World Applied Sciences Journal 22 (2): 171-180, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.22.02.2968

Impact of Biofertilizer *Trichoderma harzianum* Rifai and the Biomarker Changes in *Eruca Sativa* L. Plant Grown in Metal-Polluted Soils

Aisha M.H. Al-Rajhi

Biology Department, Faculty of Science,
Princess Nora Bent Abdularahman University, King Saudi Arabia (KSA)

Abstract: This study was designed to elucidate the role of *Trichoderma harzianum* Rifai as biofertilizer for improving the *Eruca sativa* plant growth in the presence of metals mixture. At the same time using the changes in plant metabolism as biomarker for evaluating the toxicity of metals on plant in the presence of these biofertilizers. *E. sativa* plants cultivated in soil inoculated with *T. harzianum* as a biofertilizer and were exposed to different concentrations (50, 100 and 200 ppm) of copper and zinc mixture as sulfate salts. The fatty acid composition of *Eruca sativa* could be used as an additional biomarker of soil contamination by metals mixture. Saturated (Palmitic and stearic acid) and unsaturated (oleic acid) fatty acid contents could be detected in the plant cultivated in soil at different treatments with *T. harzianum* in presence of different mixtures of copper and zinc concentrations, while other fatty acids were found to be affected by metals in the soil. Behenic acid was detected only at high concentration (100 and 200 ppm) in absence of *T. harzianum inoculation.* Stearic, arachidic and lignoceric acids could be detected at high concentrations with *T. harzianum* inoculation. Inoculation of *T. harzianum* in the soil improved the plant growth and minimizes the toxic effect of metals mixture. At the same time an increase in the content of chlorophyll (a) and (b) could be detected. Addition of *T. harzianum* to cultivated soil minimizes the absorbed amount of metals in plant, where the percentage of copper was 6.04 and 4.69% with uninoculation and inoculation of *T. harzianum*, respectively. On the other hand, copper and zinc mixture decreased the percentage of Mg^{+2} metal in plant and the addition of *T. harzianum* increased their percentage, where their percentage was 4.92% in presence of 100 ppm of metals mixture without *T. harzianum* and becoming 7.08% with *T. harzianum*. Exposure of *Eruca sativa* plant to high concentrations of metals mixture (100 ppm) without *T. harzianum* induced a significant increase in catalase and peroxidase enzymes activities.

Key words: *Trichoderma harzianum* · *Eruca sativa* · Biofertilizer · Biomarker changes · Metal- polluted soils

INTRODUCTION

Saudi Arabia is an arid country which is located at 16°N and 32°E. Climate is characterized by long, hot, dry summer and mild, cool and short winter. The agricultural lands of Saudi Arabia, which are coarse textured containing salts to varying degrees and mostly irrigated with saline groundwater, are not considered suitable for some of the commonly grown crops [1, 2]. Saline soils are wide-spread in Saudi Arabia. The dominant ions that contribute to salinity in these soils are Na^{+}, Ca^{2+} and Mg^{2+}) [1]. Growth activity and pathogenicity of the plant pathogenic fungi varied significantly under salinity stress [2]. *Eruca sativa* belongs to the Brassicaceae family grown as a minor oil crop and for the preparation of some traditional medicines and remedies [3]. Fatty acids composition in *Eruca sativa* oil was reported by Dorado *et al*. [4] and Chakrabarti and Ahmad [5]. A biomarker is defined as an observable or measurable change in an organism at a molecular, biochemical, cellular, physiological, or behavioral level related to its exposure to at least one chemical pollutant [6]. Marina *et al*. [7] stated that lipid biomarker might provide an early indication of a plant's exposure to metals and the potential bioavailability of metals and could facilitate or strengthen the diagnosis of soil contamination. Based on previous results reported by Nouairi *et al*. [8], it was anticipated that fatty acid composition might be a

Corresponding Author: Aisha M.H. Al-Rajhi, Biology Department, Faculty of Science,
Princess Nora Bent Abdularahman University, King Saudi Arabia (KSA).

significant target of heavy metal exposure. Although copper is an essential element for all living organisms as enzyme cofactor and key participant in several metabolic pathways, at elevated concentrations it becomes toxic [9]. An excess of this metal may lead to detrimental effects on photosynthesis, chlorophyll synthesis, fatty acid metabolism and carbohydrate synthesis [10, 11]. Many plants synthesizing and accumulating oxidized fatty acid derivatives in response to biotic or a biotic stress have been reported by Farmer and Davoine [12].

Remediation of water contaminated by heavy metals is an acute environmental and technological issue in many countries, especially those with a water deficit. Today, due to fast development of industry and production of different toxic compounds containing heavy metals, the environment surrounding these industries are heavily polluted and cause destruction of living ecosystem in these area [13]. Metal concentrations in soil range from less than one mg/kg (ppm) to high as hundred thousand mg/kg, whether due to the geological origin of the soil or as a result of human activity [14]. Excess concentrations of some heavy metals in soils such as Cu (II), Ni (II) and Zn (II) caused the disruption of natural terrestrial ecosystems [15]. Some heavy metals at low doses are essential micronutrients for plants, but in higher doses they may cause metabolic disorders and growth inhibition for most of the plants species [16]. In naturally polluted environments, the microbe's response to heavy metals toxicity depends on the concentration and the availability of metals and on the action of factors such as the type of metal, the nature of medium and microbial species [17]. Fungi and yeast biomasses are known to tolerate heavy metals [18-20]. They are a versatile group grow under various extreme conditions of pH, temperature and nutrient availability, as well as high metal concentrations [21]. They offer the advantage of having cell wall material which shows excellent metal-binding properties [22]. Generally, microbial biomasses have evolved various measures to respond to heavy metals stress via processes such as transport across the cell membrane, biosorption to cell walls, entrapment in extra-cellular capsules, as well as precipitation and transformation of metals [23]. Fungal tolerance towards a mixture of metals is of high importance both for fungal survival and their application for industrial purposes [24]. In a multicomponent system, soil microorganisms (*Pseudomonas putida, Trichoderma harzianum*) could compete effectively with soil minerals and solid organic matter to accumulate Zn, Cd and Hg [25, 26].

A number of plant-associated microbes are free-living and strongly beneficial to plants. A fungus in the genus *Trichoderma* [27] has evolved multiple mechanisms that result an improvements in plant resistance to disease and plant growth and productivity. *Trichoderma* spp. increases the uptake and concentration of a variety of nutrients (copper, phosphorus, iron, manganese and sodium) in roots in hydroponic culture, even under axenic conditions [28]. This uptake increase indicates an improvement in plant active-uptake mechanisms. *Trichoderma harzianum* T-22 and probably other *Trichoderma* spp. can solubilize various plant nutrients, such as rock phosphate, Cu, Mn and Zn, that can be unavailable to plants in certain soils. T-22 reduces oxidized metallic ions to increase their solubility and also produces siderophores that chelate iron [29]. Microbial interactions play very important roles in sustainable agriculture through the integrated nutrient supply. It is important to lay major emphasis on the application of efficient microbial inocula [30]. Beneficial interactions of these biofertilizers in both legumes and cereals have been reported by Saxena and Tilak [31] 1994. *Trichoderma* spp. increased growth of plants such as wheat cucumber, tomato radish and soybean [32, 33, 34]. Harman [35] reported that *T. harzianum* was as effective as a commercial rooting hormone in inducing rooting of plant such as tomato and potato. In both academic research and commercial practice, strain *T. harzianum* T-22 has been shown to increase root development in maize and numerous other plants. Adams *et al*. [36] stated that the cracl willow trees inoculated with *T. harzianum* T22 could be used to increase the rate of re-vegetation and phytostabilization of metal-polluted sites. Samplings grown with *T. harzianum* T22 produced shoots and roots that were 40% longer than those of the controls and shoots were 20% longer than those of samplings grown with ectomycorrhiza. Al-Rajhi [37] reported that the growth parameters including emergence, root depth, dry biomass of shoots and roots of wheat plants (*Triticum aestivum* L.) were significantly increased at all utilized treatments of the antagonist *Trichoderma harzianum* Rifai. Also, the infection rate by *Rhizoctonia solani* (AG8) was significantly decreased by using the different treatments of the antagonistic whereas pigment contents, soluble sugars, soluble proteins and free amino acids were significantly increased in the tested wheat cultivar. Proline accumulation by the host due to *Rhizoctonai solani* infection had a significant defensive role in wheat cultivar against the pathogen. Perveen and Bokhari [38] studied antagonistic activity of *Trichoderma*

harzianum and *Trichoderma viride* isolated from soil of ate palm field against *Fusarium oxysporum*. They reported that antagonistic interactions showed excellent activity of *T. viride* against *F. oxysporum in vitro* condition. According to Kabata-Pendias and Pendias [39], the elements capable of soil pollution are cadmium, copper, mercury, lead, zinc, chromium and nickel. These metals adsorb on the surface of soil profile and thus can accumulate in the plants. Plants respond to heavy metals toxicity in a variety of different ways, such responses include immobilization, exclusion, chelation and compartmentalization of metal ions [40].

The aim of the present study is to elucidate the role *T. harzianum* Rifai improving the *Eruca sativa* as biofertilizer for plant growth in the presence of metals mixture. At the same time using the changes in plant metabolism as biomarker for evaluating the toxicity of metals on plant in the presence of these biofertilizer.

MATERIALS AND METHODS

Seed Source: Seeds of rocket (*Eruca sativa* L.) used in these experiments were purchased from a local market. These were surface-sterilized with 96% ethanol for 1 min, washed, dipped in 0.1% $HgCl_2$ for 10 sec and then thoroughly washed with sterile distilled water according to the method described by El-Abyad *et al*. [41].

Soil Sampling: The region from which the soil sample was collected lies 17 km from Riyadh at khorais R.d., Saudi Arabia, Eastern region [37]. The soil used in the greenhouse experiments was collected from the top 6 inches of one of the rocket fields. This was brought to the laboratory where it was air-dried for 7 days, then sieved through a 2-mm sieve to be used in the experiments, the soil sample was analyzed chemically for the estimation of total soluble salts and organic matter contents, according to the method described by Johnson *et al*. [42]. Temperature, water content and pH-value were also estimated.

The Antagonist (Biofertilizer): *Trichoderma harzianum* Rifai used in this study was obtained from the culture collection of Microbial Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt [37]. The isolate of *T. harzianum* Rifai was maintained on potato dextrose agar medium at 27±1°C.

Polluted Metals: All polluted metals used in this experiments' of analytical grade and were purchased from Merck chemicals.

Greenhouse Experiment and Growth Conditions: These experiments were conducted in 13 cm diameter, 15 cm length round pots, each containing 1 kg autoclaved clay-sandy soil (2:1). Twenty seeds of *Eruca sativa* L. were sown in each pot and three pots were used for each treatment. The pots were kept in the greenhouse (temperature 27±3°C) for 22 days. Plants were irrigated every three days with 100 ml containing different concentrations of copper and zinc sulfate mixture (50, 100 and 200 ppm). At the same time, spore suspension (10^6/ml) of the biofertilizer *T. harzianum* was added to cultivated soil. Then, the plants were harvested and growth parameters were determined. Uninoculated or inoculated pots with spore suspension of the biofertilizer *T. harzianum* only were used as control.

Plant Growth Parameters: After 22 days from planting, the plants were removed, washed with tap water and the following measurements were made, root length (cm), root and shoot fresh weight (g).

Elemental Analysis: One gram of dry plant including shoot system was grinded and inoculated on scanning electron microscope grade and the percentage of metals content was measured with x ray (Scanning Electron Microscope JSM-500LV, with coated in SPi-MoDule. SPUTTER Coated).

Quantitative Determination of Chlorophylls and Antioxidant Enzymes: Chlorophyll content was determined according to Vernon and Seely [43] with using the following equations:

Chlorophyll a (mg/g tissue) = 11.63 (A665) – 2.39 (A649).

Chlorophyll b (mg/g tissue) = 2.11 (A649) – 5.18 (A665).

Where (A), denotes the reading of the optical density

Catalase (CAT) and Peroxidase activity was assayed according to the method of Kar and Mishra [44].

Fatty Acids Detection: The shoot samples (5g) were cut into small pieces and extracted twice with 65 ml chloroform-methanol (1:1 v/v). The extracts were combined and diluted with water until two layers were obtained. The lower layer was evaporated under vacuum and the obtained total lipophilic extracts were kept at -30°C. The concentrated extract was placed in GC auto sampler vials until they were analyzed. A Varian Star 3400 Cx Ion Trap GC/MS Shimadzu GC-MS-QP 5050 A. software class 5000. Searched library: Wiley 229

LIB.Column: DBI, 30m, 053 mm ID, 1.5 um film. Carrier gas: Helium (flow rate 1 ml/min.) Ionization mode: El (70 ev). Temperature program: 70°C (static for 2 min) then gradually increasing (at a rate of 2°C/min) up to 220°C (static for 5 min). Detector injector temperature 250°C. The chromatographs were compared and individual peaks were identified by comparing mass spectra to the library references.

Statistical Analysis: All data were subjected to statistical analyses according to the procedures reported by Snedecor and Cochran [45]. Treatments were compared by the least significant difference test (LSD) at both P<5% and P=1% levels of probability.

RESULTS

Physico-Chemical Characteristics of Soil Sample: During this investigation, the soil temperature at the time of sampling (Winter season of 2011) was 21°C, the pH value of the tested sample was 8.3, the water content was 52.1%, the total soluble salts was 1.9% and the organic matter content was 2.46. Growth of *Eruca sativa* at seedling stage under different treatments of soil inoculated with *T. harzianum* and amended wit different concentrations of copper and zinc mixture was studied. The length of *Eruca sativa* roots and shoot system increased in the soil inoculated with *T. harzianum* and amended with mixture of Cu and Zn particularly at 50 ppm (Table 1) but at high concentration (100 ppm) of metals mixture and in the presence of *T. harzianum* the root length was decreased unlike the fresh weight of roots, However, application of mixture of Cu and Zn concentration (50 and 100 ppm) in soil without *T. harzianum* reduced the length and fresh weight of shoot and root system. At the same time, inoculation of *T. harzianum* in the soil increased the content of chlorophyll (a) and (b) and were increased markedly when inoculated with *T. harzianum* in presence of low concentration of Cu and Zn mixture (50 ppm) but decreased with high concentration (100 ppm) and were more affected in the soil containing (100) without *T. harzianum*. Exposure of *Eruca sativa* plant to high concentrations of metals mixture (100 ppm) without *T. harzianum* induced a significant increase in enzyme activities catalase and peroxidae. On the other hand, the reduction in their activities was observed in plant cultivated in soil inoculated with *T. harzianum* and amended with high concentration of metal mixture (Table 2).

The saturated (palmitic and stearic) and unsaturated (oleic) fatty acids contents were detected in the plant cultivated in soil at different treatments with *T. harzianum* in presence of different concentrations of mixture of copper and zinc. The palmitic and stearic acid concentrations increased in plant cultivated in soil inoculated with *T. harzianum* (from 35.29 and 18.47% to 46.51 and 32.51%, respectively) but were decreased in plant cultivated in soil containing mixture of copper and zinc metals and uninoculated with *T. harzianum* (Table 3). On the other hand, the presence of *T. harzianum* with different concentrations of metals mixture in the soil having antagonistic effect on the percentage of oleic acid where their percentage was 64.11, 64.52 and 58.57% in plant cultivated in soil amended with different concentration of metals mixture, 12.92, 0.56 and 5.33% in

Table 1: Growth parameters of *Eruca sativa* plant cultivated in soil at different treatments

	Eruca sativa							
							LSD	
Growth parameter	Soil	Soil + T. *harzianum*	Soil + T. *harzianum* + 50 ppm Cu & Zn	Soil +T. *harzianum* +100 ppm Cu & Zn	Soil+50 ppm Cu & Zn	Soil+100 ppmCu & Zn	5%	1%
Root length (cm)	7.3	8.7	8.8	4.2	5.6	5.3	0.8	1.3
Root weight (g)	3.4	5.2	5.3	5.6	2.8	2.6	0.6	1.2
Shoot weight (g)	5.2	5.6	5.9	4.0	3.8	3.5	0.7	1.4

Table 2: Chlorophyll content (mg/g. fresh weight) and enzymes activity of *Eruca sativa* cultivated in soil at different treatments. Data expressed as the changes in the optical density/gram fresh weight/hr. for peroxidase and µ mol. H2O2/h/g fresh weight for catalase enzymes

	Chlorophyll and Enzyme of *Eruca sativa*							
							LSD	
Chlorophyll and Enzyme type	Soil	Soil + *T. harzianum*	Soil + *T. harzianum* + 50 ppm Cu & Zn	Soil + *T. harzianum*+ 100 ppm Cu & Zn	Soil+50 ppm Cu & Zn	Soil+100 ppmCu & Zn	5%	1%
Chlorophyll (a)	7.35	7.89	8.34	5.65	6.54	5.66	0.7	1.8
Chlorophyll (b)	2.32	2.37	2.98	1.5	1.62	1.90	0.8	1.9
Catalase	30.43	29.88	39.32	26.45	42.11	24.39	0.6	1.8
Peroxidase	0.076	0.071	0.089	0.053	0.034	0.032	0.5	1.7

Table 3: Fatty acids content of *Eruca sativa* cultivated in soil at different treatments

	Fatty acid (%) of *Eruca sativa*									
									LSD	
Fatty acid	Soil	Soil+ *T. harzianum*	Soil + *T. harzianum* +50ppm (Cu+Zn)	Soil + *T. harzianum* +100 ppm(Cu+Zn)	Soil + T. *harzianum*+ 200 ppm(Cu+Zn)	Soil+50 ppm(Cu+Zn)	Soil+100 ppm(Cu+Zn)	Soil +200 ppm(Cu+Zn)	5%	1%
Myristic	1.42	2.67	0.46	1.00	0.00	0.00	0.00	0.00	0.7	2.1
Pentadcanoic	0.00	1.05	0.00	0.00	0.00	0.00	0.00	0.00	0.8	1.3
Palmitic	35.29	41.27	40.00	46.51	27.52	28.34	25.19	29.40	0.9	1.5
Oleic	21.58	14.38	12.92	0.56	5.33	64.11	64.52	58.57	0.6	1.4
Stearic	18.47	26.55	24.59	21.90	32.51	4.96	6.57	7.97	0.4	1.1
Arachidic	5.90	2.96	4.67	4.76	2.78	0.00	0.00	0.00	0.7	1.8
Lignoceric	0.00	9.48	13.71	25.27	31.86	0.00	0.00	0.00	0.8	1.9
Linolic	2.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.6	1.8
Palmitooleic	2.11	0.00	0.84	0.00	0.00	0.00	0.00	0.00	0.5	1.7
Eicosenoic	0.00	0.00	0.00	0.00	0.00	2.58	0.00	0.00	0.9	2.0
Behenic	0.00	0.00	0.00	0.00	0.00	0.00	3.72	4.07	0.2	0.9
Unknown (R.t.18.545)	0.00	0.83	0.84	0.00	0.00	0.00	0.00	0.00	0.4	1.0
Unknown(R.t.24.305)	0.00	0.81	0.93	0.00	0.00	0.00	0.00	0.00	0.1	0.6
Unknown(R.t.17.985)	0.00	0.00	0.78	0.00	0.00	0.00	0.00	0.00	0.06	0.56
Unknown(R.t.28.267)	0.00	0.00	0.27	0.00	0.00	0.00	0.00	0.00	0.04	0.40

Rt= Retention time

Table 4: Effect of T. *harzianum* on the percentage of Cu & Zn in *Eruca sativa* cultivated in soil amended with different concentrations of their mixture

	Metal percentage % in *Eruca sativa*						
						LSD	
Metal type	Soil	Soil+50 ppmCu & Zn	Soil+100 ppmCu & Zn	Soil+50 ppmCu & Zn +*T. harzianum*	Soil+100 ppmCu & Zn + *T. harzianum*	5%	1%
Cu	4.01	6.04	6.10	4.69	5.88	0.5	1.2
Zn	2.14	2.57	2.50	1.90	4.41	1.4	2.5

Table 5: Percentage of metals in *Eruca sativa* cultivated in soil amended with different concentrations Cu & Zn mixture in presence of T. *harzianum*

	Metal percentage % in *Eruca sativa* cultivated in						
						LSD	
Metal Type	Soil	Soil+50 ppmCu & Zn	Soil+100 ppmCu & Zn	Soil+50 ppmCu & Zn +*T. harzianum*	Soil+100 ppmCu & Zn + *T. harzianum*	5%	1%
Mg	7.21	5.43	4.92	5.70	7.08	0.7	1.4
Si	5.70	6.74	6.46	7.25	6.78	0.6	1.5
S	11.96	9.88	12.14	10.27	9.97	0.8	1.3
Cl	14.99	15.52	15.63	18.48	18.51	0.5	1.4
K	22.56	20.51	30.80	22.32	22.37	1.2	2.5
Ca	31.42	33.28	19.58	28.40	23.68	1.0	2.7
Fe	0.00	0.0	1.84	0.97	1.30	1.3	2.4

plant cultivated in soil amended with different concentration of metals mixture and inoculated with *T. harzianum*, 21.58 and 14.38% in plant cultivated without metals in soil uninoculated and in soil inoculated with *T. harzianum* only, respectively. Arachidic and lignoceric fatty acids could not be detected in plant cultivated in soil uninoculated with *T. harzianum* and amended with different mixture concentrations. Behenic acid was detected at high concentration of metals mixture (100 and 200 ppm) without *T. harzianum* inoculation. At the same time addition of *T. harzianum* without metals mixture increased the percentage of myristic acid in plant compared with control (soil only) and in the case of metals mixture addition. Four undefined fatty acids with different retention time were detected only in the case of *T. harzianum* inoculation and at low concentration (50 ppm) of metals mixture.

In addition, *T. harzianum* of cultivated soil minimize the absorbed amount of metals mixture in plant, where the percentage of copper was 6.04 and 4.69% with uninoculated and inoculated soil with *T. harzianum* respectively. Also, the percentage of zinc decreased in plant in the case of *T. harzianum* inoculation particularly at low concentration (50 ppm) (Table 4 and Fig. 1). Addition of copper and zinc mixtures decreased the percentage of Mg^{+2} metal in plant but at the same time the addition of *T. harzianum* increased their percentage, where their percentage is 4.92% at 100 ppm of metals mixture without *T. harzianum* and becoming 7.08% with *T. harzianum* (Table 5 and Fig. 1). Generally the percentage of absorbed ions is affected by either *T. harzianum* or addition of copper and zinc mixtures to plant growth.

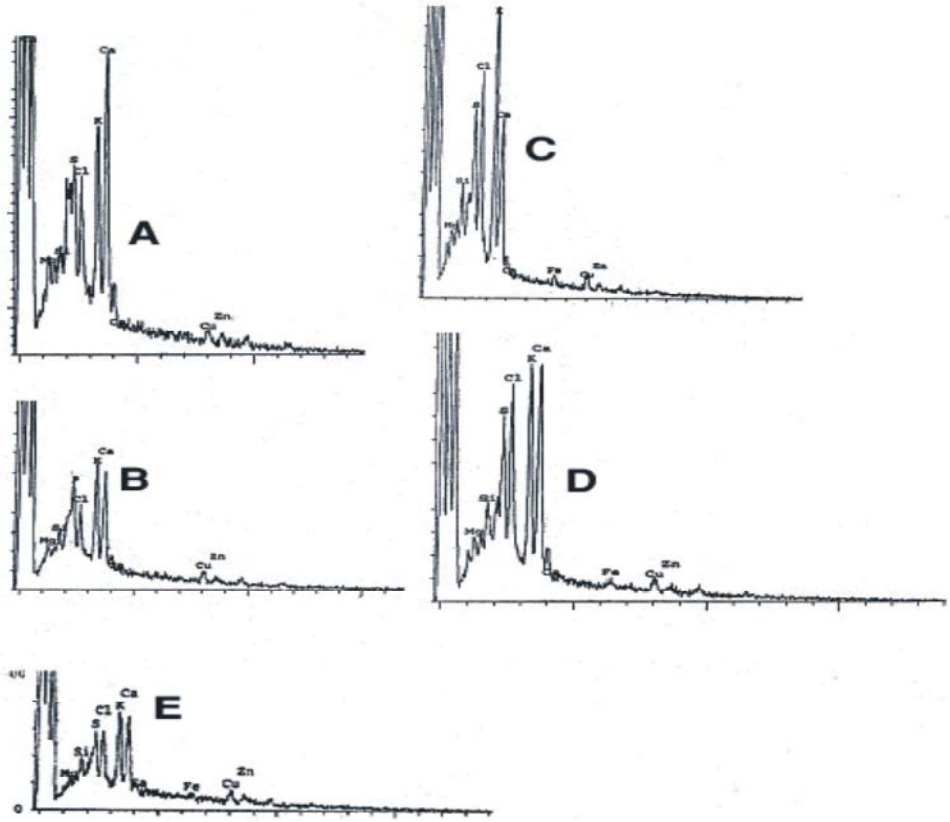

Fig. 1: Typical chromatogram of metals detected in *Eruca sativa* cultivated in (A) Soil uninoculated with *T. harzianum* and without mixture of Cu and Zn, (b) Soil with mixture of 50 ppm Cu and Zn, (C) Soil with mixture of 100 ppm Cu and Zn (D) Soil inoculated with *T. harzianum* with mixture of 50 pm Cu and Zn (E) Soil inoculated wit *T. harzianum* with mixtre of 100 ppm of Cu and Zn.

DISCUSSION

The physico-chemical characteristics of soil sample analyses were recorded by Al-Rajhi [37] and similar results were obtained by El-Nagdy [46]. The biofertilizer fungus *T. harzianum* improved growth and biomass of *Eruca sativa* cultivated in soil containing mixtures of different concentrations of copper and zinc. This beneficial soil fungus does not adversely affect the healthy root system of the plant. In this current study the inoculation of *T. harzianum* in the soil minimize the effect of copper and zinc mixtures of *Eruca sativa* growth. The result is in agreement with those obtained by Inmark [47] who stated that *T. harzianum* and other *Trichoderma* spp. could increase in yield of plant about 36% when compared to the control. The reasons why the yield could increase that can be explained by Suwan *et al*. [48] who reported that *T. harzianum* could elucidate to produce trichotoxins promoting plant. Also, Phuwiwat and Soytong [49, 50] and Soytong [51], explained that the high response for increasing the growth of Chinese radish due to the effective isolate of *T. harzianum* and *T. hamatum*. Those isolates were mixed to biological humus product.

Heavy metals are known as growth inhibitors and consequently exhibit toxicity symptoms in plants. The effect of different concentrations of copper and zinc mixture on seed germination, seedling growth and some metabolites of *Eruca sativa* plants was examined. A considerable reduction in fresh and dry matter as well as shoot and root length was obtained as a result of increasing metals concentration. Among these parameters, total root length was a more sensitive parameter than shoot length at every metals concentration. These results are in agreement with those reported by Faheed [52]. The present results demonstrate that high concentration of metals mixture decreased the content of chlorophyll a and b. Similar results with other metals were demonstrated by Kahle [53]. High concentration of selenium may inhibit photosynthesis, impair nutrient uptake and transport.

Similar results were also corresponded with those reported by Oncel *et al*. [54], who found that C (II) reduces the chlorophyll a and b in wheat.

Metal uptake by *Eruca sativa* in the present study is affected by soil inoculated with *T. harzianum* and the presence of copper and zinc mixtures, where the presence of *T. harzianum* minimize the uptake of copper and zinc, while increased the uptake of other metals such as magnesium and silicon. Magnesium increased the chlorophyll in plant. The minimization of copper and zinc uptake by plant (although the plant cultivated in soil contained high concentration of this metals), this phenomenon may explained to the ability of *T. harzianum* to accumulate these metals from the soil inside their cells and decreased their concentration in the soil. At the same time decreased the uptake of calcium. Although calcium in particular, plays many physiological roles such as signal transduction and the maintenance of cell wall and plasma membrane structural integrity. Also, plays a role in activating antioxidant enzymes [55]. Yedidia *et al*. [28] stated that *Trichoderma* spp increase the uptake and concentration of a variety of nutrients in roots of cucumber plants even under axenic conditions. *Trichoderma* spp probably have significant wide-scale uses in the remediation of pollutants in soils and water [56]. First, as has been noted earlier, highly rhizosphere competent strains of *Trichoderma*, such as *T. harzianum* strain T22, enhance root growth of a range of plants. This enhanced root growth, when combined with a plant that hyper-accumulates toxicants, will increase the volume of soil colonized by roots, including enhancement of deep root penetration [35]. Further, *Trichoderma* spp. on roots increase uptake of nitrates and other ions and may also increase uptake of various toxic metals and metalloids. Thus, it should assist in phytoextractive activities.

Catalse is an antioxidant that breaks up H_2O_2 to H_2O and O_2; catalase is a key enzyme which removes toxic peroxide [57]. In current study catalase and peroxidase activity was increased at 50 ppm of copper and zinc mixture compared with control (without metals mixture). On the other hand it decreased at 100 ppm this may be attributed to higher toxicity of metals on plant metabolism and at the same time, addition of *T. harzianum* increased their activity at 50 ppm compared with soil less *T. harzianum*. Hosseini *et al*. [58] reported the same results on *Brassica napus* plant but without *T. harzianum*. There also has been reported the induction of peroxidase activity on plants which are under stress of toxic metals such as Cu, Cd and Zn [59, 60].

Fatty acids and fatty acid-metabolites are not only major structural and metabolic constituents of the cell, but they also function as modulators of signal transduction pathways. The fatty acids of *Eruca sativa* were the most biomarker affected by soil colonized with *T. harzianum* and contained different concentrations of copper and zinc mixture, Yu *et al*. [61] stated that the differences in the fatty acids composition of lipids in birch were considered as an indicator of adverse effect of cadmium on the structure and functions of chloroplasts and therefore on photosynthesis. Generally in the present study, the fatty acids decreased in plant cultivated in soil containing high concentration of copper and zinc. Nicola *et al*. [62] indicated a relationship between metals accumulation (Zn and Cu) and the fatty acid composition of primary leaves in tomato. Other researches demonstrated that decrease of lipids was observed in Cd-stressed barley [63], tomato [64] and mustard [8] plants. Palmitic (C16:0), oleic (18:1) and stearic (18:0) acids were the major fatty acids in *Eruca sativa* cultivated in soil under different conditions variable with percentages. Their biosynthesis at all different condition expected as important role in plant metabolic pathways. In our studies, Myrestic, lignoceric and arachidic acid (20:1) were detected in plant cultivated in soil inoculated with *T. harzianum* and containing different concentrations of metals mixture, but not detected in plant cultivated in soil containing different concentrations of metals mixture only, this may be due to that *T. harzianum* minimize the effect of metals mixture on the biosynthesis of these fatty acids. Linoleic (C18:2) is the most detected fatty acid which affected by different concentrations of metals mixture till in the presence of *T. harzianum*. Unlike behenic and eicosenoic acid (20:1) they could be detected under stress only of metals mixture without *T. harzianum*. Copper stress produced alteration in the oil composition of plant [65]. Many researches stated that fatty acids changes in maize plants treated with low doses of Cd were apparently smaller. A similar effect was observed in tomato plants [66]. The more severe Cd-stress led to decreased linolenic acid (C18:3) content, as observed in other Cd-treated plants [8, 63]. In conclusion, a new approach to develop improved plant growth with using save organism *T. harzianum* in the presence of soil or water polluted with metals with concerning on the biomarker changes in plants cultivated under stress conditions.

REFERENCES

1. Al-Jaloud, A.A., M.Y. Al-Saiady, A.M. Assaeed and S.A. Chaudhary, 2001. Some halophyte plants of Saudi Arabia, their composition and relation to soil properties. Pakistan Journal of Biological Sciences, 4(5): 531-534.
2. Al-Rajhi, A.M.H., 2009. Impact of salinity stress on the free amino acid pools of some sugar beet pathogens. N. Egypt. J. Microbiol., 23: 183-196.
3. Flanders, A. and S.M. Abdul Karim, 1985. The composition of seed and seed oils of Taramira (*Eruca sativa*). J. Am. Oils Chem. Soc., 62: 1134-1135.
4. Dorado, M.P., F. Cruz, J.M. Palomar and F.J. Lopez, 2006. An approach to the economics of two vegetable oil-based biofuels in Spain. Renewable Energy, 31: 1231-1237.
5. Chakrabarti, M.H. and R. Ahmad, 2008. Transesterification studies on castor oil as a first step towards its use in Bio Diesel production. Pakistan Journal of Botany, 40(3): 1153-1157.
6. Lagadic, L., T. Caquet and J.C. Amiard, 1997. Interet d'une approche multiparametrique pour le suivi de la qualite de l'environnement, in: Lagadic, L., Caquet, T., Amiard, J.C., Ramade, F. (Eds.), Biomarqueurs en ecotoxicologie. Aspects Fondamentaux. Masson, Paris, pp: 393-401.
7. Marina, L.G., S. Blandine, L. Isabelle and B. Jean-Jacques, 2008. Development of a biomarker for metal bioavailability: The lettuce fatty acid composition. Environmental Toxicology and Chemistry, 27(5): 1147-1151.
8. Nouairi, I., W. Ben Ammar, N. Ben Youssef, D. Ben Miled Daoud, M. Habib Ghorbal and M., Zarrouk, 2006. Comparative study of cadmium effects on membrane lipid composition of *Brassica juncea* and *Brassica napus* leaves. Plant Sci., 170: 511-519.
9. Pinto, E., T.C.S. Sigaud-Kutner, M.A.S. Leitao, O.K. Okamoto, D. Morse and P. Colepicolo, 2003. Heavy metal-induced stress in algae. Journal of Phycology, 39: 1008-1018.
10. Fernandes, J.C. and F.S. Henriques, 1991. Biochemical, physiological and structural effect of excess copper in plants, The Botanical Review, 57: 246-273.
11. Ritter, A., S. Goulitquer, I. Salaun, T. Tononl, A. Correa and P. Colepicolo, 2008. Heavy metal-induced stress in algae. Journal of Bioresour. Technol., 97: 1018-1025.
12. Farmer, E.E. and C. Davoine, 2007. Reactive electrophile species. Current Opinion in Plant Biology, 10: 380-386.
13. Diels, L., M. Desmet, L. Hooberghs and P. Corbisier, 1999. Heavy metal bioremediation of soil molecule. Biotechnology, 12: 149-158.
14. Blaylock, M.J. and J.W. Huang, 2000. Phytoextraction of Metals, In: I. Raskin and B.D. Ensley (Ed.). Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment, John Wiley and Sons, Inc, Toronto, Canada, pp: 303.
15. Meagher, R.B., 2000. Phytoremediation of toxic elemental and organic pollutants. C. Op. in Plant Biol., 3: 153-162.
16. Claire, L.C., D.C. Adriano, K.S. Sajwan, S.L. Abel, D.P. Thoma and J.T. Driver, 1991. Effects of selected trace metals on germinating seeds of six plant species. Water, Air and Soil Pollution, 59: 231-240.
17. Hassen, A., N. Saidi, M., Cherif and A. Boudabous, 1998. Resistance of environmental bacteria to heavy metals. Bioresour. Technol., 64: 7-15.
18. Baldrian, P., 2003. Interactions of heavy metals white-rot fungi. Eznym. Microb. Technol., 32: 78-91.
19. Gavrilesca, M., 2004. Removal of heavy metals from the environment by biosorption. Eng. Life Sci., 4(3): 219-232.
20. Ezzouhri, L., E. Castro, M. Moya, F. Espinola and K. Lairini, 2009. Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco. African Journal of Microbiology Research, 3(2): 35-48.
21. Anand, P., J. Isar, S. Saran and R.K. Saxena, 2006. Bioaccumulation of copper by *Trichoderma viride.* Bioresour Techno., 97(8): 1018-1025.
22. Gupta, R., P. Ahuja, S. Khan, R.K. Saxena and H. Mohapatra, 2000. Microbial Biosorbents: Meeting challenges of heavy metal pollution in aqueous solutions. Curr. Sci., 78(8): 967-973.
23. Malik, A., 2004. Metal bioremediation through growing cells. Environ. Int., 30: 261-278.
24. Levinskaite, L., 2001. Stimulation effect of nickel, cadmium and chromium (VI) on soil micromycetes. Biologiq, 4: 13-15.
25. Ledin, M., C. Krantz-Rulcker and B. Allard, 1996. Zn, Cd and Hg accumulation by microorganisms, organic and inorganic soil components in multi-compartment systems. Soil Biol. Biochem., 28: 791-799.

26. Singh, M. and O.P. Sharma, 2012. *Trichoderma*–A savior microbe in the era of climate change. IJABR, 2(4): 784-786.
27. Harman, G.E., C.R. Howell, A. Viterbo, I. Chet and M. Lorito, 2004a. *Trichoderma* species-Opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol., 2: 43-56.
28. Yedidia, I., A.K. Srivastva, Y. Kapulnik and I. Chet, 2001. Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. Plant Soil, 235: 235-242.
29. Altomre, C., W.A. Norvell, T. Bjorkman and G.E. Harman, 1999. Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. Appl. Environ. Microbiol., 65: 2926-2933.
30. Elliott, L.F. and J.M. Lynch, 1995. The international workshop on establishment of microbial inocula in soils: Cooperative research project on biological resource management of the Organization for Economic Cooperation and Development (OECD). Am. J. Alt. Agric., 10: 50-73.
31. Saxena, A.K. and K.V.B.R. Tilak, 1994. Interaction among beneficial microorganisms. Indian J. Microbiol., 34: 91-106.
32. Hyakumachi, M., 1994. Plant growth promoting fungi from rhizosphere with potential for disease suppression. Soil Microorganism, 44: 53-68.
33. Meera, M.S., M.B. Shivanna, K. Kageyama and M. Hyakumachi, 1994. Plant growth promoting fungi from zoysiagrass rhizosphere as potential inducers of systemic resistance in cucumbers. Phytopathol., 84: 1399-1406.
34. Rengel, Z. and P. Marschner, 2005. Nutrient availability and management in the rhizosphere exploiting genotypic differences. New Phytol., 168: 305-312.
35. Harman, G.E., 2000. Myths and dogmas of biocontrol. Changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Dis., 84: 377-393.
36. Adams, P., F.A.A.M. De-Leij and J.M. Lynch, 2007. *Trichoderma harzianum* Rifai 1295-22 mediates growth promotion of cracl willow (*Salix fragilis*) samplings in both clean and metal contaminated soil. Microbial Ecology, 54(2): 206-313.
37. Al-Rajhi, A.M.H., 2008. Biological control of *Rhizoctonia* root-rot of wheat by *Trichoderma harzianum* Rifai *in vivo*. Bull. Fac. Sci. Cairo Univ., 76(C): 63-89.
38. Perveen, K. and N.A. Bokhari, 2012. Antagonistic Activity of *Trichoderma harzianum* and *Trichoderma viride* isolated from soil of date palm field against *Fusarium oxysporum*. Afr. J. Microbiol. Res., 61(13): 3348-3353.
39. Kabata-Pendias, A. and H. Pendias, 1993. Biogeochemia pierwiastkow sladowych, Wydawnictwo Naukowe PWN, Warszawa, pp: 400, (in Polish).
40. Cobbett, C.S., 2000. Phytochelatins and their roles in heavy metal detoxification. Plant Physiol., 123: 825-32.
41. El-Abyad, M.S., M.A. El-Sayad, A.R. El-Shanshoury and S.M. El-Sabbagh, 1993. Towards the biological control of fungal and bacterial disease of tomato using antagonistic *Streptomyces* sp. Plant Soil, 149: 185-195.
42. Johnson, L.F., E.A. Curl, J.H. Bond and H.A. Fribourg, 1959. Methods for Studying Soil Microflora. Plant Diseases Relationships. Burgess Publ. Co. Minn. USA.
43. Vernon, L.P. and G.R. Seely, 1966. The Chlorophylls. Academic Press, New York, London.
44. Kar, M. and D. Mishra, 1976. Catalase, Peroxidase and Polyphenoloxidase activity during rice leaf senescence. Plant Physiology, 57: 315-319.
45. Snedecor, G.W. and W.G. Cochran, 1967. Statistical Methods. Oxford and J.B.H. Publishing Com. 6th Edition, pp: 593.
46. El-Nagdy, M., 2000. Occurrence of zoosporic organisms in the Western region of Saudi Arabia. Microbiology, 28(4): 103-196.
47. Inmark, S., 2000. Application of Biological Products for growing Super sweet Corn. Special Problem. Department of Plant Pest Management Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok.
48. Suwan, S., M. Isobe, S. Kanokmedhakul, N. Lourit, K. Kanokmedhakul and A. Vassilev, 2004. Cadmium-induced changes in chloroplast lipids and photosystem activities in barley plants. Biologia Plantarum, 48: 153.
49. Phuwiwat, W. and K. Soytong, 1999a. Growth and yield response of Chinese radish to application of *Trichoderma harziamum*. Thammasat Int. Science Technology, 4(1): 6-8.

50. Phuwiwat, W. and K. Soytong, 1999b. Potential of *Trichoderma hamatum* on Chinese radish growth promotion. Proc. of the 5th Asia-Pacific Biochemical Engineering Conference, Phuket, Thailand, 15-18 November (CD-ROM Proceedings). Pycology, 39: 1008-1018.
51. Soytong, K., 2004. Handbook of Biologic Agriculture. KMITL Press, Bangkok. Structural effects of excess copper in plants. Botanical Review, 57: 246-273.
52. Faheed, F.A., 2005. Effect of lead stress on the growth and metabolism of *Eruca sativa* M. seedlings. Acta Agronomica Hungarica, 53(3): 319-327.
53. Kahle, H., 1988. Wirkung von Blei und Cadmium auf Wachstum, und Dissertations Botanicae 127, Berlin: J. Crame Minerals to ffhaushalt von Jungbuchen (*Fagus sylvatica* L.) in Sandkultur.
54. Oncel, I., Y. Kele and A.S. Ustun, 2000. Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings, Environ. Polut., 107: 1-17.
55. Kinraide, T.B., 2001. Ion fluxes considered in terms of membrane-surface electrical potentials. Australian J. Plant Physiol., 28: 607-18.
56. Harman, G.E., M. Lorito and J.M. Lynch, 2004b. Uses of *Trichoderma* spp to remediate soil and water pollution. Adv. Appl. Microbiol., 56: 313-330.
57. Lin, C.C. and C.H. Kao, 2000. Effect of NaCl stress on H_2O_2 metabolism in rice leaves. Plant Growth Regul., 30: 151-155.
58. Hosseini, R.H., M. Khanlarian and M. Ghorbanli, 2007. Effect of Lead on germination, growth and activity of catalase and peroxidase enzyme in root and shoot of two cultivars of *Brassica napus* L. J. Biological Sciences, 7(4): 592-598.
59. Cakmak, I. and W.J. Horst, 1991. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tip of soybean (*Glycine max*). Physiol. Plant., 83: 463-468.
60. Shah, K., R.G. Kumar, S. Verma and R.S. Dubey, 2001. Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedling. Plant Sci., 161: 1135-1144.
61. Yu, Kuznetsova, T., L.V. Vetchinnikova, A.F. Titov and M.K. Il'inova, 2008. Effect of cadmium on fatty acid composition of lipids in the shoots of Karelian birch cultured *in vitro*. Russian Journal of Plant Physiology, 55(5): 657-662.
62. Nicole, V., M. Mench, C. Cassagne and J. Bessoule, 2001. Fatty acid composition of tomato leaves as biomarkers of metal-contaminated soils Environmental Toxicology and Chemistry, 20(2): 382-388.
63. Yassilev, A., 2004. Cadmium-induced changes in chloroplast lipids and photosystem activities in barley plants. Biologia Plantarum, 48: 153.
64. Ben Ammar, W., I. Nouairi, B. Tray, M. Zarrouk, F. Jemal and M.H. Ghorbel, 2005. Cadmium effects on mineral nutrition and lipid contents in tomato leaves (in French). J. Soc. Biol., 199:157-163.
65. Deef, H.E., 2007. Cupper treatments and their effects on growth, carbohydrates, minerals and essential oils contents of *Rosmarinus officinalis* L. World J. of Agricultural Sciences, 3(3): 322-328.
66. Ouariti, O., N. Boussama, M. Zarrouk, A. Cherif and M.H. Ghorbal, 1997. Cadmium- and copper- induced changes in tomato membrane lipids. Phytochemistry, 45: 1343-1350.