# ASSESSMENT OF THE CHROMIUM TOLERANCE LEVEL OF CAPSICUM ANNUUM L. UNDER BIOTIC STRESS OF SCLEROTIUM ROLFSII

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**ABSTRACT:** Crosstalk between metal ions and fungal pathogens induces negative effect on growth of sensitive plants. The present study was designed to assess effect of the chromium (VI) stress on germination, seedlings growth and metal accumulation in chili (*Capsicum annuum* L.), under biotic stress of *Sclerotium rolfsii* sacc. It was found that the chili seedling accumulated more chromium and became more sensitive under combined application of *S. rolfsii* and Cr(VI), in comparison to the individual stress of either pathogen or metal. Inhibitory effect of Cr(VI) was increased at higher concentrations. Roots exhibited the highest sensitivity against the given stresses. The findings of this study are alarming for future evaluation of plant health risk associated with cultivation of edible plants in soil contaminated with fungal pathogens and toxic heavy metals.

**Keywords**: Chili, Chromium (VI), Sclerotia, *Sclerotium rolfsii***.**

*(Received 18-07-19 Accepted 02-12-19)*

## INTRODUCTION

Chili (*Capsicum annuum* L.), a solanaceous member, being highly nutritious is consumed globally as a fresh vegetable and as a spice (Sana *et al*., 2016). Presence of carotenoids, ascorbic acid, tocopherols, minerals, macromolecules and bioactive valued alkaloids (capsaicinoids) enhances its significance in pharmaceutical and cosmetic industry (Hassan *et al*., 2019). Pakistan is the third-largest producer and sixth largest exporter of chilies. It exports chilies to USA, Sri Lanka, Bangladesh, Saudi Arabia, UAE and many others countries.

A fungal disease namely collar rot caused by white rot fungus (*Sclerotium rolfsii*) is responsible for crop losses in chili in warm and humid climate (Sana *et al*., 2016). The pathogen is highly virulent due to the occurrence of persistent brown colored sclerotia, cell wall degrading enzymes and phytotoxin (oxalic acid) (Kirkland *et al*., 2005). The fungus infection in plants leads to necrosis and eventually tissues death. Infected soil is the main source of the pathogen dissemination (Soares *et al*., 2009).

The *S. rolfsii* have shown resistance to various heavy metals including widespread hexavalent form of chromium (Khurshid *et al*., 2017; Rafi *et al*., 2017). The Cr(VI) is toxic, carcinogenic and very common contaminant of Pakistani soil (Waseem *et al*., 2014) due to its great industrial applicability to several products and processes (Garcia-Hernandez *et al*., 2017).

Co-occurrence of pathogen and heavy metal, and their effect on plants is still poorly under stood (Morkunas *et al*., 2018). Occurrence of pathogen in metal-contaminated soil may induce toxic response in plants (Nafisa *et al*., 2016; Khurshid *et al*., 2017) and currently it is an extremely important area of research, when the mobility of pollutants in the environment is increasing (Morkunas *et al*., 2018). According to many previous reports, the negative cross-talk exist between heavy metal and pathogen stress responses (Fones *et al*., 2013; Llugany *et al*., 2013). Contradictory reports are also available, where plants defense system activate against pathogens by accumulating heavy metals (Boyd, 2012). Therefore, an *in vitro* study was conducted to assess the effect of co-occurrence of *S. rolfsii* and Cr(VI) on germination, seedlings growth and metal accumulation in *C. annuum*.

## MATERIALS AND METHODS

Chili var. Tatapuri seeds were surface sterilized with 1% Clorox (Nafisa *et al*., 2016). Cultural suspension of *S. rolfsii* (FCBP1409) was prepared by scrapping fungal mycelium from 7-day old pure fungal culture. Four different concentrations of Cr(VI) *i.e.* 50, 100, 150 and 200 PPM were prepared from the potassium dichromate salt as described earlier by Rafi *et al*., (2017).

Experiment was carried out following the protocol of Nafisa *et al*., (2013). Two-layers of sterile filter paper were evenly placed at the bottom of pre-sterilized Petri plates and surface-sterilized chili seeds were arranged on it. Four treatments were designed and for the first treatment, 3 mL of the cultural suspension ($1.5\times10^6$ $mL^{-1}$) of *S. rolfsii* were applied on seeds ($T_2$). In $T_3$, four different concentrations of Cr(VI)viz. 50, 100, 150 and 200 PPM were applied on seeds. In third set of the treatment, 1.5 mL of cultural suspension ($1.5\times10^6$ $mL^{-1}$) and 1.5 mL of each Cr concentration were poured over the seeds in the Petri plate ($T_4$). Control treatments ($T_1$) received distilled water (3 mL) only. Twenty-five seeds were allocated per Petri dish and data of 100 seeds were taken from each treatment. The set of four treatments were kept in quadruple in a completely randomized fashion at 30 °C for two-weeks.

The percentage of germinated seeds was recorded after emergence of seedling. Peroxidase and polyphenol oxidase activities of the two-week old seedlings were carried out following protocols of Shoaib *et al*., (2018). Length and weight (fresh and dry) of the seedlings were recorded. Data was analyzed statistically using LSD Test. Stress tolerance indices of germination (relative germination rate and relative injury rate), and seedling length and dry weight were calculated by employing formulae described by Sana (2016).

## RESULTS AND DISCUSSION

Generally, synergism of *S. rolfsii* with Cr(VI) exhibited more reduction in the studies parameters than either stress given alone. Inhibitory effect of Cr(VI) was increased due to higher metal concentrations (150 and 200 PPM).

Germination rate was not affected significantly after application of cultural suspension of *S. rolfsii* ($T_2$) as well as Cr(VI) at 50 and 100 PPM ($T_3$) when compared with control ($T_1$). However, a significant reduction of 30% and 40% in germination rate was recorded due to concentration of 150 and 200 PPM ($T_3$) over control. The relative germination rate (RGR) and relative injury rate (RIR) were declined more drastically at higher concentrations as compared to lower concentrations of the Cr(VI) (Table 1). Therefore, RGR was decreased to 56% and 52%, while RIR increased to 39% and 44% due to effect of 150 and 200 PPM, respectively. When *S. rolfsii* was given along with each of four different concentration of Cr(VI) ($T_3$), the studied parameters were affected drastically as compared to control and other treatments. Therefore, RGR was decreased from 78 to 48% and RIR was increased from 26 to 50% with increased in Cr(VI) concentrations (50-200 PPM) combined with *S. rolfsii* (Table-1).

Application of *S. rolfsii* ($T_2$) significantly decreased lengths of chili shoot and root by 34% and 22%, respectively over control. Increase in Cr(VI) concentrations (50-200 PPM) ($T_3$) more significantly reduced lengths by 40-60%, respectively as compared to control. The combination of *S. rolflii* and Cr exhibited significantly decreased shoot length by 40-60% and root length by 40-90% over control (Fig.-1).

Seedlings weight (fresh and dry) in $T_2$ was dropped by 30% as compared to $T_1$. Metal at 50 and 100 PPM insignificantly reduced seedling weight by 20%, while it was declined significantly by 40-50% due to application of 150 and 200 PPM of Cr(VI) over $T_1$. When pathogen was given along with various concentrations of metal, the seedling weight was significantly decreased by 40-60% over control (Fig.-1).

Regression analysis ($R^2 \geq 0.60$) of the tolerance indices confirmed the strong inhibitory effect of the pathogen and increasing concentrations of Cr(VI) either given alone or in combination on chili seedling (Fig.-2).

Activities of POX and PPO significantly decreased by 51% and 40%, respectively due to infection caused by *S. rolfsii* in $T_2$ as compared to $T_1$. The activity of POX improved significantly at 50 PPM and affected insignificantly at 100 PPM in $T_3$ and $T_4$ with respect to control treatment. However, at 150 or 200 PPM, the POX activity significantly dropped by 30-50% in both $T_3$ and $T_4$. Like POX, the activity of PAL boosted at low concentration (50 PPM), while significantly reduced by 30-60% in at remaining metal concentrations in $T_3$ as well as in $T_4$ in comparison to control (Fig.-3).

Chili seedling uptake more Cr(VI) at lower concentrations than at higher concentrations. When each of 50, 100, 150 and 200 PPM of Cr(VI) solutions were given to chili seedling, it absorbed 64%, 62%, 50% and 50%, respectively metal. However, when chili were grown under synergism of *S. rolfsii* and Cr(VI), the seedling accumulated 88%, 67%, 50% and 50% of the chromium after application of 50, 100, 150 and 200 PPM, respectively (Table-2).

The negative consequences of the *S. rolfsii* on germination and seedling growth in chili might be ascribed to clogging of xylem vessel by mass of fungal mycelium, while accumulation of fungal oxalate acids has been known to induce foliar wilting by manipulating guard cells (Guimaraes and Stotz, 2004).

**Table-1: Effect of *Sclerotium rolfsii* (SR) and different concentrations of Cr(VI) on relative germination rate (RGR) and relative injury rate (RIR) in *Capsicum annum.***

| Treatments | RGR (%) | RIR (%) |
|---|---|---|
| Control | 93**a** | 0i |
| SR | 84**b** | 9**g** |
| Cr(VI) 50 PPM | 91**a** | 2h |
| Cr(VI) 100 PPM | 84**b** | 9**g** |
| Cr(VI) 150 PPM | 56**de** | 39**d** |
| Cr(VI) 200 PPM | 52**e** | 44**b** |
| SR + Cr(VI) 50 PPM | 78**bc** | 16**f** |
| SR + Cr(VI) 100 PPM | 71**c** | 23**e** |
| SR + Cr(VI) 150 PPM | 65**d** | 30**c** |
| SR + Cr(VI) 200 PPM | 49**f** | 47**a** |

Values with different letters show significant difference (P≤0.05) as determined by LSD Test.

Drastic effect of Cr(VI) on germination, seedling growth and biomass was severe at 150 and 200

PPM. The results were in accordance with findings of Nagarajan and Ganesh (2004) and Khurshid *et al*., (2016, 2017). Toxicity of Cr resulted in depletion of oxygen and reserve food materials of seeds due to metal interference with hydrolytic enzymes and amylase activity of seed (Nagarajan and Ganesh, 2004; Khurshid *et al*., 2016). Reduction in seedling length and biomass might be attributed to surface accumulation of metal within seedling, and adverse effect of Cr(VI) on auxin synthesis and nitrogen metabolism (Khurshid *et al*., 2016). Seedling became more sensitive to increasing concentrations of metal to increase in metal toxicity inside plant cells.

Synergism of *S. rolfsii* and Cr(VI) induced drastic effect on germination and seedling growth could be associated with disturbance in physiological phases of the plant cell particularly at higher metal concentrations. Further, robust nature of *S. rolfsii* may proliferated in the presence of Cr(VI) concentration (Rafi *et al*., 2017), while Cr(VI) being very mobile can easily get absorbed or accumulated in through porous and weaken root (Khurshid *et al*., 2017). Net consequences of synergism drastically affected seedlings growth and biomass and more accumulation of chromium under interactive effect of pathogen and metal.

Reduction in the activities of POX and PPO in all treatments ($T_2$-$T_4$) might be associated with disproportion between ROS production and activities of enzymes (Khurshid *et al*., 2017). Such alterations might be cause loss of plant cell wall rigidity and less oxidation of phenolic compounds to stand against stress, thus plant suffered from stress.

**Table-2: Chromium concentration in *Capsicum annuum* seedlings due to effect of *Sclerotium rolfsii* (SR) and different concentrations of Cr(VI).**

| Treatments | Meta uptake by chili seedling (PPM) |
|---|---|
| **50 PPM** | 32**g** ± 0.37 |
| **SR+50 PPM** | 44**f** ± 0.52 |
| **100 PPM** | 62**e** ± 1.21 |
| **SR+100 PPM** | 60**e** ± 0.99 |
| **150PPM** | 68**d** ± 1.34 |
| **SR+150 PPM** | 75**c** ± 0.34 |
| **200 PPM** | 90**b** ± 0.78 |
| **SR+200 PPM** | 110**a** ± 1.21 |

Values in column with different letters show significant difference (P≤0.05) as determined by LSD Test.

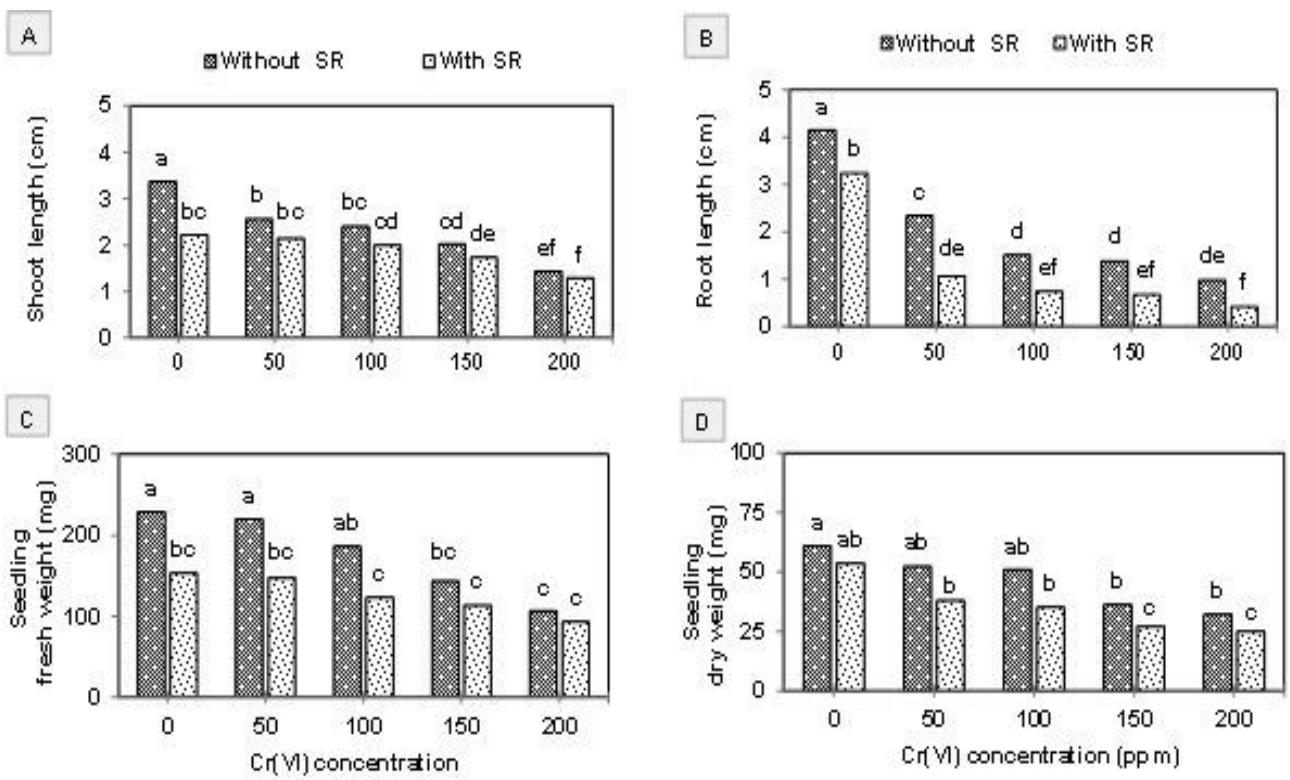


**Figure-1: A-D: Growth of *Capsicum annuum* due to biotic stress of *Sclerotium rolfsii* (SR) and abiotic stress of Cr(VI) at 15th day of germination. A: Shoot length; B: Root length C: Seedlings fresh weight; D: Seedlings dry weight. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.**

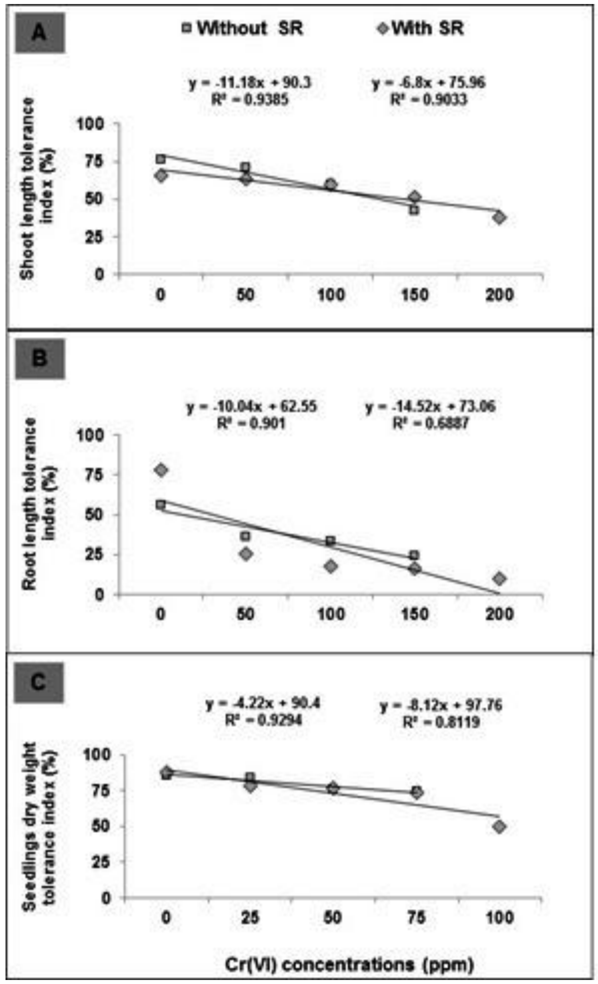


**Figure-2: A-C: Regression analysis for the relationship between tolerance indices (%) of *Capsicum annuum* due to biotic stress of *Sclerotium rolfsii* (SR) and abiotic stress of Cr(VI). A: Shoot length tolerance index; B: Root length tolerance index; C: Seedlings dry weight tolerance index.**

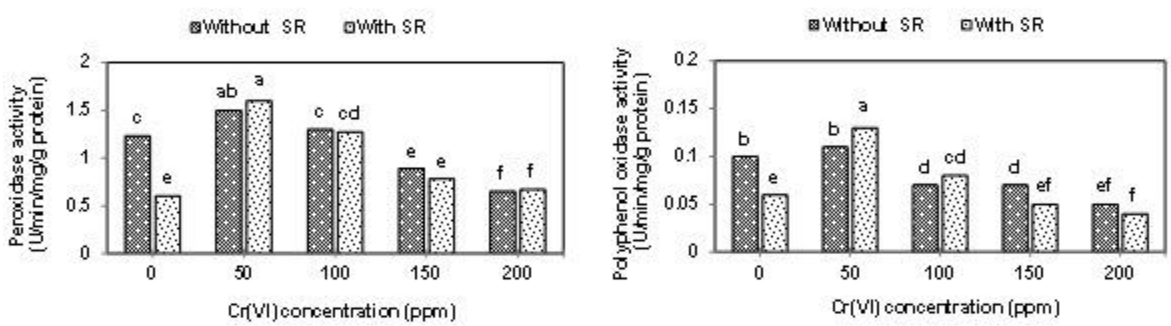


**Figure-3: Effect of *Sclerotium rolfsii* (SR) and Cr(VI) on the activities of antioxidant enzymes in *Capsicum annuum* seedlings. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.**

**Conclusion:** Simultaneous occurrence of *S. rolfsii* and various concentrations (50-200 PPM) of Cr(VI) more severely affected germination, seedlings growth and activities of defense related enzymes (POX and PPO) than either stress given alone. Inhibitory effect of Cr(VI) was increased due to higher metal concentration of 150 and 200 PPM. Amongst the plant parts, roots growth and biomass exhibited the maximum sensitivity against given stresses. Chili seedlings uptake more Cr(VI) at lower concentrations than at higher concentrations.

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