International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Mitigation of hazardous effects of mercury pollution on wheat seedlings through *Trichoderma harzianum* seed coating treatment

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Abstract

In view of increasing mercury pollution, mainly due to anthropogenic activities and consequently contaminating soils, available water and crops growing in such conditions, a research activity was planned to overcome this heavy metal pollution, so as to grow healthy and pure crops safe for man consumption. This research was performed in botany department of Nusrat Jahan College Rabwah Chenab Nagar to evaluate the potential of *Trichoderma harzianum*, fungus seed coating on two wheat cultivars namely "Shafaq-06" and "Punjab-11" in mitigating mercury stress. Seeds of wheat cultivars and *Trichoderma* fungus were taken from NARC PAKISTAN. Seeds after surface sterilization were coated with *Trichoderma* at the rate of $2x10^7$ CFU using pelgel for 24 hours and then they were air dried for 12 hours. After air drying seeds were sown in cups of sand and seedlings were harvested after thirty days of sowing. Mercury stress (M1:30mM and M2: 40mM) was applied at two leaf stage. Seedlings (roots and shoots separate) were preserved in potassium phosphate buffer (50mM) and were than subjected to different biochemical tests. Results of these tests revealed that *Trichoderma harzianum* seed coating very positively overcame mercury stress by triggering production of ROS scavenging proteins and preventing from oxidative damages.

Key words: wheat, mercury, *Trichoderma harzianum* and seed coating

Full length article **Corresponding Author*, e-mail: maleeha.umber@njc.edu.pk

1. Introduction

Heavy metal mercury has become a serious environmental pollutant due to increasing anthropogenic activities. Contamination of soil by regular deposition of such toxic element is extremely harmful for crops and resultantly overall environment [1]. It is experimentally proved fact that plants grown in mercury contaminated soils uptake this harmful element along with other elements hence damaging physiological and biochemical processes of different plants [2]. Soils if constantly exposed to this stress become permanently toxic and toxicity is transferred to ultimate fruit grown which is harmful for consumption by mankind and other animals. Mercury pollution also causes production of ROS species, causing oxidative damage to plants grown under this type of heavy metal pollution [3].

Wheat is economically very significant crop because more than thirty five percent of overall population around the globe is dependent on it for its food requirement [4]. Therefore it is very much necessary that yield of wheat should be increased and healthy. Also, issue of increasing population is placing great constrain in fulfilling food requirements of all. Hence any damage or mal growth of wheat crop is highly unbearable. In this scenario smart and appropriate physiological, genetic and environmental methodologies should be launched to grow healthy wheat crop so that food requirement can be fulfilled at its maximum, overcoming present biotic and abiotic stresses in particular mercury pollution [5].

Current study has been planned to use and check *Trichoderma harzianum* seed coating method to overcome mercury pollution and combat deteriorating effects of this

pollutant on wheat crop. *Trichoderma* is soil born fungi that belong to ascomycetes group, it is well known in growth enhancement of various plants [6]. Different scientific studies have proved *Trichoderma* species as effective bioagent in curing multiple biotic stresses and few abiotic stresses [7].

2. **Materials and Methods**

The research work was performed in department of Botany at Nusrat Jahan College Rabwah, Chenab Nagar to determine the role of *Trichoderma harzianum* in mitigating damages caused in wheat crop due to mercury pollution. Two wheat cultivars, PUNJAB-11 and SHAFAQ-06 were used for this study. Seeds of these cultivars as well as *Trichoderma harzianum* fungus were obtained from NARC PAKISTAN. All seeds were firstly surface sterilized through mercuric chloride and were than seed coated with *Trichoderma* using pelgel at the rate of $2x10^7$ CFU for twenty four hours. After completion of seed coating process, seeds were air dried for twelve hours at room temperature. Than sowing of seeds, in cups of sand took place. These cups were set in groups according to following treatments:

- Control group (without mercury stress)
- U1 group (without *Trichoderma* coating but M1:30mM Mercury nitrate stress applied)
- U2 group (without *Trichoderma* coating but M2:40mM Mercury nitrate stress applied)
- M1 group (with *Trichoderma* coating and M1:30mM Mercury nitrate stress)
- M2 group (with *Trichoderma* coating and M2:40mM Mercury nitrate stress)

Mercury nitrate stress was applied at two leaf stage. Seedlings were harvested after 30 days of germination. At the time of harvest roots and shoots were separated, washed and preserved in 50mM potassium phosphate buffer. Next step was grinding of all preserved samples using mortar pestle. The grinded samples were than centrifuged at 14000rpm for 15 minutes. The centrifuged samples were than subjected to following biochemical tests:

2.1. Total Soluble Proteins

Concentration of total soluble proteins was examined using the method of (Bradford, 1976) [8] with few amendments. The 1ml supernatant was reacted with 2ml Bradford Reagent and incubated for 15-20 min then reading was measured at 595 nm. Total soluble proteins were computed using standard graph. Bovine Serum Albumin was used as standard.

2.2. Ascorbate Peroxidase Activity (APX)

The APX working was measured using the method of Asada and Takahashi (1987) [9]. The reaction solution (1600µl) was comprised of 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 400 µl of enzyme extract. The absorbance was taken at 290 nm against the blank and the enzyme activity was represented in Umg^{-1} protein (U=change in 0.1 absorbance min-1 mg^{-1} protein).

2.3. Total Phenolic Contents

Total phenolics were evaluated with the help of Folin-Ciocalteu protocol (Wolfe *et al*. 2003) [10] with few amendments. Samples were mixed with 5ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4ml (75 g/l) of sodium carbonate. The tubes were shacked for fifteen seconds and were permitted to stand for 30 min at 40°C so that the color develops. Then absorbance was taken at 765 nm on spectrophotometer. Total phenolic content were represented as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 0.1216x$, $r^2 = 0.9365$, where x was the absorbance and y the tannic acid equivalent (mg/g).

2.4. MDA Contents

Malondialdehyde (MDA) was determined in accordance to method proposed by Dhindsa et al. (1981) [11]. In the 2ml TCA, added 2 ml of 0.6% thiobarbituric acid. It was heated at 100 degree centigrade for 20 minutes in water bath. After heating immediately cooled for 20 minutes and then centrifuged at 10000 rpm for 10 minutes. The resulting color was taken at 532 nm on spectrophotometer.

2.5. Hydrogen Peroxide Concentration

H_2O_2 concentration was determined according to the protocol of (Velikova *et al*., 2000) [12]. The 0.1ml of supernatant was added to 0.1ml of 10Mm potassium phosphate buffer (PH 7.0) and 1M IKI. The absorbance was taken 390nm. The contents of H_2O_2 in the tissue were given standard curve which constructed using a series (0, 20, 40, 60, 80 and 100 µm) of analytic reagent grade H_2O_2.

3. Results

3.1. Total Soluble Proteins

According to Figure 1 protein concentration is increased in roots and shoots of both wheat cultivars at M1 and M2 stress levels as compared to control and U1, U2, non *Trichoderma* groups. Thus this result supports *Trichoderma* efficacy in eliminating drastic effects of mercury stress and increasing protein content under mercury stress.

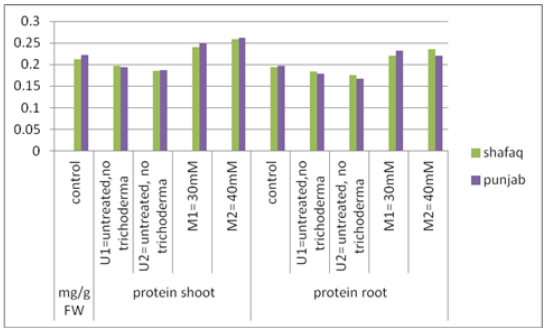

Fig. 1: Mean values of protein concentration of wheat cultivars at different treatments

3.2. Ascorbate Peroxidase Activity (APX)

Result of this test reveals that *Trichoderma* application has enhanced APX activity in both cultivars at M1 and M2 stress levels as compared to U1 and U2, control group (Figure.2). This increased APX activity activates ROS scavenging proteins.

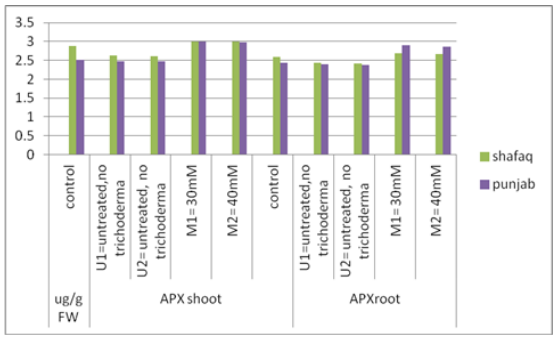

Fig. 2: Mean values of APX activity of wheat cultivars at different treatments

3.3. Total Phenolics Content

Total phenolics content prominently increased in all wheat cultivars under both stress levels in *Trichoderma* coated seedlings as compared to non coated seedlings and seedlings of control group (Figure.3). This increase suggests that under mercury stress *Trichoderma* increases phenolics, triggering ROS scavenging proteins.

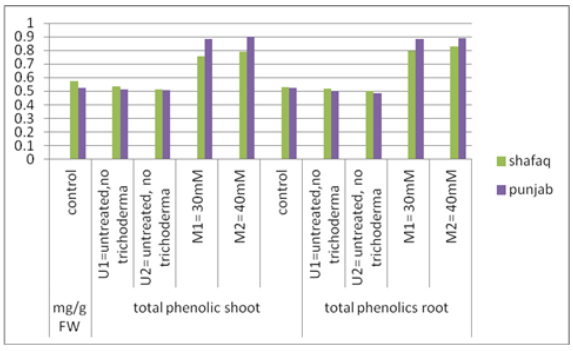

Fig. 3: Mean values of total phenolics content of wheat cultivars at different treatments

3.4. MDA Content

According to Tab. 1. MDA content in *Trichoderma* treated seedlings of both wheat cultivars decreased under mercury stresses of both levels. While non *Trichoderma* coated seedlings showed an increase in MDA content on exposure to stresses of both levels.

Tab. 1: Mean values of MDA content in shoots and roots of wheat cultivars at different treatments

Unit: umol/gFW	SHAFAQ	PUNJAB
Control Shoot	0.132	0.127
U1=Untreated (no *Trichoderma*)	0.142	0.167
U2=Untreated (no *Trichoderma*)	0.165	0.172
M1=30mM	0.125	0.124
M2=40mM	0.119	0.106
Control Root	0.104	0.121
U1=Untreated (no *Trichoderma*)	0.139	0.147
U2=Untreated (no *Trichoderma*)	0.147	0.159
M1=30mM	0.109	0.112
M2=40mM	0.096	0.103

3.5. Hydrogen Peroxide Concentration

Table 2 shows decline in H_2O_2 concentration under mercury stresses in *Trichoderma* coated seedlings as compared to non coated and control group seedlings indicating that *Trichoderma* induces ROS scavenging protiens under stress conditions.

Tab. 2: Mean values of hydrogen peroxide in shoots and roots of wheat cultivars at different treatments

Unit: umol/gFW	SHAFAQ	PUNJAB
Control Shoot	0.235	0.225
U1=Untreated (no *Trichoderma*)	0.279	0.247
U2=Untreated (no *Trichoderma*)	0.288	0.255
M1=30mM	0.216	0.196
M2=40mM	0.202	0.187
Control Root	0.228	0.23
U1=Untreated (no *Trichoderma*)	0.2606	0.268
U2=Untreated (no *Trichoderma*)	0.267	0.275
M1=30mM	0.214	0.193
M2=40mM	0.203	0.181

4. Discussions

In view of our results, it is apparent that *Trichoderma harzianum* has pronouncedly overcame mercury stress. *Trichoderma* coated seedlings increased protein content even in stress while non coated seedlings could not cope up with stress and showed decline in protein content. This increase in coated seedlings may be due to activation of certain growth hormones like auxins, gibberalins which promote healthy growth even in stress conditions. Rasool et al. (2013) [13] also found simillar results in increament of protein concentration with *Trichoderma* treated seedlings under saline conditions. Furthermore enhanced concentrations of total phenolics content and ascorbate peroxidase on exposure to mercury stress in *Trichoderma* seed coated wheat cultivars as

compared to non coated seedlings proved induction of stress-related proteins such as glutathione S-transferase (GST), glutathione-dependent formaldehyde dehydrogenase (FALDH), and peroxidase by *Trichoderma*. These proteins work as scavengers and whenever in stress conditions free radicals are boosted, these proteins deteriorate them and hence prevent oxidative damage. Shoresh and Harman (2008) [14] also found same impact of this fungus in maize crop. Due to activation of GST, FALDH and other such proteins, hydrogen peroxide and malondialdehyde content is reduced in *Trichoderma* treated seedlings during mercury stresses while this decline was not seen in non-coated seedlings. This proves that *Trichoderma harzianum* activates stress related proteins which inhibit H_2O_2 and MDA preventing cells from lipid peroxidation and oxidative damages. Hence concluded that *Trichoderma harzianum* seed coating is very effective and cheap mean to grow crops under mercury stress by activating ROS scavengers.

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