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EFFECT OF ASCORBIC ACID, SALICYLIC ACID, YEAST EXTRACT, THYME OIL AND MYCORRHIZAL INOCULATION ON HEAVY METALS-AFFECTED OR SEWAGE SLUDGE – AMENDED SOYBEAN PLANTS.

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ABSTRACT

Heavy metals may be introduced in agricultural soil by many sources, one of which is sewage sludge application as a fertilizer. Heavy metals in polluted soil can negatively affect plant growth and productivity. Aiming at exploring means to alleviate heavy metals stress on soybean plant, the present study was performed with the objective to test the alleviating effect of ascorbic acid, salicylic acid, yeast extract, thyme oil as well as inoculation with arbuscular mycorrhizal fungi on soybean plants challenged with either cadmium, cobalt or sewage sludge. Results indicated that though all had beneficial effect manifested by maintaining yield and reducing its content from heavy metals as well as elevating stress-related metabolites, especially phenols, application of thyme oil or yeast extract and inoculation with arbuscular mycorrhizal fungi were the most effective and are recommended to be employed to mitigate heavy metals stress on soybean in heavy metals-affected soils.

INTRODUCTION

Huge quantities of waste water are produced worldide due to everexpanding urban population. As a result of waste water treatment, sewage sludge (SS) is generated. SS can be used agriculturally as a fertilizer (Ailincai *et al*., 2009; Arriagada *et al*., 2009; Ghanavati *et al*, 2012), which is considered as an environment-friendly mean of waste disposal (Oudeh *et al*., 2002). Nevertheless, sluge application on agricultual land may introduc potential contaminants such as heavy metals (HMs) (Arriagada *et al*., 2009).
The presence of (HMs) in the atmosphere, soils and water may lead to bioaccumulation of the heavy metals in the food chain which can be highly dangerous and cause serious biological problems to all living organisms. These adverse effects on legume plants were reported for Cd (Sandalio *et al*., 2001; Chen *et al*., 2003) and Co (Jayakumar, 2007; Jayakumar *et al*., 2009 a, b). Moreover, HMs can negatively affect the symbiotic relationship of the plant with arbuscular mycorrhizal fungi (Arriagada *et al*., 2009; Ghanavati *et al*, 2012).

Looking for ways and means to improve crop production from HMs - stressed plants, many attempts were investigated either through the application of AOs (Yamaguchi *et al*., 1999; Drazic and Mihailovic, 2005) or inoculation with the AMF (Repetto *et al*., 2003; Lins *et al*., 2006).
There is an evidence that AsA plays an important role in the removal of ROS toxicity (Conklin, 2001) in addition to its roles in plant growth and development. So, it has been employed to alleviate HMs -induced toxicity in some plant species (Guo *et al*., 2005). Significant roles for antagonizing stress effects on plants were also ascribed to SA (Pal *et al*., 2002; Drazic and Mihailovic, 2005).

Salycilic acid (SA) is considered as a hormone-like substance, which plays an important role in a number of key plant physiological processes (Hayat *et al*., 2007). In addition, it provides protection against biotic and a biotic stresses (Joseph and Sujatha, 2010). SA was reported to induce salt tolerance in many plant species (Stevens *et al*. 2006; Gunes *et al*., 2007).

Essential oils are secondary plant metabolites that have been recently employed to modulate some aspects of plant biology. They have been employed to induce plant biotic stress resistance (Ahmad *et al*., 2011; Hong *et al*., 2011; Pereira *et al*., 2012). And to mitigate peroxynitrite-induced oxidative stress (Prieto *et al*., 2007).

Yeast extract is considered a rich source of phytohormones (especially cytokinins), vitamins, enzymes, amino acids, and minerals (Khedr and Farid, 2000; Mahmoud, 2001). Consequently, it was utilized to enhance growth and production of some plant species (El-Desuki and El-Greadly, 2006; El-Tohamy and El-Greadly, 2007;; Mady, 2009; Nassar *et al*., 2001).
Arbuscular mycorrhizal fungi (AMF) have been implicated in many aspects of plant-heavy metals interactions. Inoculation with AMF was employed to reduce toxicity of elevated Zn concentrations (Andrade *et al*., 2009) and to decrease cadmium toxicity by its immobilization in soil (Janouskova *et al*., 2006). In SS-amended soil, AMF colonization was beneficial in reducing heavy metals concentrations in SS-affected plants (Abdel-Aziz *et al*., 1997) and had the potential to modify the risks for plants from metal in sludges (Oudeh *et al*., 2002). In addition, inoculation with AMF is advocated in SS-amended soil as natural mycorrhization was reported to be suppressed (Arriagada *et al*., 2009; Ghanavati *et al*, 2012).

The objective of the present study was to assess the effects of AsA, SA, yeast extract, and thyme oil as well as inoculation with AMF on yield and certain biochemical constituents of soybean plants fertilized with SS or affected by Cd or Co.

MATERIALS AND METHODS

A field experiment was carried out at the Agricultural Research Station, Fac.
Agric., Mansoura University during 2011 season. Soybean Seeds (cv. Giza 22) were kindly supplied by the Plant Breeding Section , Field Crops research Institute, ARC , Ministry of Agric. , Giza, Egypt. were used in this experiment Seeds were presoaked in solutions of either salicylic acid (SA), 300 mg/l ; ascorbic acid (AsA), 300 mg/l ; yeast extract , 100 mg/l or thyme oil , 3 ml/l and cultivated on May 16th after inoculation with ryzobium <u>*Glomus macrocarpium*</u> in the experimental soil of which the main mechanical and chemical analyses are shown in Table (1), and to which Cobalt (as Cobalt Chloride –hexa-hydrate), Cadmium (as Cadmium Sulphate) , and Sewage sludge were added at the rate of 0.2 gm kg^{-1} soil, 0.1 gm kg^{-1} soil, and 21.5 gm kg^{-1} soil, respectively. The analysis of the applied sewage sludge is shown in Table (2). After seedling establishment, mycorrhizal inoculums, A pure culture of the AMF *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe,

was injected in the soil in the root region at the rate of 5 ml per plant providing spores ml^{-1} . Plants were sprayed two times, 20 and 50 days after sowing, with the same applied antioxidants and at the same rates. Using automatic atomizers after adding Tween 20 (v/v) as a wetting agent at 0.05 % . All the normal cultural practices were applied as recommended by the Ministry of Agriculture for growing soybeans .

Recorded parameters

Certain stress-related biochemical constituents as well as yield and its quality , as evidenced by its content from heavy metals were determined as follows:

Photosynthetic Pigments

Determined in the terminal leaflet of the 4^{th} leaf from plant top 80 and 140 DAS. Fresh leaf samples (0.5 gm) were extracted by methanal for 24 h at laboratory temperature after adding traces from sodium carbonate (Robinson *et al.*,1983), then total chlorophylls and carotenoids were determined spectrophotometrically and calculated by the equations introduced by Mackinny (1941).

Total ascorbate determination:

Total ascorbate content was determined 80 DAS. 0.5 g of leaf was ground in 50 ml of 2% (w/v) metaphosphoric acid using mortar and pastel and centrifuged for 30 min at 13 000 rpm at 4°C. The ascorbate content (µ.mol / g FW) was measured in the supernatant at 25°C . The absorbance of the red color was measured at 520 nm (Omaye *et al.,* 1979).

Total phenols determination:

Total phenols were determined 80 DAS.1g of dry of ground leaves was macerated in 10 ml 80% ethanol for at least 24 hours at 0°C, the alcohol was clarified, the remained residue was re-extracted with 10 ml 80% ethanol 3 times. At the end, the clarified extract was completed to 50 ml using 80% ethanol. The colorimetric method of Folin-Denis as described by Daniel and George (1972) was employed for the determination of phenolic compounds .Quantities were determined by reading the developed blue color at 725 nm. Using 0.5 ml 80% ethanol and reagents only as a blank (Daniel and Georg,1972 and A.O.A.C.,1967).

Table (1). Mechanical and chemical analysis of the used soil.

Coarse sand %	11.3
Fine sand %	27.4
Silt %	26.1
Clay %	32.5
$CaCO_3$ %	2.8
Organic matter %	2.0
Total N%	0.11
Available P ppm	14
Exchangeable K ppm	214
TSS %	0.21
Available Cd ppm	0.74
Available Cr ppm	46.0

*** Mechanical analysis followed the pipette method using sodium hydroxide as a dispersing agent (Piper, 1950). The contents of Cd, Cu and Pb were determined in DTPA (Diethylene triamine pentacetic acid) soil extract (1:2 w/v) by atomic absorption spectrophotometry. Other soil chemical analysis were carried out according to Jackson (1967).**

Table(2): The analysis of the used sewage sludge.

Sample	%
Organic matter	36.03
Carbon	20.59
Total N	1.05
Total P	0.442
Total K	0.47
SAMPLE	**PPM**
Available p	0.993
Available k	0.1130

Seed yield

At the end of experimental period (15 th October) , pods were collected to determine the seed yield.

Cobalt and Cadmium content in the plant and seeds :

400 mg of dried powdered plant material were wet digested in a mixture (3 ml) of supra-pure nitric and perchloric acids (2:1V/V.) on a hot plate (120 c) up to complete digestion (Cascato *et al* .,1997). eavy metals were determined by atomic absorption spectrophotometry (Perkin-Elmer 2380.

RESULTS

1- Chemical constituents :

1-1-Total chlorophylls :

Data presented in table (3) show the effect of applied stress alleviators (SAs) to heavy metals (HMs)- treated or sludge (SS)-amended soybean plants on total chlorophylls content in the leaves .As evident from the data at both sampling dates that SAs increased while HMs and / or their combinations with SAs decreased total chlorophylls content. SS slightly decreased this parameter

Table (3) : Effect of some heavy metals (Cobalt , Cadmium or Sewage sludge) and antioxidants (ASA , SA , Yeast ext. , thyme oil or Myco.) as well as their combinations on total chlorophyll (mg/gm fresh weight) of soybean plant at 80 and 140 days from sawing.

Groups / Treatment	Control	%	Cobalt	%	Cadmium	%	Sewage sludge	%	Mean	%
First sampling date(80 days from sowing)										
Control	10.9	100	6.9	63	7.4	68	10.2	94	8.9	100
Ascorbic acid	11.1	102	10.2	94	10.2	94	11.1	102	10.7	120
Salicylic acid	11.6	106	8.6	79	7.5	69	10.5	96	9.6	108
Yeast extract	11.1	102	8.7	80	11.7	107	10.7	98	10.6	119
Thyme oil	11.7	107	10.2	94	8.6	79	10.8	99	10.3	117
Myco.	11.8	108	11.0	101	7.6	70	11.7	107	10.5	119
Mean	11.4	100	9.3	81	8.8	77	10.8	95		
2nd sampling date(140 days from sowing)										
Control	16.9	100	11	65	10	59	15.6	92	13.4	100
Ascorbic acid	20.5	121	18	107	11.6	69	19.6	116	17.4	130
Salicylic acid	17.5	104	11.6	69	10.5	62	16	95	13.9	104
Yeast extract	17	101	16	95	11.6	69	15.07	89	14.9	111
Thyme oil	16.5	98	17.9	106	10	59	16.5	98	15.2	114
Myco.	16.5	98	16.3	96	11	65	14.6	86	14.6	109
Mean	17.5	100	15.1	86	10.8	62	16.2	93		

1-2-Total carotenoids :

Data presented in table (4) show the effect of applied SAs to HMs-treated or SS-amended soybean plants on total chlorophylls content in the leaves .Data show that SAs increased while HMs decreased total carotenoids content. The interaction treatments of AsA , SA and Yeast extract with Cadmium , Cobalt or Sewage sludge slightly increased total carotenoids content in soybean leaves especially in the second sampling data . So, it could be mentioned that applied SAs could alleviate the harmful effect of heavy metals on carotenoids contents. .

Table (4) : Effect of some heavy metals(Cobalt , Cadmium or Sewage sludge)and antioxidants (ASA , SA , Yeast ext. , thyme oil or Myco.) as well as their combinations on carotein (mg/gm fresh weight) of soybean plant at 80 and 140 days from sawing.

Groups / Treatment	Control	%	Cobalt	%	Cadmium	%	Sewage sludge	%	Mean	%
First sampling date(80 days from sowing)										
Control	1.3	100	1.1	85	0.8	58	1.2	92	1.1	100
Ascorbic acid	1.2	92	1.2	91	1.2	92	1.6	121	1.3	118
Salicylic acid	1.1	82	1.2	92	1.1	85	1.2	95	1.2	106
Yeast extract	1.6	121	1.3	97	1.2	95	1.4	108	1.4	125
Thyme oil	1.1	85	1.3	103	0.9	68	1.2	92	1.1	104
Myco.	1.2	92	1.1	85	1.2	89	1.4	105	1.2	111
Mean	1.2	100	1.2	97	1.1	85	1.3	107		
2nd sampling date(140 days from sowing										
Control	0.39	100	0.31	80	0.40	103	0.27	69	0.34	100
Ascorbic acid	0.45	116	0.47	121	0.45	116	0.46	117	0.46	134
Salicylic acid	0.41	105	0.60	155	0.47	121	0.45	116	0.48	142
Yeast extract	0.45	116	0.40	103	0.46	119	0.37	95	0.42	123
Thyme oil	0.37	95	0.36	93	0.47	121	0.39	100	0.40	116
Myco.	0.52	134	0.27	68	0.31	80	0.37	94	0.37	107
Mean	0.43	100	0.40	93	0.43	99	0.38	89		

1-3-Total ascorbic acid :

Data presented in table (5) show the effect of applied SAs to HMs-treated or SS-amended soybean plants on total AsA content in the leaves .
The data show that heavy metals treatments as well as SS application increased total AsA contents compared with control plants. Within SAs treatments, mycorrhizal inoculation treatment decreased total AsA content while the other treatments almost didn't affect this parameter.

Table (5) : Effect of some heavy metals (Cobalt , Cadmium or Sewage sludge)and antioxidants (ASA , SA , Yeast ext. , Thyme oil or Myco.) as well as their combinations on ascorbic acid content (mg/100gm f.wt) of soybean plant at 80 and 140 days from sawing.

Groups / Treatment	Control	%	Cobalt	%	Cadmium	%	Sewage sludge	%	Mean	%
Control	46.2	100	61.5	133	69.2	166	61.4	133	61.5	100
Ascorbic acid	61.5	133	46.2	100	69.2	166	76.9	166	65.4	106
Salicylic acid	46.2	100	61.5	133	69.2	166	61.5	133	61.5	100
Yeast extract	61.5	133	61.5	133	69.2	166	61.5	133	65.4	106
Thyme oil	46.2	100	76.9	166	69.2	100	76.9	166	61.6	100
Myco.	46.2	100	46.1	100	69.2	133	30.7	66	46.1	75
Mean	51.3	100	59.0	115	69.2	135	61.5	120		

1-4- Total phenol content

Data presented in table (6) show the effect of applied SAs to HMs-treated or SS-amended soybean plants on total phenols content in the leaves Data show that Cd and Co induced soybean plants to produce more phenols, and the effect was more pronounced in case of Cd. Fertlization with SS didn't affect phenols content. In addition, treatment with yeast extract or thyme oil enhanced plant total phenols content. When the interaction effects are considered, data show that all SAs increased total phenols in Cd- and Co-affected plants whereas only yeast extract and thyme oil increased this parameter in SS-affected plants. Yeast extract combined with any of cadmium or sewage sludge were more effective in this connection .

Table (6) : Effect of some heavy metals (Cobalt , Cadmium or Sewage sludge) and antioxidants (ASA , SA , Yeast ext. , thyme oil or Myco.) as well as their combinations on total phenol content (total phenol : mg/100g f.wt) of soybean plant at 80 and 140 days from sawing.

Groups / Treatment	Control	%	Cobalt	%	Cadmium	%	Sewage sludge	%	Mean	%
Control	0.097	100	0.110	114	0.119	123	0.108	112	0.113	100
Ascorbic acid	0.101	105	0.119	123	0.123	127	0.102	105	0.111	103
Salicylic acid	0.115	119	0.120	125	0.124	128	0.097	101	0.114	106
Yeast extract	0.114	118	0.128	133	0.138	143	0.144	149	0.131	121
Thyme oil	0.122	127	0.115	119	0.129	133	0.122	127	0.122	113
Myco.	0.112	116	0.119	124	0.133	138	0.109	113	0.118	110
Mean	0.110	100	0.119	108	0.128	116	0.114	103		

2- Pods yield and quality:

2-1- Yield

As data presented in table (7) indicate that the applied SAs increased pod yield of soybean. Thyme oil and Mycorrhiza were the most effective in this respect. On the other hand, Co decreased whereas Cd and SS didn't affect pod yield. The interaction data indicate that Thyme oil and Mycorrhiza were the most effective in mitigating the harmful effect of heavy metals on soybean yield .

Table (7) : Effect of some heavy metals (Cobalt , Cadmium or Sewage sludge) and antioxidants (ASA , SA , Yeast ext. , thyme oil or Myco.) as well as their combinations on pods yield weight (gm) /plant of soybean plant.

First sampling date(140 days from sowing)					
Groups / Treatment	Control	Cobalt	Cadmium	Sewage sludge	Mean
Control	226	148	217	205	199
Ascorbic acid	261	175	279	250	241
Salicylic acid	243	225	256	278	251
Yeast extract	300	243	254	216	253
Thyme oil	301	357	319	343	330
Myco.	309	324	330	342	326
Mean	273	245	276	272	
LSD at 5%	Anti : 17		HM: NS	Inter: 34	

2-2- Seed cadmium and cobalt content :

All applied SAs decreased cadmium content in the seed which was elevated as the result of the addition of Cd and Co as well as SS (Table 8). The least Cd content in the seed of Cd-trated plants was recorded in those treated with thyme oil. This was also true regarding seed Co content, but the treatment of yeast extract was the most effective in reducing Co content in the seeds of Co-treated plants (Table 9).

Table (8) : Effect of some heavy metals (Cobalt , Cadmium or Sewage sludge) and antioxidants (ASA , SA , Yeast ext. , thyme oil or Myco.) as well as their combinations on cadmium.

Groups / Treatment	Control	%	Cobalt	%	Cadmium	%	Sewage sludge	%	Mean	%
Control	0.1250	100	0.1250	100	0.6250	500	0.2500	200	0.2813	100
Ascorbic acid	0.0000	0	0.0000	0	0.3750	300	0.0000	0	0.0938	33
Salicylic acid	0.0000	0	0.0000	0	0.5000	400	0.0000	0	0.1250	44
Yeast extract	0.0000	0	0.1250	100	0.3750	300	0.0000	0	0.1250	44
Thyme oil	0.0000	0	0.1250	100	0.2500	200	0.0000	0	0.0938	33
Myco.	0.0000	0	0.0000	0	0.3750	300	0.0000	0	0.0938	33
Mean	0.0208	100	0.0625	300	0.4167	1996	0.0417	200		

Table (9) : Effect of some heavy metals (Cobalt , Cadmium or Sewage sludge) and antioxidants (ASA , SA , Yeast ext. , thyme oil or Myco.) as well as their combinations on Cobalt content (ppm) in seed oil of soybean plant .

Groups / Treatment	Control	%	Cobalt	%	Cadmium	%	Sewage sludge	%	Mean	%
Control	0.250	100	0.625	250	0.250	100	0.500	200	0.406	100
Ascorbic acid	0.125	50	0.375	150	0.000	0	0.000	0	0.125	31
Salicylic acid	0.125	50	0.250	100	0.125	50	0.375	150	0.219	54
Yeast extract	0.000	0	0.125	50	0.125	50	0.250	100	0.125	31
Thyme oil	0.125	50	0.500	200	0.250	100	0.125	50	0.250	62
Myco.	0.125	50	0.500	200	0.250	100	0.250	100	0.281	69
Mean	0.125	100	0.396	317	0.167	133	0.250	200		

DISCUSSION

In the present investigation, both Cd and Co affected negatively soybean plants growth (data not shown) and depressed plant yield concomitant with decreasing vital plant metabolites such as chlorophylls and carotenoids whereas induced the stress-related metabolites AsA and phenolics. Cadmium toxicity can easily cause oxidative stress in plant cells, because Cd can effectively trigger the synthesis/accumulation of reactive oxygen species (ROS),which can cause cellular damage or lipid peroxidation (Shah*et al*., 2001) and inhibit or promote the activities of antioxidant enzymes involved in the oxidative defense system depending on the level of stress imposed. A common response to Cd is an increased consumption of glutathione (Schützendübel *et al*., 2001;) for phytochelatins production which protec from heavy metals. The idea that Cd, and perhaps also other toxic metals, act in cells through a depletion of antioxidative defences is further supported by the observation that glutathione reductase, ascorbate peroxidase and catalase activities were inhibited at time scales similar to those found for the depletion of GSH .

Cadmium causes oxidative stress probably through indirect mechanisms such as interaction with the antioxidative defense system, disruption of the electron transport chain or induction of lipid peroxidation. The activation of lipoxygenase an enzyme that stimulates lipid peroxidation, has been reported after cadmium exposure [Smeets,etal,2005].

As an important component of the plant antioxidant defence system, AsA reduces H_2O_2 accumulation and lipid peroxidation, hence maintains membranes integrity and decreases electrolytes leakage under stress conditions (Guo *et al*., 2005). AsA -preventive role against HMs-induced electrolytes leakage may maintain the osmotic potential of plant cells, hence their expansion growth (Alaoui-Sossé *et al*., 2004). AsA may also protect chlorophyll against photoxidation and acts as a cofactor utilized in the xanthophylls cycle (Conklin, 2001). This cycle, in which violaxanthin is converted to zeaxanthin across the thylakoid membranes, is thought to be involved in non-photochemical quenching of excess light energy in photosystem II (Demmig-Adams and Adams, 1990)

There are many reports which show that SA can ameliorate the injurious effects of heavy metals on plants (Mishra and Choudhuri, 1999; Zhou *et al*., 2007, 2009). For example, exogenously applied SA ameliorated the adverse effects of Pb and Hg on cellular membranes in some rice cultivars (Mishra and Choudhuri, 1999). Popova *et al*. (2009) showed that exogenous application of salicylic acid can attenuate cadmium toxicity in pea seedlings. Such alleviating effect of SA was also examined in soybean seedlings grown under Cd stress (Drazic and Mihailovic, 2005). Protective effects of SA include up-regulation of anti-stress processes and recovery of growth processes after the stress is over (Sharikova*et al*., 2003). Several reports show that SA can induce antioxidant activity under multiple stresses such as UV, heat, and salt (Tissa *et al*., 2000). In addition, SA-induced protection of plants from oxidative injury caused by metals including Cd is mainly linked to enhanced accumulation of antioxidant enzymes (Wang *et al*.,2006). Guo *et al*. (2007) reported that exogenously applied SA improved rice Cd tolerance by accelerating the activities of enzymes involved in the antioxidant defense system. It has also been observed that alteration in photosynthesis with exogenous SA under environmental stresses could be due to either non-stomatal or stomatal factors (Dubey, 2005). SA may also maintain ionic homeostasis which is usually disturbed under HMs stress (Ramos *et al*., 2002).

Yeast extract is considered a rich source of phytohormones (especially cytokinins), vitamins, enzymes, amino acids, and minerals (Khedr and Farid, 2000; Mahmoud, 2001). So, application of yeast extract may compensate for vital growth factors which is usually depressed under HMs stress (Chaoui and El-Ferjani, 2005).

The beneficial role of thyme oil in attenuating HMs stress may be due to its antioxidant activity. In this context, thymol and carvacrol had the potential to prevent the formation of the reactive species, toxic products and to mitigate peroxynitrite-induced oxidative stress (Prieto *et al*., 2007).

Beneficial effects of AMF-inoculation to HMs-stressed plants were previously recorded (Medina *et al*., 2005; Ouziad *et al*., 2005). reported that mycorrhizal inoculation may play an important role in increasing metal tolerance of plants (Gaur and Adholey, 2004). AMF-colonized plants are more resistant to not only HMs-stress but also to other stresses caused by drought, salinity and pathogen’s attack (Ouziad *et al*., 2005). AMF-induced plant growth and HMs tolerance may be mediated through improving the supply of mineral nutrients of low mobility in the soil, particularly P (Vivas *et al*., 2003). Accoridng to Smith and Read (1997), AMF’s fine hyphae not only enhance the supply of mineral nutrients but also, effectively, mobilize water to host plants. The alleviation effects of AMF against HMs stress may also due to complex and not easily-resolved interactions between the symbiotic partners (Ouziad *et al*., 2005). Hence, AMF-induced alleviation of plant stress may also be founded on other aspects. According to Sanchez-Blanco *et al*., 2004), water-stressed Rosmarinus officinalis plants had higher leaf and stem water potentials, root hydraulic conductivity, and osmotic adjustment when inoculated with *G. deserticola* compared with non-inoculated plants. Moreover, according to Wu and Xia (2006) mycorrhization improved photosynthetic activity, stomatal conductance, photochemical efficiency of PSII, and enhanced chlorophyll content in plants under water stress compared to the non-mycorrhizal plants. Enhanced osmotic adjustment and photosynthesis were also founded the growth enhancement of *G. versiforme*–inoculated Citrus tangerine compared with non-incoulated plants growing under water stress conditions (Wu and Xia, 2006).

CONCLUSION

1- Natural substances such as thyme oil and yeast extract could be used as an economic, environment friendly method to alleviate plant heavy metals stress in heavy metals-affected soils and to reduce the accumulation of the metals in the economic, consumed plant organs.
2- Mycorrhization of large-scale cultivations should be developed to make possible seed inoculation with the mycorrhizal symbioant which will extend the use of Arbuscular mycorrhizal fungi for reducing HMs stress on economic plants.

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تأثير حامض الأسكوربيك، حامض السالسيلك، مستخلص الخميرة، زيت الزعتر و الميكوريزا على نباتات فول الصويا النامية تحت إجهاد العناصر الثقيلة أو المسمدة بالحمأة

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تلوث التربة بالعناصر الثقيلة يمكن أن يحدث من خلال العديد من المصادر ومن بينها إضافة الحمأة كسماد. زراعة النباتات فى التربة الملوثة بالعناصر الثقيلة يمكن أن يؤثر سلبا على نموها وإنتاجيتها. ونظرا لأهمية البحث عن وسائل لتقليل هذا الأثر السلبى على نبات فول الصويا كأحد المحاصيل الهامة فلقد أجريت هذة الدراسة بهدف تقييم الأثر المخفف لكل من حامض الأسكوربيك، حامض السالسيلك، مستخلص الخميرة، زيت الزعتر و الميكوريزا على نباتات فول الصويا النامية تحت إجهاد العناصر الثقيلة ، الكادميوم والكوبالت، أو المسمدة بالحمأة. ولقد أوضحت نتائج الدراسة أنة بالرغم من أن كل هذة المواد قد أدت غلى زيادة قدرة النباتات على تحمل الإجهاد الناشئ عن تأثرها بالكادميوم والكوبالت، متمثلا بصفة خاصة فى زيادة المحصول وتقليل محتواة من العناصر الثقيلة، وكذا فى زيادة المحتويات البيوكيماوية ذات العلاقة بتحمل الإجهاد، وبصفة خاصة الفينولات، فلقد كانت معاملات مستخلص الخميرة و زيت الزعتر و إضافة الميكوريزا هى الأفضل تأثيرا فى هذا الشأن ومن ثم فإنة يمكن التوصية بتطبيق هذة المعاملات عند زراعة فول الصويا فى الأراضى المتأثرة بالعناصر الثقيلة لزيادة قدرة النبات على تحمل الإجهاد.

قام بتحكيم البحث

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