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AMELIORATION OF LEAD EFFECTS ON *Phaseolus vulgaris* YIELD, YIELD COMPONENTS AND CHEMICAL CONSTITUENTS OF DRY SEEDS BY SOME BIO-TREATMENTS

BY

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ABSTRACT

The effect of some biotreatments i.e.; spraying with yeast (*Saccharomyces cerevisiae*) Y (0.4 g/l), adenosine triphosphate ATP (20 ppm) and vitamin C V (200 ppm) have been investigated on the model polluted dry bean plants. The model pollution of (10 ppm) lead acetate Pb effect has been studied on the growth, dry seed yield and yield components of dry bean (*Phaseolus vulgaris*) cv. Giza 6 seeds. The experiments were done in Mallawy Agricultural Research Station, Minia Governorate during two successive Nily seasons of 2001/02 and 2002/03. The content of some minerals, seed lead content, vitamin C amount, the phenolic content and the reducing power activity have been studied in the dry bean. The following results have been found:

1- The polluted control Pb plants showed unexpected increasing -compared to control- for number and weight of pods/plant, weight of seeds/plant, weight of 100 seeds and total seed yield (ton/fed). In dry seeds, the model of pollution Pb showed high content of Na, Fe, Mn compared to control. The reducing power –as indicator for the antioxidative activity- has been induced for the polluted plants Pb comparing to the control.

2- The biotreated plants showed increasing –compared to control- for number of branches/plant, number and weight of pods, weight of seeds/plant and weight of 100 seeds, and total seed yield (ton/fed). Increasing has been shown for the biotreated plants in Fe and Mn content compared to control. Treated plants with ATP increased the phenolic content (77.8%) and the reducing power (8.5%), while yeast Y increased the reducing power by 6.3%.

3- Spraying the biotreatments for the polluted plants –compared to the polluted plants Pb- increased plant height, number of branches/plant, number and weight of pods/plant, weight of seeds/plant and weight of 100 seeds, and total seed yield (ton/fed). The Y+Pb treatment increased the seed content of Na, Zn, Fe, Ca, Mg and Mn. All the biotreatments increased the phenolic content of the polluted plants Pb, while did not enhance the reducing power for the polluted plants Pb. The treatment ATP+Pb showed increasing (22.2%) for vitamin C content.

4- Pb didn't accumulate in seeds, moreover only plants treated with yeast for the polluted Y+Pb and nonpolluted Y plants have Pb content 8.5 and 2.0 ppm, respectively.

The authors would like to recommend spraying plants with vitamin C (200 ppm) or ATP (20 ppm) for enhancing the yield and seed quality of *P. vulgaris*. Whereas, using the yeast extract (0.4 g/l) showed Pb accumulation in seeds. So that, the researchers suggest that spraying vegetables with yeast extract should be minimized.

Key words: lead, pollute, *Phaseolus vulgaris*, reducing power, phenol, yeast, ATP, Vitamin C.

INTRODUCTION

In the last century, the levels of heavy metals have dramatically increased in the environment as a result of human industry activities. Heavy metals can damage many cellular components including proteins, membrane lipids and nucleic acids (Halliwell and Gutteridge, 1989). They affected also on various physiological and biochemical processes in plants. Heavy metals, including lead, are not essential for plant growth, but affect on plant metabolism. For the toxicological significance of lead, the acceptable lead level was reduced from 500to100 ng/g (Health and Human Services, 1994). Solliway *et al.* (1996) added that the mechanism of lead poison contributes to induction of oxidative stress, production of reactive oxygen species and decreasing the antioxidative defense systems.

Oxidative stress is known to be generated by a number of environmental factors as toxic metals and air pollutants. Rucinska *et al.* (1999) stated that at higher lead concentrations, the formation of both free radicals and reactive oxygen species is beyond the capacity of the antioxidant system, which in turn may contribute to the reduced root growth. The root system partially defends the above-ground parts from lead and other heavy metals. Deng *et al.* (2004) showed that metal accumulation by wetland plants differed among species, populations and tissues. Stefanov *et al.* (1993) pointed out that more than 90% of the lead incorporated was concentrated in *P. vulgaris* roots, whereas only 45% of the lead appeared in *Z. mays* roots and the remaining was in the leaves. Cvetanovska *et al.* (1998) found that Pb accumulation was greater in roots than in shoots. High Pb concentrations affected on the accumulation of essential elements. Hernandez *et al.* (2002a) found that *P. vulgaris* species had a similar capacity of Pb accumulation in shoots which occurred in concentrations proportional to the Pb content in the substrate.

Geebelen *et al.* (2002) mentioned that elevation of Pb-EDTA concentration reduced chlorophyll a and b content in leaves, visible effects on root morphology and shoot length and reduced the content of Ca, Fe, Mn and Zn taken up by plants, probably due to ion leakage as a result of observed toxicity. While, elevation of ethylene-diamine-tetra-acetic acid (EDTA) concentration increased, chelation of divalent cations resulted in reduced plant uptake of Zn, Cu, Fe and Mn.

Badiani *et al.* (1994) showed that the antioxidant variations were associated with the possible intermittent overproduction of partially reduced O_2

forms, whose formation could have been stimulated by natural daily changes in environmental factors likely to cause oxidative stress. Piechalak *et al.* (2002) published that the largest amount of lead was accumulated in the roots of *P. vulgaris*. This was composed of homophytochelatins as detoxicative-phytochelatin system.

Some bio-treatments showed in some studies to be valuable in vegetables growing. Yeast showed to be nutritional, protector and induce thermo-tolerance for vegetables under stress conditions (Spencer *et al.*, 1983). The yeast suspension contains hormones, sugars, amino acids, nucleic acids, protein, phospholipids, vitamins and minerals. Active dry yeast is high also in natural plant growth regulators such as cytokinins. The reducing agent vitamin C (L-ascorbic acid) has a role in scavenging free radicals in plant and animal. It also prevents many infections diseases (Secretin, 1974). On the other hand, Adenosin triphosphate (ATP) has a role in establishing thermo-tolerance for energizing the active metabolic ATP dependent process (Thomas *et al.*, 1999).

The aim of our work is to study the effect of lead acetate on the *P. vulgaris* growth, yield and Pb accumulation in seeds. The model of pollution also tested on other biological metals i.e. Zn, Ca, Fe, Mg, Mn, Na, and K for the food quantity and controlling heavy metals in plants by biotreatments. The phenolic content, vitamin C of *P. vulgaris* seeds and the reducing power as indicator for the antioxidative activity have been studied in the polluted and the treated plants. The biochemical treatments i.e. yeast, ATP and vitamin C as plant environmental stress adaptors, and inhibitors the polluted role of lead acetate have been studied in the various experiments.

MATERIALS AND METHODS

Two field experiments were conducted in Mallawy Agricultural Research Station, Minia Governorate during the two successive Nily seasons of 2001/2002 and 2002/2003 to study the influence of some treatments on growth, yield, yield components and some chemical constituents of *P. vulgaris* cv Giza 6. The physical and chemical properties of the experimental soil were mentioned (Table 1). Treatments were arranged in a randomized complete block design with three replicates. Each plot consists of three rows. The experimental plot area was 7.20 m^2, each row was 4.0 m long and 0.60 m wide. Seeds were sown at one side of the row spacing 7.5 cm apart, on September 3^{rd} 2001 and September 5^{th} 2002.

Table (1): The physical and chemical properties of the experimental soil

Properties	2001/02	2002/03
Organic matter	1.35	1.45
Available N%	0.90	0.95
Available K (ppm)	70.5	74.22
Available P (ppm)	5.4	5.65
E.C. (m moh/cm)	0.6	0.65

Texture: clay loam, soil samples were taken from 25 cm soil depth. E.C. electrical conductivity

Three replicates for each one in the eight treatments were investigated in our study as follows:

- Control plants spraying with water and without lead or biotreatments.
- Lead acetate Pb (10 ppm) was sprayed once –as a model for common pollution- after 30 days from sowing.
- Plants spraying with active dry yeast extract Y (0.4 g/l) after 30, 45 and 60 days from sowing.
- Plants spraying with vitamin C V (200 ppm) after 30, 45 and 60 days from sowing.
- Plants spraying with ATP (20 ppm) after 30, 45 and 60 days from sowing.
- Plants (Y+Pb) spraying with lead acetate (10 ppm) after 30 days from sowing, then the next day the same plants sprayed by yeast extract (0.4 g/l) and after 45 and 60 days from sowing.
- Plants (V+Pb) spraying with lead acetate (10 ppm) after 30 days from sowing, then the next day the same plants sprayed by vitamin C (200 ppm) and after 45 and 60 days from sowing.
- Plants (ATP+Pb) spraying with lead acetate (10 ppm) after 30 days from sowing, then the next day the same plants sprayed by ATP (20 ppm) and after 45 and 60 days from sowing.

Yeast preparation (Y):

Commercial soft yeast was grown and multiplied efficiently during conductive aerobic and nutritional conditions to produce devolve beneficial bioconstituents, i.e. carbohydrate, sugars, proteins, amino acids, fatty acids, hormones and etc. This procedure was modified by Shady (1970) and Spencer *et al.* (1983). Analysis of prepared yeast stock solution was as follows; total proteins (5.3%), total carbohydrate (4.7%) (AOAC, 1965), N (1.2%), P (0.13%), K (0.30%), Ca (0.02%), Mg (0.013%), Na (0.01%), Fe (0.13 ppm), Mn (0.07 ppm), Zn (0.04 ppm), Cu(0.04 ppm), B (0.016 ppm), Mo (0.001 ppm), Pb (2.0 ppm) (Nelson, 1944), IAA (0.5 mg/ml) and GA (0.3 mg/ml) (Vogel, 1975).

ATP:

Chemical agent adenosin triphosphate was produced from Chemical Gommhoria Co., Egypt in crystallized powder, and the stock solution was kept at 4 °C.

Vitamin C (L-Ascorbic acid): was freshly prepared at 200 ppm concentration.

The common cultural practices for *P. vulgaris* L. production were followed. Ten plants from every plot at 80 days after sowing were taken randomly to determine plant height and number of branches/plant. At harvest time, twenty plants were taken randomly from each plot to determine number, weight of pods and weight of seeds per plant. Weight of 100 seeds and dry seed yield per plot was calculated and converted to ton per feddan (ton/fed) [Plot=1/500 fed].

Determination of the mineral content:

The content of potassium, calcium and magnesium (as macro elements) and zinc, manganese and iron (as micro elements) and sodium (as trace elements) have been measured as ppm in the dry seeds. Lead was measured according to the method reported by Allen *et al.* (1997) using inductively coupled plasma (400) emission spectrometry. Lead content has been measured as well in yeast for the result requirement. The minerals have been determined using Atomic Absorption Spectrophotometer at Chemical Analysis Laboratory, Agricultural Research Center, Giza according to the method of Nation and Robinson (1971).

Preparation the extracts:

The fresh dry seeds from each treatment were fine crushed. From each powder group, 100 g were dissolved in 1 liter of methanolic HCl. The solutions were concentrated by rotary evaporator at 40°C to 5 ml, and kept at 4°C for further determinations.

Determination of total phenolic compounds (TPC):

The phenolic compounds concentration was measured according to the method of Taga *et al.* (1984) and calculated using tannic acid and gallic acid as standards. The dry samples for different treatments in methanolic HCl (0.05 ml) were added to 2 ml 2% Na_2CO_3. After 2 min, 50% Folin-Ciocalteu reagent (0.1 ml) was added to the mixture, then it was left for 30 min. Absorbance at 750 nm was measured using a spectrophotometer. Results are the mean of three samples, were expressed as grams percentage of tannic acid or gallic acid equivalent per 100 g dry weight.

Determination of vitamin C:

Determination of L-ascorbic acid concentration in the methanolic extracts has been performed using the indophenol method. 2,6-dichlorophenol indophenol (50 mg) in 250 ml destilled water was used as described by Mondy and Ponnampalam (1986). The mean values were reported from three replicates.

Reducing power:-

The reducing power of treatments was determined according to the method of Oyaizu (1986), with some modifications. Extracts (0.05 ml) in distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion of trichloro acetic acid (2.5 ml, 10%) was added to the mixture, which was then centrifuged at 300 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1 %), and the absorbance of three replicates were measured at 450 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Statistical analysis:

The data were statistical analyzed for variance procedure among the means of the two seasons and three replicates for treatment means using LSD test as described by Gomez and Gomez (1984) using SPSS package. Data of the mean values ± SD and the differences were considered significant at $P < 0.05$.

Concerning to the minerals, phenolic content, vitamin C content and reducing power, the means (average of two seasons) and standard deviation were calculated for all data (triplicates determination) according to the procedures of statistical analysis system (M-Stat).

RESULTS AND DISCUSSION

Plant growth:

Data in Table (2) show that spraying the plants with either of lead acetate at 10ppm as a model pollution Pb or with ATP (20ppm), vitamin C (200ppm) and active dry yeast (0.4g/l), in a single form insignifecantly decreased the plant height compared with the control during both seasons of growth. However, spraying the polluted plants with vitamin (V+Pb) or yeast (Y+Pb) significantly increased plant height compared with the polluted Pb and the control treatment. Xian (1989) found that crop growth was restricted in polluted compared with non-polluted soils. In this respect, ATP recorded the lowest values in both seasons of growth.

Mean values of the number of branches per plant showed decrease for the polluted plants Pb compared to the control and other treatments. Sprayed the polluted plants with biotreatments increased the number of branches compared to the polluted plants Pb. Vitamin C has the highest branches/plant in the two seasons, followed by the yeast Y. Fathy and Farid (1996) mentioned that spraying with yeast extracts improved growth and yield of common bean (*P. vulgaris*).

Table (2): Effect of spraying the polluted and nonpolluted plants with some treatments on vegetation growth of *P. vulgaris* cv. Giza 6 in Nily 2001/02 and 2002/03

Treatments	Plant height (cm)		No.branches/plant		No.pods/plant		Weight pods/ Plant (g)	
	2001/02	2002/03	2001/02	2002/03	2001/02	2002/03	2001/02	2002/03
Cont.	41.6	42.17	5.77	5.67	11.0	11.01	21.07	23.72
Cont. /Pb	41.07	40.84	5.23	5.18	12.44	11.14	28.97	29.08
ATP	37.28	38.7	5.44	6.34	14.63	14.0	26.18	26.34
ATP /Pb	45.31	45.77	6.34	5.34	13.78	13.87	30.38	27.26
Yeast	40.85	42.04	6.74	7.44	13.73	13.84	33.17	29.37
Yeats /Pb	49.86	49.13	6.37	6.13	14.71	13.87	31.72	31.99
Vit.C	40.84	42.13	7.5	7.72	14.9	15.33	37.67	37.27
Vit.C /Pb	48.5	48.71	6.61	6.58	14.71	14.71	32.37	29.83
LSD 5%	4.38	3.619	1.104	1.868	2.391	1.561	4.419	6.991

Number and weight of pods per plant:

The values tended to increase by the model pollution Pb compared to control (Table 2). All biotreatments exhibited significant increase for the number of pods per plant over the control in both seasons and over Pb in the second season. The treatments increased significantly the weight of pods per plant compared to the control in the first season, and by vit.C an Y+Pb in the second season. The highest mean value of the weight of pods per plant was observed with the vitamin C treatment in both seasons. Similar results were reported by Abdel

Naem *et al.* (2002) for tomato plants. They showed that various treatments as well exhibited significant increased of the tomato fruits weight per plant. In agreement, Shalata and Neumann (2001) reported that vit.C increased the resistance to salt stress and reduce lipid peroxidation.

Weight of seeds/plant and of 100 seeds:

Polluted plants Pb increased weight of seeds/plant, 100 seeds in both seasons compared to control. Weight of seeds/plant values for the treatments have significantly differences as in Table (3). Vitamin C V has the highest value in the two measurements in both seasons. Vitamin C significantly increased the weight of seeds per plant compared to control. Spraying the polluted plants with the biotreatments enhanced the seed weight comparing to polluted plants Pb. All of the biotreatments significantly increased the weight of 100 seeds comparing to control. In agreement, Fathy and Farid (2000) showed similar result. Spraying the polluted plants with ATP (ATP+Pb) or yeast (Y+Pb) significantly increased the values compared to the polluted plants Pb for weight of 100 seeds.

Total seed yield:

All treatments improved the yield, but yeast and vitamin C (24.4 and 48.7%, respectively) significantly increased the total yield in the second season comparing to control. Vitamin C V was the highest in both seasons (51.5 and 48.7%, respectively) comparing to control (Table 3). In agreement, Youssef (2000) stated that antioxidant substances improved the potato yield and quality. Abdel Naem *et al.* (2002) reported that ATP (20ppm) and vit.C (200ppm) increased tomato fruit number/plant, fruit weight and yield ton/fed.

Spraying the polluted plants with the tested treatments increased the yield comparing to the polluted plants P. The reason may due to high Zn content in control (Table 4). In agreement, Chaoui *et al.* (1997) found that bean treated for 96 h with either 5 μM Cd or 100 μM Zn showed reduction in the growth. Hernandez *et al.* (2002b) found that at 200 ppm Pb increased the biomass production, chlorophyll concentration and the total protein content in all plants. However, at 600 ppm Pb biomass production and chlorophyll content decreased to levels below the control. In agreement, total yield (ton/fed.) of seeds value –in our study- has been increased at 10 ppm Pb. Vitamin C V+Pb decreased the yield for the pollute plants compared to the unpolluted V. However, no significant difference has noticed between the yields of polluted treated groups.

It is worthy to say that unpolluted control had the lowest value in five growth measurements. In meanwhile, the polluted control induced higher number and weight of pods/plant, weight of seeds and total seed yield. On the other hand, vitamin C as reducing agent succeeded to show the highest values in the five from seven growth measurements. Mishra and Choudhuri (1998) mentioned that ascorbic acid alleviated the metal adverse effects on rice seedling growth.

Mineral content:

In polluted plants Pb, Pb did not accumulate in tested seeds (Table 4) but -as expected- accumulated in root (Stefanov *et al.*, 1993 & Cvetanovska *et al.*, 1998). The yeast treated polluted plants Y+Pb had Pb 8.5 ppm in seeds followed by the yeast Y treated plants (2,0 ppm). That's may be due to tight Pb binding sites in yeast (Marciniec *et al.*, 1989) or to the high content of Pb from 0.21 to 3.01 mg/kg in yeast (Cibulka *et al.*, 1992). In correlated, analysis was found that yeast contains 2 ppm lead. Moreover, the relative resistance of yeast species to various metallic and metalloid ions was proved (Berdicevsky *et al.*, 1993). For one of these reasons, yeast might encourage Pb accumulation in plant seeds. Pb high content increased Na, Fe, Ca and Mn contents in polluted dry bean seeds treated with yeast Y+Pb. While, sprayed the polluted plant with ATP A+Pb showed the highest Mg content.

Table (3): Effect of spraying the polluted and nonpolluted plants with some biotreatments on yield and yield components of *P. vulgaris* cv. Giza 6 in Nily 2001/02 and 2002/03

Treatment	Weight of seeds/plant (g)		Weight of 100 seeds (g)		Total seed yield (ton/fcd.)	
	2001/02	2002/03	2001/02	2002/03	2001/02	2002/03
Cont.	17.73	18.36	57.37	58.75	1.347	1.395
Cont. /Pb	19.63	21.13	59.69	59.45	1.492	1.606
ATP	20.57	20.77	60.99	60.83	1.563	1.578
ATP /Pb	22.98	21.47	61.78	61.65	1.746	1.627
Yeast	20.87	23.17	62.13	61.61	1.587	1.735
Yeats /Pb	23.89	24.45	61.2	61.3	1.816	1.858
Vit.C	26.85	27.11	62.08	61.75	2.041	2.075
Vit.C /Pb	24.85	23.7	60.88	59.7	1.889	1.802
LSD 5%	4.267	5.923	1.285	0.755	0.691	0.241

The importance economy was proved for snap bean's nutritional value. Calcium content –for instance- was in high value (Table 4) in agreement with Quintana *et al.* (1996). They stated that snap bean has a high Ca content reached 5 mg/g dry matter. On the other hand, polluted plant P showed -in the present study- the lowest Ca content.

Model pollution P (10 ppm) has decreased K, Ca, Mg and Zn contents, while increased Na, Fe and Mn contents (Table 4) comparing to control. Moreover, control showed the highest content in K and Zn comparing to the other groups. In agreement, Hardiman *et al.* (1984) stated that uptake of heavy metals was associated with a decrease in zinc content in plants.

Spraying plants with vitamin C V decreased the Na K, Mg and Zn content compared to control. Vitamin C –as well- is able to reduce redox-active metals such as Cu and Fe.

Comparing to control, spraying with ATP, yeast and vitamin C increased (Fe, Ca, Mn & Fe, Mg, Mn & Fe, Ca, Mn respectively). On the other hand, spraying the polluted plants with ATP, yeast or vitamin C increased (Zn, Ca, Mg & Na, Zn, Fe, Ca, Mg, Mn & Zn, Ca, Mg) respectively comparing to the polluted plants. Micro elements (Zn, Mn) which defined as those minerals recommended with daily intakes in mgs-tens of mgs range, proved to be in a small amount.

Table (4): The mineral content (ppm) in dry weight of seeds for the different treatments

Treatments	Pb	Na	K	Zn	Fe	Ca	Mg	Mn
Cont.	trace	520.2	**3244**	**19.5**	*45.25*	354	23.7	*7.0*
Cont.Pb	trace	583.5	3142	12.5	56.5	*278.75*	*11.5*	11.5
ATP	trace	496.2	3122	14.0	50.25	359	22.4	7.75
ATP.Pb	trace	5.401	2940	15.0	52.25	357.25	**28.9**	7.25
Yeast	**2.0**	*495*	*2783*	*12.25*	47.75	326.25	26.7	12.25
Yeast.Pb	**8.5**	**672.0**	2813	14.25	**57.75**	**392.5**	17.9	**13.75**
Vit.C	trace	509.0	2940	13.25	50.75	375	19.5	11.5
VitC.Pb	trace	560.2	3093	14.25	47.75	387.5	13.7	7.75

Phenolic content:

The methanolic seed extracts have been investigated for % of total phenolic content measured as tannic and as gallic acid (Figure 1). Decreasing the phenolic content for control treated with lead Pb (0.04, 1.0 g/100g as tannin and gallic respectively) has been shown. ATP treated plants showed increasing phenolic contents (0.16, 4.0 g/100g as tannic and gallic respectively) 77.8% comparing to control (0.09, 2.4 g/100g as tannic and gallic respectively), following by treating plant with vitamin C V (0.089, 2.3 g/100g as tannic and gallic respectively) then with yeast Y (0.08, 2.1 g/100g as tannic and gallic respectively). In this respect, Abdel Naem *at al.* (2002) found that ATP, yeast or vitamin C increased the tomato phenolic content.

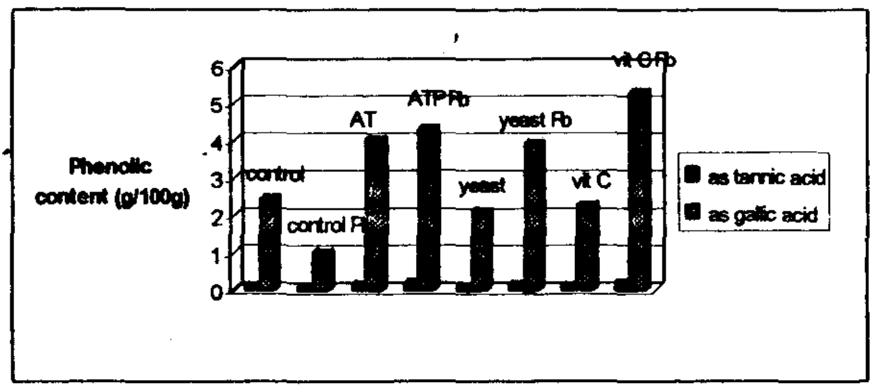

Figure (1): The phenolic content g/100 g dry seeds as Tannic acid and as Gallic acid for the different treatments. Each value is expressed as mean ± standard deviation (n=3).

Proteggente *et al.* (2002) stated that the antioxidant capacities of aqueous/ methanolic extracts were contributed from polyphenols, simple phenols and the ascorbate component. The role of decreasing the phenol content with increasing lead was studied by Stefanov *et al.* (1995). They pointed that lead is bound to non-lipid compounds, probably to proteins and phenols in leaves, pericarp and seeds.

Lead polluted plants P –except for control- induced phenol composition. Lead with vitamin C V+Pb (0.2, 5.3 g/100g as tannic and gallic respectively), followed by ATP+Pb (0.2, 4.3 g/100g as tannic and gallic respectively) then yeast Y+Pb (0.1, 3.9 g/100g as tannic and gallic respectively) increased the phenolic contents. This phenomenon has been noticed by Chaoui *et al.* (1997), who suggested that some antioxidant enzymes can be activated, notably in upper plant parts, in response to oxidative stress induced by Cd and Zn. Generally, we can say that these bio-treatments could ameliorate the phenolic content with this concentration of the polluted stress (10 ppm Pb).

Vitamin C content:

L-ascorbic acid (vitamin C) is the most powerful reducing agent found in plants, and showed an important role in scavenging free radicals. The treated lead samples showed decreasing the vitamin C (mg/lit) content than samples without lead in all of the seed methanolic extracts (Figure 2). Control and ATP (without lead) showed the highest vit. C (371.8 mg/lit). They were followed by vitamin C V treated, and polluted ATP A+Pb (314.6 mg/lit). Polluted ATP+Pb increased the vitamin content 22.2% compared to polluted control Pb. The lowest vitamin C contents (257.4 mg/lit) were that for control Pb, yeast Y+Pb, and vitamin C V+Pb (all treated with lead acetate).

Abdel Naem *et al.* (2002) found that vitamin C content for tomato sprayed with vitamin C was higher than spraying with ATP or yeast. Conklin *et al.* (1996) showed that ascorbate concentration effects on reactive oxygen species detoxification. They implicated the ascorbate role in defense against environmental stresses.

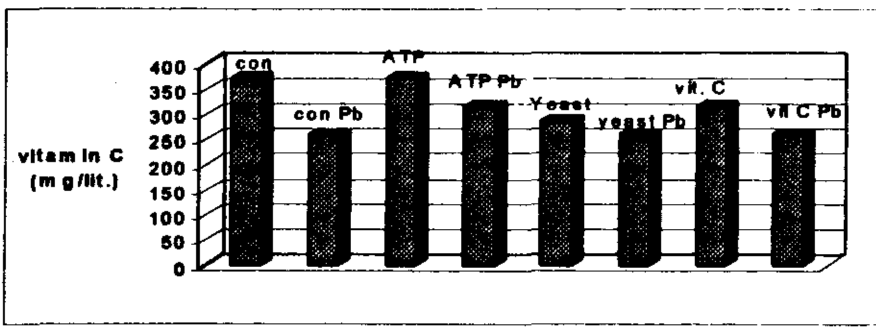

Figure (2): Vitamin C content (mg/lit) in dry weight. Each value is expressed as mean ± standard deviation (n=3).

Reducing power activity:

The reducing power for sample methanolic extracts was shown in Figure 3. The results indicated that treatments with ATP (1.162 absorbance), or yeast Y (1.138 abs.) increased the reducing power than control (1.071 abs.). On the other hand, treatment with vitamin C V (1.007 abs.) decreased the reducing power than control. The treatments with ATP or Y have higher reducing power 8.5 and 6.3% respectively. The extracts high in reducing power may produce products react as electron donor with free radicals to convert them to more stable products or terminate radical chain reaction (Yen and Chen, 1995).

On the other hand, treatments failed to increase the reducing power for the polluted plant Pb extract (1.186 abs.). The activities for vitamin C, yeast or ATP were 1.123, 1.087 and 1.082 respectively. The high activity for polluted control Pb by low Pb concentration (10ppm) might stimulate metabolic process. Patra *et al.* (2004) suggested forming metallothioneins and phytochelatins inside cells participate in excess metal storage and detoxification. When the system overloaded, oxidative stress defence mechanisms are activated.

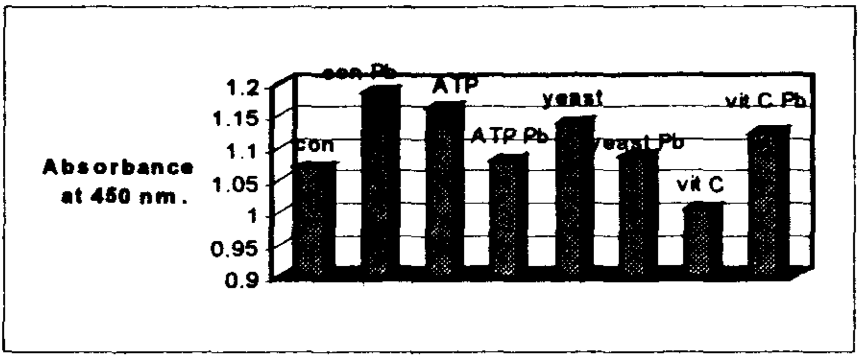

Figure (3): The reducing power for different methanol treatments extracts. Each value is expressed as mean ± standard deviation (n=3).

Either lead treated plants for control Pb or vitamin C V+Pb induced the reducing power compared to unpolluted ones. It's interesting to notice that increasing reducing power for treatments with polluted vitamin C V+Pb than V correlated with the phenolic content (Figure 1) in the phenolic content measurement. In agreement, Proteggente *et al.* (2002) found correlation between the FRA (Ferric Reducing Ability) and ORAC (Oxygen Radical Absorbance Capacity) assays with the total phenolic and vitamin C contents.

ATP (20ppm) increased both of the phenolic content and reducing power compared to control, suggesting for enhancing the seed quality. Especially, the Pb affinity to react with phosphate groups and active groups of ADP or ATP is a possible mechanism. In an explanation of the mechanism, Tiwari (2001) mentioned to the metal chelating activity for the plant flavonoids and their binding metal on their molecules. On the other hand, Stefanov *et al.* (1995) found that the radicals formed as a result of metal ion breakdown from treatment with lead acetate, decreased the activity of the antioxidative complex in pepper seeds.

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تحسين تأثيرات الرصاص (على ناتج محصول الفاصوليا والمكونات الكيميائية للبذور الجافة) ببعض المعاملات الحيوية

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لقد تمت دراسة تأثير الرش بالأدينوزين تراى فوسفات وفيتامين ج (٢٠ و ٢٠٠ جزء/المليون على التوالى) والخميرة (٤و٠ جم/لتر) على نباتات الفاصوليا جيزة ٦ (خلال الموسمين ٢٠٠٢-٢٠٠٣) بمحطة البحوث الزراعية بملوى. وكذلك دراسة المعاملات الحيوية على النباتات المعاملة بخلات الرصاص (١٠ جزء/المليون) كنموذج للتلوث الطبيعى ووجدت النتائج كالتالى:

١- أظهرت النباتات المعاملة بالرصاص زيادة غير متوقعة -مقارنة بالكونترول غير المعامل- لعدد ووزن قرون النبات ووزن بذور النبات ووزن ١٠٠ بذرة والناتج الكلى (طن/الفدان). وفى تقدير المعادن زاد الصوديوم والحديد والمنجنيز فى وجود خلات الرصاص كمصدر للتلوث. وزادت كذلك القدرة المضادة للأكسدة لمستخلص البذور الملوثة عن الكونترول.

٢- المعاملات الحيوية للنباتات (الكونترول) -مقارنة بالكونترول غير المعامل- زادت عدد أفرع النبات وعدد ووزن قرون النبات ووزن بذور النبات ووزن ١٠٠ بذرة والناتج الكلى (طن/الفدان). المعاملات أيضا زادت من محتوى الحديد والمنجنيز. والمعاملة بالATP زاد المحتوى الفينولى(٧٧،٨ %) بينما زادت القدرة المضادة للأكسدة بالمعاملة بال ATP (٨،٥%) أوالخميرة (٦،٣%).

٣- المعاملات الحيوية للنباتات المعاملة بالرصاص حسنت وزادت طول النبات وعدد أفرع النبات وعدد ووزن قرون النبات ووزن بذور النبات ووزن ١٠٠ بذرة والناتج الكلى (طن/الفدان) (مقارنة بالمعاملة بالرصاص). المعاملة بالخميرة حسنت من محتوى البذور للصوديوم والزنك والحديد والكالسيوم والماغنسيوم والمنجنيز وكل المعاملات زادت من المحتوى الفينولى مقارنة بالنبات الملوث. المعاملة بال ATP زادت من محتوى فيتامين ج (٢٢،٢%). بينما لم تظهر المعاملات تحسن فى القدرة المضادة للأكسدة لمستخلصات النباتات الملوثة.

٤- لم يحدث تراكم للرصاص بالبذور عدا فى البذور المعاملة بالخميرة (٢ جزء فى المليون) وكذلك البذور الملوثة المعاملة بالخميرة (٨،٥ جزء فى المليون).

وبناء على هذه النتائج نوصى بأستخدام كلا من المعاملتين فيتامين ج (٢٠٠ جزء فى المليون) أوATP(٢٠ جزء فى المليون) وذلك لزيادة الأنتاج مع تحسين الجودة للبذور. فى حين أن المعاملة بمستخلص الخميرة (٤و٠ جم/لتر) أدت الى تراكم الرصاص ببذور النباتات.