Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

GROWTH OF A SOIL-BORNE PLANT PATHOGEN *SCLEROTIUM ROLFSII* UNDER CHROMIUM(III) STRESS

Nighat Sana, Amna Shoaib, Arshad Javaid*
Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.

A B S T R A C T

Tolerance of plant pathogens to heavy metal stress is an important area of research that has been rarely explored in Pakistan. The current research work was carried out to investigate the impact of Cr(III) on growth of *Sclerotium rolfsii* Sacc., the cause of collar rot of chilli (*Capsicum annuum* L.). Laboratory bioassays were conducted in using solid as well as liquid malt extract growth medium amended with 14 different concentrations (0, 5, 10, 20, 30, ...,100, 150, 200, 300, 400, 500 ppm) of Cr(III). Growth medium flasks with different concentrations of Cr(III) were inoculated with the pathogen and incubated for 7 days at 25±2 ^{o}C. Results showed that radial growth of *S. rolfsii* on solid medium and its biomass on liquid medium were significantly decreased with the increase in concentrations of Cr(III) up to 100 ppm, while fungus was unable to grow at concentrations above 100 ppm. There was a linear relationship (R^2 = 0.9242) between Cr(III) concentrations and fungal biomass on liquid medium, and a non-linear relationship (R^2 = 0.9264) between Cr(III) concentrations and fungal radial growth on solid growth medium.

Keywords: Chromium, heavy metal, fungal growth, *Sclerotium rolfsii*.

INTRODUCTION

Information regarding the effects of heavy metals on growth and activity of phytopathogenic fungi is scanty. Toxicity assessment studies on specific organism help to explore the organism's sensitivity to a particular toxicant and hidden facts concerning the ecosystem health and policies to protect it (Rani *et al*., 2011). Generally, fungi have been reported as dominant community in metalliferous soil due to their innate ability to take up the pollutants as nutrients through absorption or accumulation (Gadd, 1993). It has been documented that different fungal species and even isolates of the same species exhibit differential growth and physiological responses to various heavy metals and their concentrations in the medium (Zetic *et al*., 2001). Many fungal species of the genera namely *Alternaia, Fusarium, Trichoderma, Aspergillus* and *Penicillium* are categorized as metal-tolerant fungi (Nazina e*t al*., 2002).

Sclerotium rolfsii is a robust fungus of warm and humid climates that causes serious crop losses worldwide including Pakistan (Yaqub and Shezad, 2011; Al-Askar *et al*., 2013; Javaid and Iqbal, 2014). The fungus can withstand with harsh environmental conditions and can survive in soil for several years by forming scleotia. Over 500 plant species comprised of both cultivated and wild ones are attacked by this fungus and infected plants show severe wilting and rotting (root, stem and foot) symptoms followed by plant collapsing (Arunasri *et al.,* 2011). Collar rot of chilli caused by *S. rolfsii* is a very important fungal disease of chilli growing in parts of Khyber Pakhtun Khwa and Punjab. This disease is well recognized and areas have been restricted for chili cultivation due to this pathogen (Saleem *et al*., 1997, 1998; Irfan-ul-Din *et al*., 2012).

Many areas of Pakistan are contaminated with heavy metals including chromium (Cr). A higher level of Cr ranging from 40-2000 ppm, has been reported in soil and water of different areas in Pakistan (Mushtaq *et al*., 2010; Iqbal *et al*., 2011; Akhtar *et al*., 2014). Metallurgy, refractory, fungicide, electroplating, tannery, wood preservation and pigment manufacturing industries are major sources of Cr pollution (Caglieri *et al*., 2006; Nath *et al*., 2005; Babel and Opiso, 2007). Cr lethality to different forms of life have been

* Corresponding Author:
Email: arshadjpk@yahoo.com

extensively investigated but its interaction with plant pathogenic fungi like *S. rolfsii* is an important area of research that needs to be explored (Anjisha *et al.,* 2012). The occurrence of Cr not only affects microbial flora but also alters microbial respiration. It is also toxic and mutagenic to various microorganisms (Das and Mishra, 2008). Therefore, current *in vitro* study was conducted to investigate the effect of Cr(III) on growth of *S. rolfsii*, isolated from chili plants suffering from collar rot disease.

MATERIALS AND METHODS

Pathogen and Pathogenicity test: *S. rolfsii* was isolated from infected plants of chilli. Diseased portions were cut into 0.5 cm pieces and surface sterilized by 0.1% sodium hypochlorite solution for 2 min followed by thorough washing with sterilized water. These pieces were placed on 2% malt extract agar (MEA) medium in 9-cm diameter Petri plates and incubated at 25±2 °C. Emerging fungal colonies were purified on fresh MEA Petri plates and identified as *S. rolfsii* on the basis of morphological characteristics.

Pathogenicity of *S. rolfsii* was checked by growing the chilli var Golla Peshawari plants in the pathogen inoculated soil. Soil was inoculated by mixing freshly prepared conidial suspension (30 mL) in 500 g sterilized soil filled in plastic pots followed by sowing of chilli seeds. Soil without inoculation served as control. The pots were kept under controlled environmental conditions at soil moisture level of 40-50% at 25 °C and regularly monitored for disease development. The characteristic wilting symptoms caused by the *S. rolfsii* on the test plant were confirmed on 45th day after germination.

Preparation of Cr(III) solution: Stock solution of 1000 ppm Cr(III) was prepared by dissolving chromium nitrate [$Cr(NO_3)_3$. $9H_2O$] (Merk, Germany) in distilled water. Further dilutions of 5, 10, 20,…, 100, 150, 200,…, 500 ppm were prepared by adding appropriate quantity of sterilized distilled water to the stock solution.

Laboratory bioassays: To assess the impact of Cr(III) on growth of *S. rolfsii,,* both MEA and malt extract broth (MEB) were prepared with each of fourteen different Cr(III) concentrations i.e. 5, 10, 20,…, 100, 150, 200,…, 500 ppm. Both solid and broth growth media were sterilized by autoclaving at 121 °C for 30 minutes. Chloromycetin was added under aseptic conditions to avoid bacterial contamination. Metal-amended solid and broth medium were inoculated with 5 mm disc from actively growing fungal culture. Inoculated plates were incubated at 25+2 °C for 7 days. Treatments without addition of Cr(III) solution was labeled as control. Each treatment was replicated 3 times and experiment was performed using a completely randomized design. After one week incubation period, radial colony diameter of the fungus was measured in agar plates and percentage inhibition of mycelial growth due to different metal concentrations was calculated using the following formula:

$$Growth\ inhibition\ (\%) = \frac{DC - DT}{DC} \times 100$$

Where: DC = Fungal colony diameter in control; DT = Fungal colony diameter in Cr (III) amended medium.

In case of ME broth, dry weight of mycelial biomass was recorded. Dry weight was taken by drying fungal biomass along with pre weighed filter paper in a drying oven at 60 °C for 24 h.

Statistical analysis: Data regarding radial growth and biomass of the fungus were analyzed by one way ANOVA. Treatment means were delineated by Tukey's HSD test at 5% level of significance using computer software Statistics 8.1. Relationship between different concentrations of Cr(III) and fungal radial growth/biomass was found out by drawing trend lines using MS Excel program.

RESULTS AND DISCUSSION

Data regarding the effect of different concentrations of Cr(III) on radial growth of *S. rolfsii* on MEA medium is presented in Figure 1A-C. In case of lower concentrations of Cr(III) i.e. 10 and 20 ppm, there was an insignificant reduction in radial growth of the fungus over control. However, further increase in Cr(III) concentrations significantly decreased the fungal growth. Fungal growth was significantly reduced by 8-20% due to 30-60 ppm concentrations, and by 23-67% due to 80-100 ppm concentrations as compared to control. Growth of the fungus was completely arrested with increase in concentration from 200 to 500 ppm. Regression analysis (R^2 = 0.9242) showed a non-linear relationship between growth of the fungus and metal concentrations. Data concerning the effect of different concentrations of Cr(III) on fungal biomass in ME broth medium is presented in Figure 2A-C Dried biomass of *S. rolfsii* was declined by 4-14% at concentration range of 10-50 ppm. However, it was not significantly different with respect to control. The inhibitory action of Cr(III) against *S. rolfsii* was significantly increased on increasing metal ions concentrations in the medium above 50 ppm. Fungal biomass was significantly declined by 34-94% at metal concentrations of 50-100 ppm over control.

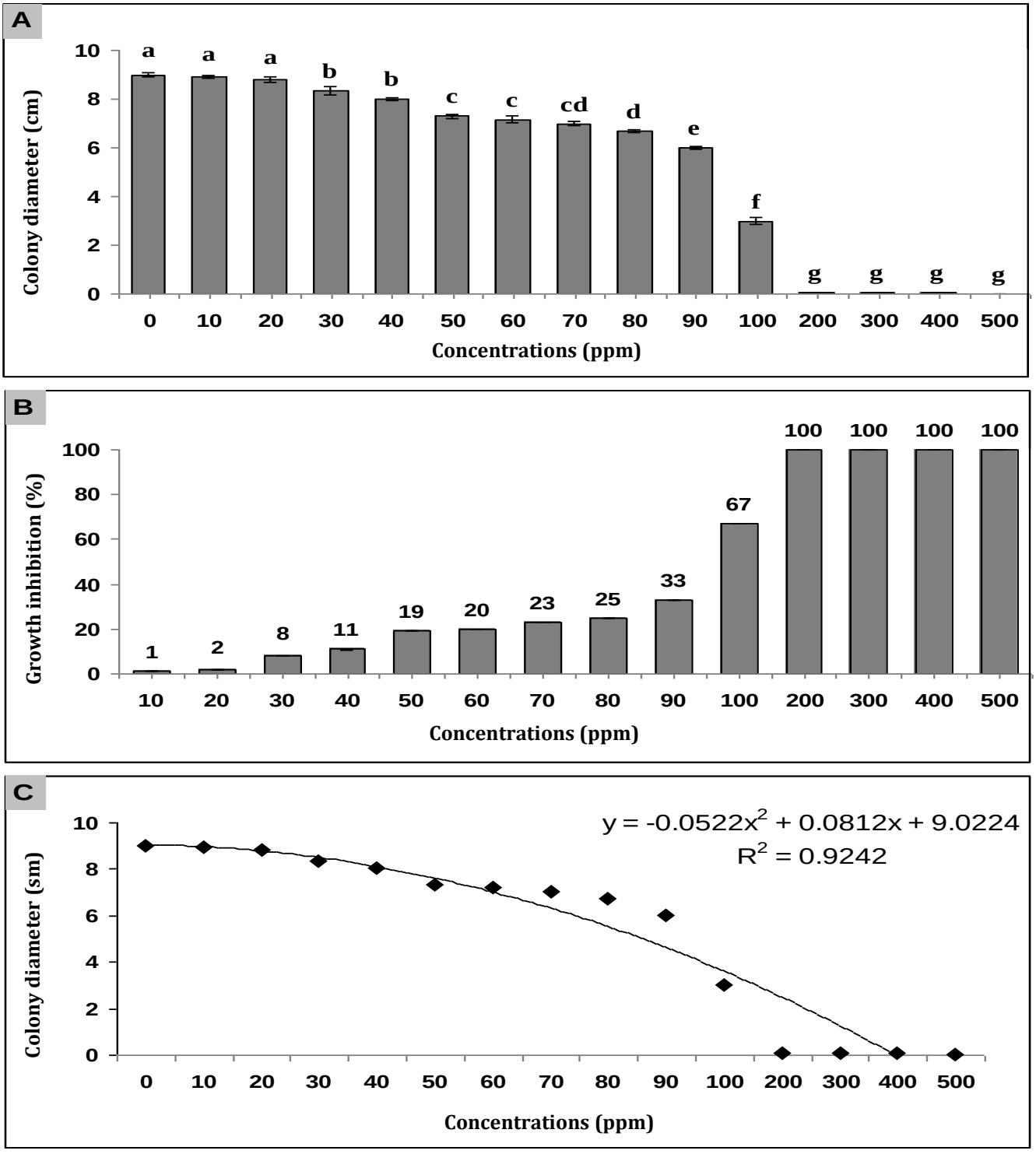

Figure 1A-C. Effect of different concentrations of Cr(III) on radial growth of *Sclerotium rolfsii* on malt extract agar medium.

A: Effect on radial growth;

B: Percentage growth inhibition over control;

C: Regression analysis for the relationship between different concentrations of Cr(III) and radial growth of *Sclerotium rolfsii.* Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

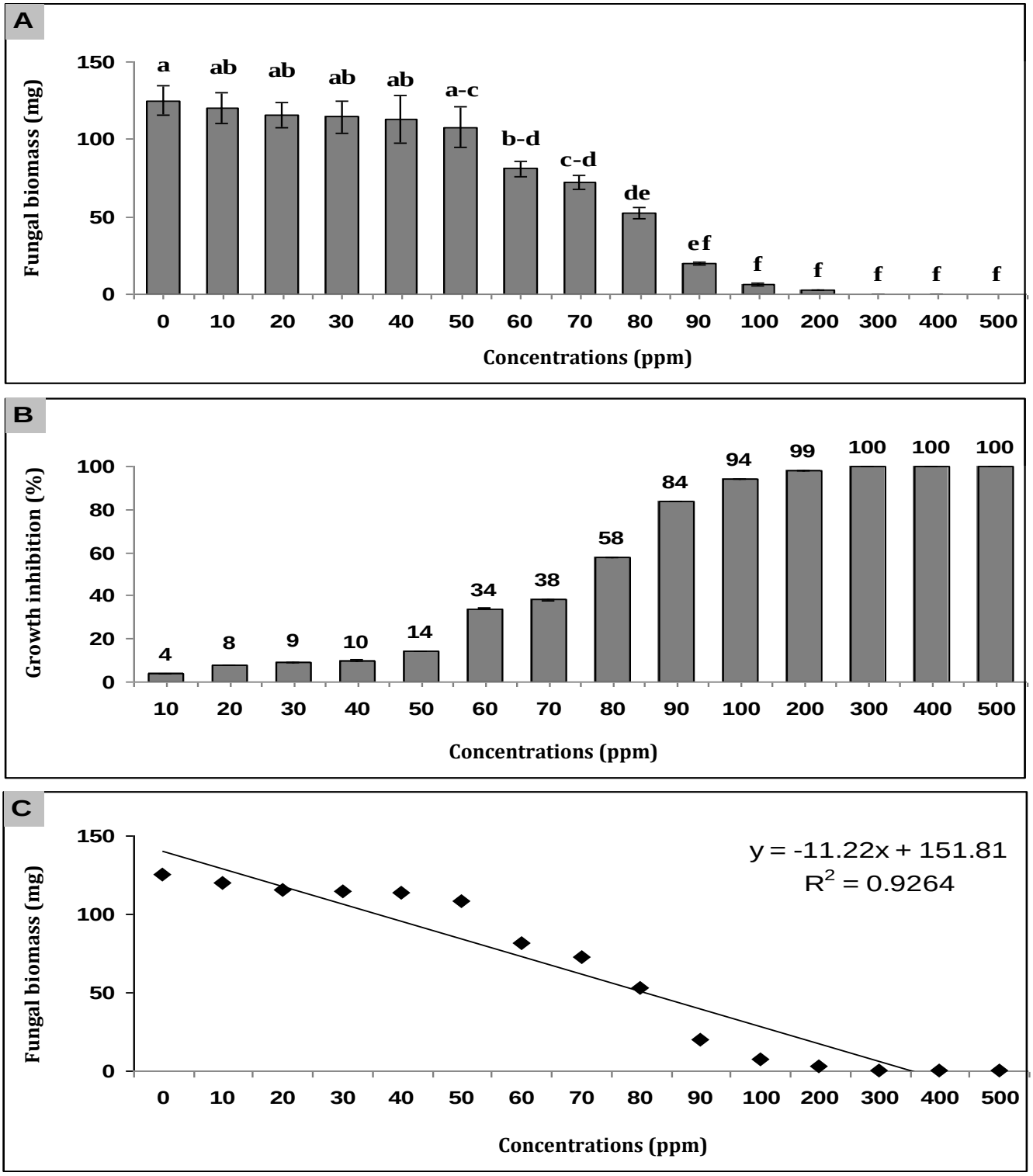

Figure. 2 A-C. Effect of different concentrations of Cr(III) on biomass of *Sclerotium rolfsii* in malt extract broth medium.
A: Effect on fungal biomass;
B: Percentage biomass inhibition over control;
C: Regression analysis for the relationship between different concentrations of Cr(III) and radial growth of *Sclerotium rolfsii.* Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test.

At a metal concentration of 200 ppm, there was 99% reduction in fungal biomass. Further increase in metal concentration in the medium completely arrested the fungal growth. Linear regression analysis (R^2 = 0.9264) confirmed inhibitory effect of increasing concentrations of Cr(III) on growth of the fungus (Figure 2 C). At lower concentrations of the metal solution, non-significant reduction in growth of the fungus could be owing to minute requirement of the fungus for Cr(III) for its functionality and adaptation to heavy metal stress (Zang *et al*., 2008). Reduction in growth was accompanied by intense aerial mycelia due to increase in Cr(III) concentration within range of 30-100 ppm. Besides, loops and connective filaments develop, together with an increase of hyphal branching indicated increasing concentration of the metal in fungus (Lilly *et al*., 1992). Absence of growth at metal concentration above 100 ppm could probably occurred by toxic effect of the metal and saturation of metal binding sites in the fungus (Amatussalam *et al*., 2011). Internalization of the metal in cytosol likely to cause the loss in cell integrity and impairment of cell functions (Pal *et al.*, 2010). Therefore, disruption in normal mitochondrial electron transport chain, glycolysis and oxidative phosphorylation could possibly decrease the fungal growth (Capobianco *et al*., 1998). Moreover, displacement of essential metal with the contaminant one results in mutation of biomolecule along with failure of fungus to perform normal activities at higher doses (Ochiai, 1997). Whereas, no growth of fungus at higher range of metal concentrations possibly occurred due to non-sporulation of *S. rolfsii* mycelium under extreme toxicity level or prolongation of lag phase.

The present study concludes that *S. rolfsii* can tolerate Cr(III) stress up to 100 ppm of the metal concentration in soilless growth medium. However, in soil medium, this phenomenon may be different to some extent. Further studies are therefore, required to investigate such metal-fungal pathogen interactions in soil medium.

LITERATURE CITED

Akhtar, S., S. Iram, M. Mahmood ul Hassan, V. Suther, R. Ahmad. 2014. Heavy metal concentration in peri-urban soils and crops under untreated wastewater. Int. J. Sci. Eng. Res. 5: 523.

Al-Askar, A.A., Y.M. Rashad, W.M. Absulkhair. 2013. Antagonistic activity on an endemic isolate of *Streptomyces* tendae RDS 16 against phytopathogenic fungi. Afr. J. Microbiol. Res. 7: 509-516.

Amatussalam, A., M.N. Abubacker, R.B. Rajendran. 2011. *In situ Carica papaya* stem matrix and *Fusarium oxysporum* (NCBT-156) mediated bioremediation of chromium. Indian J. Exp. Biol. 49**:** 925-31.

Anjisha, R., Maharshi, V.S. Thaker. 2012. Growth and development of plant pathogenic fungi in define media. Eur. J. Exp. Biol. 2: 44-54

Arunasri, P., T.V. Chalam, N.P.E. Reddy, S.T. Reddy, R. Reddy. 2011. Investigation on fungicide selectivity of *Trichoderma spp* and S*clerotium rolfsii* (collar rot pathogen) in crossandra. Int. J. Appl. Biol. Pharm. Technol. 2: 290-293.

Babel, S., E.M. Opiso. 2007. Removal of Cr from synthetic wastewater by sorption into volcanic ash soil. Int. J. Environ. Sci. Technol. 4: 99-107.

Caglieri, A., M. Goldoni, O. Acampa, 2006. The effect of inhaled chromium on different exhaled breath condensate bio-markers among chrom-plating workers. Environ. Health Perspect 114: 542-546.

Capobianco, J.O., D. Zakula, D.J. Frost, R.C. Goldman, L. Li, L.L. Klein, P.A. Lartey. 1998. Cellular accumulation, localization and activity of a synthetic cyclopeptamine in fungi. Antimicrob. Agents Chemother. 42: 389.

Das, A., S. Mishra. 2008. Hexavalent Chromium (VI): Health hazards and environmental pollutant. J. Environ. Res. Develop. 2: 86-92.

Gadd, G.M., 1993. Interaction of fungi with toxic metals. New Phytol. 124: 25-60.

Iqbal, M.A., M.N. Chaudhay, S. Zaib, M. Imran, K. Ali, A. Iqbal, 2011. Accumulation of heavy metals (Ni, Cu, Cd, Cr, Pb) in Agricultural soils and spring seasonal plants, irrigated by industrial waste water. J. Environ. Technol. Manage. 2: 37-40.

Irfan-ud-Din, F. Raziq, S. Hussain, Saifullah. 2012. Occurrence of Phytophthora root rot (*Phytophthora capsici leonian*) of pepper in Peshawar and Malakand divisions. Sarhad J. Agric. 28: 443-450.

Javaid, A., D. Iqbal. 2014. Management of collar rot of bell pepper (*Capsicum annuum* L.) by extracts and dry biomass of *Coronopus didymus* shoot. Biol. Agric. Hort. 30: 164-172.

Lilly, W.W., G.J. Wallweber, T.A. Lukefahr. 1992. Cadmium absorption and its effects on growth and mycelial morphology of the basidiomycete fungus, *Schizophyllum commune*. Microbios 72: 227-37.

Mushtaq, N., K.S. Khan. 2010. Heavy metals

contamination of soils in response to waste water irrigation in Rawalpindi region. Pak. J. Agric. Sci. 47: 215-224.

Nath, K., S. Saini, Y.K. Sharma. 2005. Chromium in tannery industry effluent and its effect on plant metabolism and growth. J. Environ. Biol. 26: 197-204.

Nazina, T.N., A.A. Grigoryan, Y. Xue, E.V. Novikova, M.V. Ivanov. 2002. Phylogenetic diversity of aerobic saprtrophic bacteria isolated from Daqing Oil Field Microbiol. 71: 91-97.

Pal, T.K., S. Bhattacharyya, A. Basumajumdar. 2010. Improvement of bioaccumulation of cadmium by *Aspergillus niger* as a function of complex nutrient source. J. Indian Chem. Soc. 87: 391-394.

Rani, M.J., M.C.J. Milton, M. Uthiralinganm, R. Azhaguraj. 2011. Acute toxicity of mercury and chromium to *Clarias batrachus* (Linn.). Bioresourc. Bull. 5: 368-372

Saleem, A., M. Ansar, I. Ahmad. 1998. Root and collar rot of chillies caused by *P. capsici* L. A new record for Pakistan. Pak. J. Agric. Res. 27: 155-156.

Saleem, A., M.H. Bokhari, K. Hameed, M. Ansar. 1997. Fungi associated with root and collar rot disease of chillies in different parts of the Punjab Pakistan. Pak. J. Phytopathol., 9: 80-84.

Yaqub, F., S. Shahzad. 2008. Effect of seed pelleting with *Tichoderma* spp., and *Gliocladium* virens on growth and colonization of roots of sunflower and mung bean by *Sclerotium rolfsii*. Pak. J. Bot. 40: 947-954.

Zhang, Y., Liu, M., Shi, X., and Zhao Z. Dark. 2008. Septate endophyte (DSE) fungi isolated from metal polluted soils: their taxonomic position, tolerance, and accumulation of heavy metals *in vitro*. J. Microbiol. 46: 624-632.

Zetic, V.G., V.S. Tomas, S. Grba, L. Lutilsky, D. Kozlek. 2001. Chromium uptake by *Saccharomyces cerevisiae* and isolation of glucose tolerance factor from yeast biomass. J Biosci. 26: 217-223.