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Full Length Article

Growth of *Pisum sativum* under Single or Combined Action of *Sclerotium rolfsii* and Copper [Cu(II)]

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Abstract

A study was performed to investigate the influence of *Sclerotium rolfsii* (pathogen of southern blight diseases) on germination and growth of pea (*Pisum sativum* L.) under Cu(II) toxicity stress. Experiments were conducted in petri plates (9-cm diameter) lined with sterilize filter papers inoculated with *S. rolfsii* (spore suspension 2.0×10^6) and different concentrations of Cu(II) solution viz. 25, 50, 75 and 100 mg L^{-1}. Germination and different growth parameters were declined by 10–70% in treatments inoculated with pathogen alone. There was 10–50% reduction in these parameters either due to effect of metal alone or combined with the pathogen. Root growth showed more susceptibility to both stresses than shoot growth. In another petri plate bioassay, effect of Cu(II) on growth of *S. rolfsii* was checked, Cu(II) suppressed the growth of fungus by 10–50% at concentrations of 25–100 mg L^{-1}. Further pots and field trials are required to confirm the results of petri plate and recommendation to the farmers. © 2013 Friends Science Publishers

Keywords: Copper; Heavy metal; Pea growth; *Sclerotium rolfsii*; Southern blight disease

Introduction

Pea (*Pisum sativum* L.), native to central and southeast Asia, is an annual, self-pollinated, rapid-growing and cool season crop of family Fabaceae. This fourth-most widely grown grain legume in the world has a net production of 8.3 million tons (FAO, 2008). In Pakistan too, peas are widely grown and ranked second amongst the food legumes after chickpea (Shahid *et al*., 2011). These are cultivated on 10.48 thousand hectares having gross yield of 82 thousand metric tons (Anonymous, 2007-2008). Green peas are rich in carbohydrates, proteins, fats, minerals, vitamins A, B and C in a reasonable amount along with water soluble fiber and antioxidants (Choudhary, 1990; Hussein *et al*., 2006; Noreen *et al*., 2009).

Southern blight disease caused by *Sclerotium rolfsii* Sacc. has a long history in agriculture as a notorious and devastating soil-borne pathogen of many vegetable crops including peas thus imparts massive yield reduction along with low quality products (Arunasri *et al*., 2011). The pathogen attack on more than 500 cultivated and wild plants species in 100 families including all the agricultural and horticultural crops and cause wilt, root rot, stem rot and foot rot (Ciancio and Mukerji, 2007). Moreover, it has an ability to thrive under extensive environmental situations including a broad pH range, high moisture and maximum mycelial growth between 25–35°C. Water, infected soil, infected seedlings, agricultural machines and farming tools are major source of dissemination of *S. rolfsii* (Beute and Rodriguez-Kabana, 1981).

Besides, biotic factors, a dramatic transition in agricultural production systems in many parts of the world has occurred through the contamination of food products and food chain by hazardous heavy metals (Qishlaqi and Moore, 2007; Wei and Yang, 2010; Tomas *et al*., 2012). Toxic level of copper (Cu) in agricultural soil through application of fungicides, pesticides, herbicides, fertilizers and sewage sludge (Gunkel *et al*., 2007), ultimately reached plants and now become worldwide public health trepidation. The highest acceptable content of Cu in soil is 100 mg/kg and range of 5-30 mg/kg dry weight is considered as a physiological need of Cu for plants (Kabata-Pendias, 1996). Cu toxicity manifests as inhibitor of root growth (elongation and branching), leaf chlorosis, reduces trans-root potential, defective flowering, decreased number and size of leaves and reduced germination (Cook *et al*., 1997; Inmaculada, 2005; Puig *et al*., 2007).

Both pathogen and metal are known to adversely affect plant growth and health. However, studies are scarce about the combined effect of pathogen and metal stress on plant growth. It is hypothesized that the combined effect of these two factors may be more severe as compared to the effect of either alone. The present research work was undertaken to check the damaging impact of *S. rolfsii* and Cu(II) on germination and seedling growth of pea.

Materials and Methods

Metal Treatments

Copper sulfate ($CuSO_4.5H_2O$) was used for preparation of

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Cu(II) solution. A stock solution of 1000 mg L^{-1} of metal was prepared by dissolving 4.6 g of the salt in 1000 mL of double distilled water. Working concentrations (25, 50, 75 and 100 mg L^{-1}) were prepared by diluting stock solution.

Influence of Cu(II) on *P. sativum*

To check the toxic effect of Cu(II) on seed and seedling growth of pea, petri plate assays were performed according to method of Li and Yang (2006) with slight modification. Surface-sterilized seeds of *P. sativum* were placed in pre-sterilized petri plates (9-cm diameter) lined with sterilized Whatman No.1 filter papers. There were 25 seeds in each petri plate. In first treatment, seeds were moistened with 3 mL of spore suspension (2.0×10^6) of *S. rolfsii*, second set of treatment received 3 mL of each of four concentrations viz. 25, 50, 75 and 100 mg L^{-1} of Cu. In third set, 3 mL of different concentrations of Cu containing 2.0×10^6 fungal spores were poured in each petri plate. Petri plates supplied with 3 mL of distilled water served as control. All the plates were incubated at 25±2°C for 15 days in a completely randomized design in growth chambers with 10 h light period daily. Percentage of germination and infected seeds (by *S. rolfsii*) was recorded. Data regarding length, fresh and dry weight of both root and shoot were recorded after 15 day of incubation. Dry weight was measured by placing seedlings at 80±1°C in an oven until constant weight was observed.

Influence of Cu(II) on *S. rolfsii*

Another set of experiment was performed to check the effect of Cu(II) on *S. rolfsii* growth. Basal medium for the growth of fungus was prepared by adding malt extract agar (2% MEA) and each of four concentrations (25, 50, 75 and 100 mg L^{-1}) of metal in water followed by autoclaving and pouring in petri plates. The metal-amended medium were inoculated aseptically with 5 mm diameter inoculum-disc of the test fungus, obtained from healthy growing fungal cultures and incubated at 25±2°C for 7 days. The medium with inoculums disc but without any metal served as control. Radial colony diameter of the fungus was measured and percentage inhibition of mycelial growth by the metal concentrations was calculated using the formula:

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

Where, MGI = Mycelial growth inhibition; DC = Colony diameter in control; DT = Colony diameter of test fungi.

Data obtained from different treatments were compared through mean values. All means were tested for a significant difference utilizing Tukey's Range test or Tukey's HSD (Honestly Significant Difference) (Richard, 2008).

Results

Influence of Cu(II) on Seedling Growth of Pea

The effect of all the treatments significantly suppressed plant growth and biomass at the 15th day of incubation. In general, the maximum reduction in plant growth was recorded in treatments inoculated with *S. rolfsii* alone followed by parallel reduction by metal applied alone or combined with pathogen. Amongst the plant parts, root growth and biomass exhibited the maximum sensitivity to both pathogen and metal stresses as compared to rest of growth parameters (Table 1).

Inoculation of *S. rolfsii* resulted in significantly high rate (80%) of infected pea seeds as comparison to rest of the treatments and control (Fig. 1). The adverse effect of *S. rolfsii* was also severe on seed germination (Fig. 2) and seedling growth when compared to other treatments and control (Table 1). Thus, there was significantly greater reduction of 30%, 13-40% and 50-60% in germination rate, coleoptiles and hypocotyls growth parameters, respectively as compared to control.

Plant flourished under Cu(II) stress alone exhibited significant reduction of 6-20%, 2-11%, 8-25%, 11-31%, 14-35%, 25-52% and 20-27% in germination rate, shoot (length, fresh and dry weight) and root (length, fresh and dry weight), respectively as compared to control.

When pea seeds were exposed to combine stress of pathogen and Cu(II), the infected seeds and germination rate significantly reduced up to 53-40% and 6-26%, respectively with increasing metal dose from 25 to 100 mg L^{-1} (Fig. 1 and 2). The combine effect of metal and pathogen resulted in a significant decline of 6-26%, 7-15%, 8-25%, 21-31%, 14-45%, 30-51% and 35-48% in germination, shoot length, shoot fresh weight, dry weight, root length, root fresh weight and dry weight in comparison to control. However, there was non-significant difference in root, shoot growth and biomass of the treatments under simultaneous stresses of different concentration of Cu(II) and fungal pathogen (Table 1).

Influence of Cu(II) on Growth of *S. rolfsii*

Cu(II) exhibited antifungal activity and suppressed the growth (cm) of *S. rolfsii* by 10-50% at metal concentration of 25-100 mg L^{-1}, respectively (Fig. 5).

Discussion

Inoculations of treatments with *S. rolfsii* significantly decreased seed germination, shoot and root growth and biomass of *P. sativum*. Likewise, previously a high reduction in length and fresh weight of shoot and root, nodulation, pods and yield in plants inoculated with *S. rolfsii* have been reported (Khalequzzaman, 2003; Blum and Rodriguez, 2004; Ansari, 2005). The pathogenic effect of

Table 1: Effect of *Sclerotium rolfsii* (SR) and Cu(II) on seedling growth of *P. sativum*

Treatments	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Control	1.70 a	0.28 a	0.040 a	4.70 a	0.39 a	0.050 a
SR	1.48 c	0.20 b	0.025 c	2.35 e	0.17 b	0.020 d
Cu(25 mgL^{-1})	1.66 ab	0.26 ab	0.036 ab	4.00 ab	0.29 ab	0.042 ab
Cu(50 mgL^{-1})	1.65 ab	0.25 ab	0.035 a-c	3.90 bc	0.27 b	0.041 ab
Cu(75 mgL^{-1})	1.64 ab	0.23 ab	0.032 a-c	3.20 cd	0.22 b	0.040 a-c
Cu(100 mgL^{-1})	1.51 a-c	0.21 ab	0.028 bc	3.10 de	0.18 b	0.038 b-d
SR + Cu(25 mgL^{-1})	1.57 a-c	0.25 ab	0.031 a-c	2.85 de	0.26 b	0.030 b-d
SR + Cu(50 mgL^{-1})	1.49 a-c	0.23 ab	0.030 bc	2.68 de	0.22 b	0.030 b-d
SR + Cu(75 mgL^{-1})	1.48 bc	0.22 ab	0.028 bc	2.65 de	0.21 b	0.030 b-d
SR + Cu(100 mgL^{-1})	1.48 bc	0.21 ab	0.026 bc	2.59 de	0.19 b	0.020 b-d

In column values, with different letters show show significant difference (P≤0.05) as determined Tukey's HSD Test

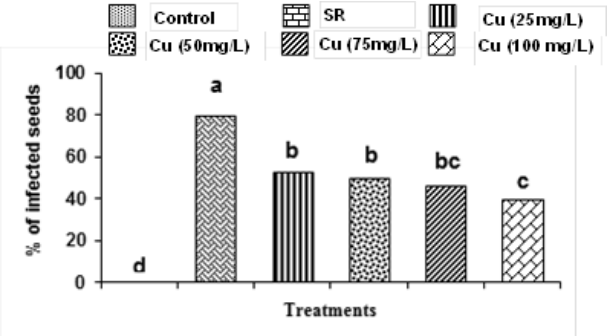

Fig. 1: Effect of *Sclerotium rolfsii* (SR) and Cu(II) on rate of infected seeds

Values with different letters at their top show significant difference (P≤0.05) as determined Tukey's HSD Test

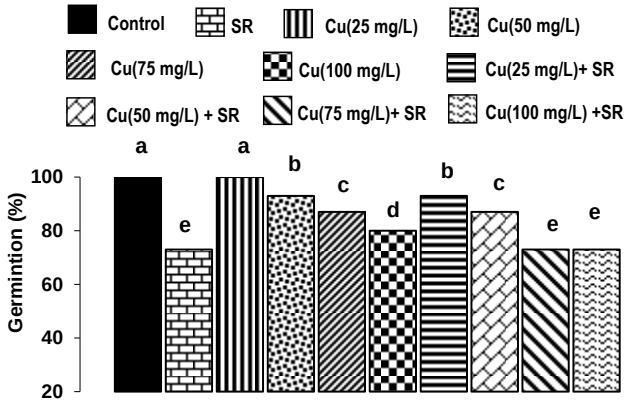

Fig. 2: Effect of *Sclerotium rolfsii* (SR) and Cu(II) on germination of *Pisum sativum*

Values with different letters at their top show significant difference (P≤0.05) as determined Tukey's HSD Test

S. rolfsii probably be correlated with production of variety of enzymes by the fungus, that might exhibit inhibitory action on different physiological and metabolic functions of the plant through disturbing level of oxidative enzymes, consequently plant growth and biomass declined (Karaman and Matavuly, 2005).

The reduction in germination rate and growth parameters due to action of metal alone is attributed to toxic concentration of metal as a dominant factor affecting the plant development (Adriano, 1986). Oxygen depletion at higher metal concentration could be another possibility of stunt root growth. It has been reported that nutrients by and large are absorbed against concentration gradients through the utilization of respiratory energy. When roots were totally submerged in metal-contaminated condition, it probably caused depletion in the oxygen amount thus result in slow growth of root (decrease cell division and cell elongation) by the inhibitory action of Cu(II) (Godzik, 1993).

Under simultaneous action of both, it might be expected that fungus would not either grow or flourish due to antifungal action of Cu, therefore expected loss in plant growth and biomass was not exceeded 50%. So, drastic effect on plant growth could be possible due to either detrimental effect of Cu(II) on plant or puncturing and penetration of roots outer cell layer by some of the *S. rolfsii* spores (Sadana *et al*., 1983), thus providing more absorption sites for metal.

Cu(II) exhibited antifungal activity and suppressed the growth of *S. rolfsii* on increasing metal concentration. These findings were supported by earlier workers, who recognized usefulness of Cu based-pesticides against soil-borne fungal pathogens (Everett *et al.*, 2007). Furthermore, action of Cu(II) on *S. rolfsii* could be assume as fungistatic rather than fungitoxic.

The present study concludes that *S. rolfsii* and copper either present alone or in combination have detrimental influence on seeds and seedlings of pea. The adverse influence of Cu(II) on the test plant was increased with elevating metal concentrations in the range of 25-100 mg L^{-1}.

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