control; respectively. The thinner

leaflets induced by pollution stress with cadmium could be attributed to the decrease induced in thickness of both palisade and spongy tissues as well as in the dimensions and components of main midvein bundle. The decrements below the control were 7.1, 11.8, 32.6 and 37.7% for palisade tissue, spongy tissue, length of midvein bundle and width of midvein bundle; respectively. Also, the number of xylem rows/midvein bundle, number of vessels/midvein bundle and vessel diameter in leaflets of stressed plants were decreased by 27.3, 30.6 and 28.6% less than those in leaflets of normal plants; respectively.

In this respect, Fouda and Arafa (2002) stated that soybean 'Giza 21' plants grown under pollution stress with 150 ppm cadmium produced thinner leaflet blades due to the decrements induced in thickness of mesophyll tissues and in size of the midvein bundle as compared to the control, being in agreement with the present findings.

As to the effect of natural extracts, it is clear that the application of yeast or garlic extract on soybean plants grown under stress of 100 ppm cadmium caused enhancement in leaflets structure of polluted plants and the best results in this respect were obtained from treatment with 60 ml active yeast extract/L. which caused recovery of the reduction occurred in most of the histological characters under investigation where their mean values reached or surpassed the level of control.

It could be stated that these natural extracts had the ability to minimize the harmful effect of cadmium on anatomical structure of soybean leaves. The treatment of active yeast extract induced negligible decrease in thickness of midvein as well as in length of midvein bundle of leaflet blades of polluted soybean plants by 4.5 and 5.1% less than the control; respectively. By contrast, such treatment increased thickness of lamina by 4.6% more than the control. Such increase in lamina thickness could be attributed to the increments induced in thickness of both palisade and spongy tissues by 7.1 and 3.2% more than the control; respectively. Likewise, yeast extract treatment increased width of main midvein bundle, number of xylem rows/midvein bundle and number of vessels/midvein bundle of leaflet blades of polluted soybean plants. The increments over the control were 17.9, 18.2 and 12.2% for width of midvein bundle, number of rows/midvein bundle and number of vessels/midvein bundle; respectively. However, mean diameter of vessel was decreased by 19.1% below the control.

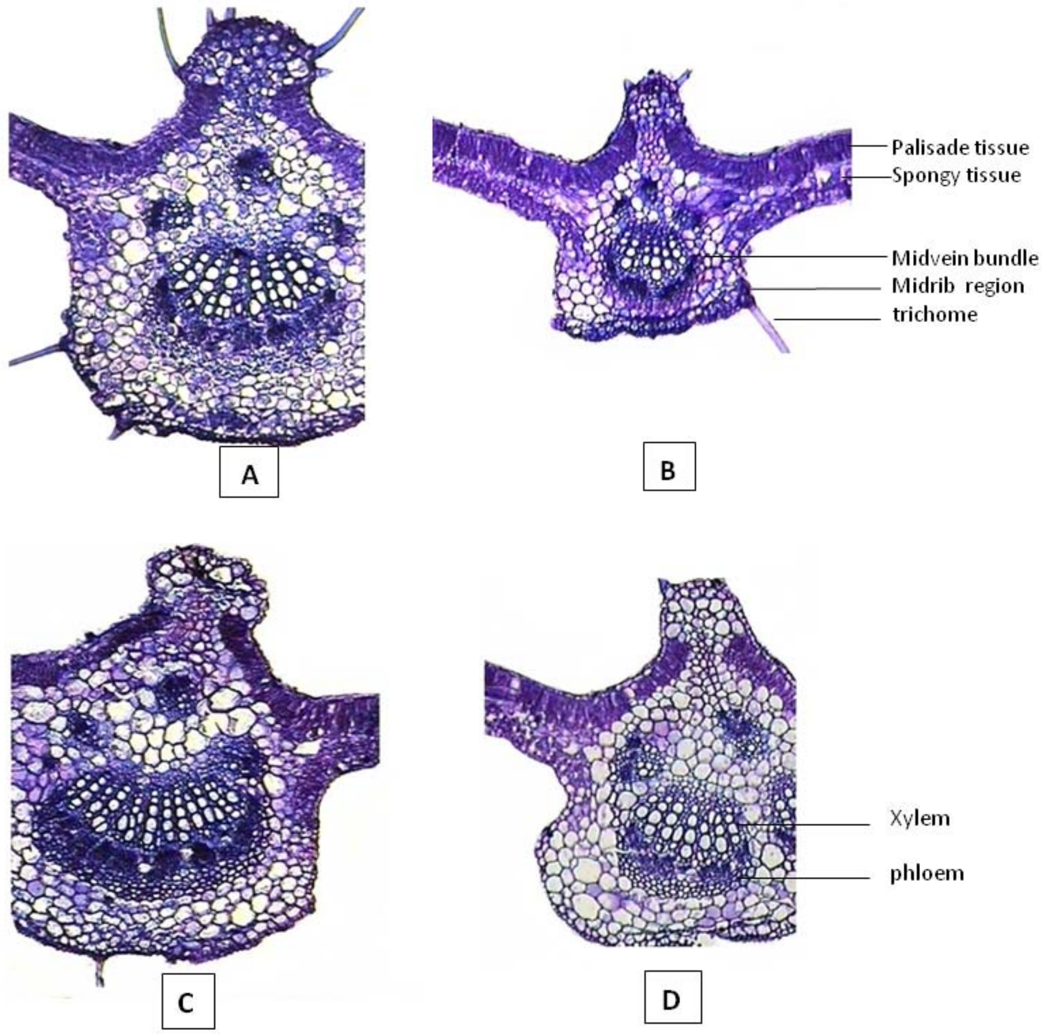

Fig. 1: Transverse sections through the terminal leaflet blade of the eighth compound leaf developed on the main stem of soybean ' Giza 35 '(aged 75 days) grown under pollution stress with 100 ppm cadmium and affected by foliar spray with 60 ml/L active yeast extract or with 30 ml/L garlic cloves extract. (x 100)

A- From control plant (normal plant).
B- From plant grown under pollution stress with 100 ppm cadmium.
C- From polluted plant with 100 ppm cadmium and sprayed with 60 ml/L active yeast extract.
D- From polluted plant with 100 ppm cadmium and sprayed with 30 ml/L garlic cloves extract.

As to the effect of garlic extract, it is realized that such treatment alleviate the adverse effect of cadmium on anatomical structure of leaflet blades of soybean 'Giza 35' plant. The thickness of both midvein and lamina was decreased by 14.3 and 3.0% less than those of the control; respectively. The decrements observed could be attributed to the decrease induced in thickness of both palisade and spongy tissues as well as in the dimensions and components of main midvein bundle. The decrements below the control were 2.4, 4.3, 16.9 and 19.2% for palisade tissue, spongy tissue, length of midvein bundle and width of midvein bundle; respectively. Also, the number of xylem rows/midvein bundle, number of vessels/midvein bundle and vessel diameter in leaflets of stressed plants which affected by garlic extract treatment were decreased by 18.2, 16.3 and 7.1% less than those of control plant; respectively.

III- Yield characters:

The combined data of two successive summer seasons of 2010 and 2011 for yield characters of soybean 'Giza 35' grown under stress of pollution with different levels of cadmium and treated with either yeast or garlic extract and the results of their statistical analysis are given in Table (3).

Table 3: Effect of yeast or garlic extract on some yield characters of soybean 'Giza 35' grown under stress of pollution with different levels of cadmium (Average of two seasons, 2010 and 2011 combined)

Treatments	Number of pods/plant	Number of seeds/plant	Weight of 100 seeds (g)	Seed yield (g)/plant
Control (Tap water)	62.8 A	132.4 A	16.53 A	21.89 A
50 ppm Cd	56.0 C	116.5 B	15.11 BCD	17.60 C
100 ppm Cd	48.4 D	100.6 D	13.85 DE	13.93 D
200 ppm Cd	37.9 E	78.2 E	12.52 E	9.79 E
50 ppm Cd + Yeast extract	64.6 A	133.8 A	16.49 A	22.06 A
100 ppm Cd + Yeast extract	59.7 ABC	124.7 AB	15.46 AB	19.28 B
200 ppm Cd + Yeast extract	50.4 D	104.1 CD	13.92 CD	14.49 D
50 ppm Cd + Garlic extract	62.2 AB	129.9 A	16.51 A	21.45 A
100 ppm Cd + Garlic extract	57.3 BC	118.5 B	15.28 ABC	18.11 BC
200 ppm Cd + Garlic extract	48.2 D	99.7 D	13.87 DE	13.83 D
L.S.D. (0.05)	5.3 pods	10.6 seeds	1.38 g	1.56 g

Means having the same letter(s) are not significantly different at 0.05 level.

It is realized from Table (3) that all assigned concentrations of cadmium induced significant decreases in number of pods/plant, number of seeds/plant, specific seed weight and seed yield/plant of soybean 'Giza 35'. Worthy to note that the significant decreases in yield characters got higher as the concentration of cadmium increased in irrigation water. Thus, the highest significant decrease in any of the investigated characters was recorded at the higher used concentration of 200 ppm cadmium, being 39.6, 40.9, 24.3 and 55.3% less than the control for number of pods/plant, number of seeds/plant, weight of 100 seeds (specific seed weight) and seed yield/plant; respectively.

These results are in accordance with those recorded by Xian (1989) on kidney beans as well as by Fouda and Arafa (2002) on soybean plant.

As to the effect of natural extracts, it is noted that the application of yeast or garlic extract on soybean plants grown under stress of 50 and 100 ppm cadmium caused a significant recovery of the reduction occurred in all investigated yield characters and their mean values reached the level of control plants without significant difference in most cases. Also, it was found that natural extract of either yeast or garlic minimized the harmful effect of cadmium at higher used concentration of 200 ppm on yield characters of soybean 'Giza 35'. Generally, the best results in this respect were obtained from treatment with yeast extract then by treatment with garlic extract without significant difference between them. Thus, it could be stated that these natural extracts could alleviate the negative effect of cadmium and make a progressive improvement on the yield of soybean. Such increase in yield by natural extract treatments could be attributed mainly to the enhancing effect of natural extracts on fruit setting and the increment observed in number of fruits/plant, number of seeds/plant and specific seed weight. The positive effect of these extracts on plant growth may contribute also to the increase in plant productivity. Similar results were also reported by El-Gamal and Hammad (2003).

IV- Physiological characters:

All chemical determinations of physiological characters were carried out in the second season of 2011 on fresh green leaves, at the age of 75 days, and on mature dried seeds, at harvest time, of treated and untreated plants of soybean 'Giza 35'.

A- Chemical analysis of leaves:

Photosynthetic pigments, total sugars and phytohormones as well as cadmium were determined quantitatively in leaves of treated and untreated plants. Results are presented in Table (4).

Photosynthetic pigments:

Data presented in Table (4) clearly show that the mean value of Chl.a, Chl.b and carotenoids in leaves of cadmium polluted soybean plants were decidedly lower than those of control plants. Moreover, increasing cadmium concentration in irrigation water decreased gradually photosynthetic pigments and the maximum decrease was observed at 200 ppm cadmium, being 31.5, 34.4 and 31.0% less than the control for Chl.a, Chl.b and carotenoids; respectively. These results are in agreement with those reported by Gil *et al.* (1995) on tomato and Fouda and Arafa (2002) on soybean. In this respect, Fodor *et al.* (1998) found that the inhibition effect of heavy metals on Fe uptake and transport to plant leaves may be a result also in reducing chlorophyll synthesis and causes chlorosis.

Table 4: The effect of yeast or garlic extract on photosynthetic pigments, total sugars, phytohormones and cadmium concentrations in leaves of soybean 'Giza 35' (aged 75 days) grown under stress of pollution with different levels of cadmium in the second season of 2011

Treatments	Photosynthetic pigments (mg/g F.W.)			Total sugars (mg/g F.W.)	Phytohormones (μg/100 g F.W.)			Cadmium ppm
	Chl. a	Chl. b	Carotenoids		IAA	GA_3	ABA	
Control (Tap water)	3.141	1.088	1.437	8.797	15.749	21.678	14.047	0.27
50 ppm Cd	3.015	1.072	1.396	8.114	14.286	20.251	14.662	17.90
100 ppm Cd	2.724	0.985	1.174	6.581	12.543	17.528	17.141	28.60
200 ppm Cd	2.152	0.714	0.991	5.244	9.974	14.983	21.525	42.50
50 ppm Cd + Yeast extract	3.216	1.092	1.471	8.814	15.775	21.722	14.112	4.85
100 ppm Cd + Yeast extract	3.112	1.086	1.405	8.221	14.518	20.314	14.593	13.92
200 ppm Cd + Yeast extract	2.824	0.993	1.211	6.713	12.621	17.626	17.617	20.75
50 ppm Cd + Garlic extract	3.172	1.089	1.446	8.779	15.751	21.693	14.108	4.16
100 ppm Cd + Garlic extract	3.024	1.076	1.389	8.164	14.422	20.244	14.642	15.23
200 ppm Cd + Garlic extract	2.751	0.981	1.176	6.623	12.607	17.153	17.805	22.48

Data in Table (4) also indicate that yeast as well as garlic extracts were able to decrease the harmful effect of cadmium and improve the concentration of photosynthetic pigments in leaves of cadmium polluted soybean plants. The best results in this concern were obtained from treatment with yeast extract followed by garlic extract. Such promotional effects of natural extracts upon the formation of chlorophyll might be due to the active role of such agents in the pathway of synthesis of α-amino levulinic acid, the precursor of chlorophyll biosynthesis, (Stroev, 1989). In this respect, El-Gamal and Hammad (2003) found that both garlic and yeast extracts caused an increase in the concentration of photosynthetic pigments in leaves of cadmium polluted tomato plants, being in agreement with the present findings.

1- Total sugars:

It is obvious from Table (4) that all adopted concentrations of cadmium decrease the concentration of total sugars in leaves of soybean 'Giza 35'. The decrease in concentration of sugars got higher as the concentration of cadmium increased in irrigation water. The highest decrease of sugar concentration was detected at 200 ppm cadmium, being 40.4% less than the control. The negative effect of cadmium on total sugars level in leaves of soybean plants was previously reported by Fouda and Arafa (2002). Such effect might be attributed to the deleterious effect of cadmium on the photosynthestic pigments and consequently the inhibition induced in photosynthesis process which lead to reduction in formation of sugars within plant tissues (Burzynski, 1987 and Poskuta *et al.,* 1988).

It is realized from Table (4) that the application of yeast or garlic extract seems to alleviate the unfavourable effect of cadmium on concentration of total sugars in leaves of soybean 'Giza 35'. Similar results were also recorded by El-Gamal and Hammad (2003) on tomato plants.

2- Phytohormones:

Data presented in Table (4) clearly show that all assigned concentrations of cadmium decreased the concentration of IAA and GA_3 in soybean leaves. Whereas, the concentration of ABA was increased due to cadmium treatments. Worthy to note that the effect of cadmium on phytohormones got higher as the concentration of cadmium increased reached its maximum at the high used level of 200 ppm Cd in irrigation water. It is obvious that the application of yeast or garlic extract on soybean plants grown under stress of pollution with cadmium minimized the harmful effect of such heavy metal on the concentration of phytohormones in leaves of soybean 'Giza 35'.

3- Cadmium:

It is realized from Table (4) that the concentration of cadmium in leaves of cadmium polluted soybean plants was decidedly higher than that of control plants. Moreover increasing cadmium concentration in irrigation water increased gradually the concentration of cadmium in leaves of soybean 'Giza 35'.

These results are in accordance with those reported by El-Ghinbihi (2000) on common bean and by El-Nabarawy (2002) on spinch. Likewise, Fouda and Arafa (2002) conformed these findings on soybean plants.

Data also indicated that yeast and garlic extracts were able to decrease the harmful effect of cadmium treatment on cadmium accumulation in leaves of cadmium polluted soybean plants.

B- Chemical analysis of seeds:

Chemical analysis was done to determine the percentage of crude protein and total lipids as well as cadmium concentration (ppm) in seeds of treated and untreated plants of soybean 'Giza 35'. The results of these determinations are presented in Table (5).

1- Crude protein:

Data presented in Table (5) reveal that the percentage of crude protein in seeds of cadmium polluted soybean plants was decidedly lower than that in seeds of control plants. Moreover, increasing cadmium concentration in irrigation water decreased gradually the percentage of crude protein and the maximum decrease was recorded at the high used concentration of 200 ppm Cd. These results are in agreement with those recorded by El-Ghinbihi (2000) on common bean and by Fouda and Arafa (2002) on soybean.

Results in Table (5) also reveal that foliar application with yeast or garlic extract was sufficient for decreasing the harmful effect of cadmium and improve the percentage of crude protein in seeds of cadmium polluted soybean plants.

Table 5: The effect of yeast or garlic extract on crude protein %, lipids% and cadmium concentration (ppm) in seeds of soybean 'Giza 35', at harvest time, grown under stress of pollution with different levels of cadmium in the second season of 2011

Treatments	Crude protein %	Total lipids %	Cd (ppm)
Control (Tap water)	35.18	20.51	0.19
50 ppm Cd	33.92	20.51	8.84
100 ppm Cd	29.57	18.22	17.52
200 ppm Cd	24.18	14.61	26.29
50 ppm Cd + Yeast extract	34.97	21.84	3.72
100 ppm Cd + Yeast extract	33.64	20.63	9.45
200 ppm Cd + Yeast extract	30.15	18.44	15.83
50 ppm Cd + Garlic extract	34.62	21.69	4.06
100 ppm Cd + Garlic extract	32.98	20.38	9.75
200 ppm Cd + Garlic extract	29.73	18.15	16.14

1- Total lipids:

It is clear from Table (5) that all assigned concentrations of cadmium decreased the percentage of total lipids in seeds of cadmium polluted soybean plants. Worthy to note that the decrease in percentage of total lipids got higher as the concentration of cadmium increased in irrigation water.

It is obvious that foliar application with yeast or garlic extract seemed to alleviate the unfavourable effect of cadmium on the percentage of total lipids in seeds of soybean 'Giza 35'.

2- Cadmium:

It is noted from Table (5) that the accumulation of cadmium in seeds of cadmium polluted soybean plants was increased progressively with increasing cadmium concentration in irrigation water. It is clear that foliar application with active yeast extract (60 ml/L) or with garlic cloves extract (30 ml/L) induced prominent decrease in cadmium concentration in seeds of cadmium polluted soybean plants.

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