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# Phyto-Pathogens The Threat for Environment

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## Abstract

Soil borne fungi can reduce the yield and quality in vegetable crops. In the present study, the diseased samples of tomato plant i.e., root, shoot and soil were collected from the field near Institute of Agricultural Sciences, University of the Punjab Lahore. Different fungi like *Penicillium* spp., *Mucor*, *Alternaria alternata*, *Macrophomina phaseolina* and *Fusarium oxysporum* were isolated from the root, shoot and soil sample. The isolated fungi were further control by applying copper oxychloride at different level i.e., 10, 20, 30, 40 and 50%. The results revealed that *Fusarium oxysporum* was acting as pathogenic fungi in the field and causing wilt symptoms in plants. While, the higher doses of copper oxychloride showed significant reduction in each fungal growth. Further studies are required to check the impact of copper oxychloride on the growth of tomato plant.

## INTRODUCTION

The pathogenic micro-fungal floras of field soils cause root rots, seedling damping-off and vascular wilt diseases in plants (Lichtenzveig *et al.*, 2006). Soil borne pathogens are adapted to survive and grow in bulk soil but rhizosphere is the playground where a fungus establishes parasitic and symbiotic relationship with plants (Raaijmakers *et al.*, 2009). Soil borne fungal pathogens infect number of plants. The most important genera include *Alternaria, Armillaria, Aspergillus, Chaetomium, Cylindrocladium, Fusarium, Geotrichum, Penicillium, Phytophthora, Pythium, Rhizoctonia* and *Sclerotinia* (Azaz, 2003). Trichoderma genus is also soilborne but it is non-pathogenic to plants (Ahmad *et al.*, 1987). The species of *Armillaria, Cylindrocladium, Phytophthora, Pythium, Rhizoctonia* cause root rot diseases characterized by decay of true root system. Stem, collar and head rots are caused by species of *Fusarium, Rhizoctonia, Sclerotina, Sclerotium, Phytophthora* and occasionally *Aspergillus niger*, all produce symptoms of wilting and death of leaves as well as whole plant. Species of *Fusarium, Pythium, Phytophthora, Rhizoctonia* and *Sclerotium* fungi affect the seeds during germination, pre-emergence or post-emergence phases of seedling establishment (Lichtenzveig *et al.*, 2006). Fungi in genus *Trichoderma* is common in soil and root ecosystems act as a bio-control agent against plant pathogens. It has been known since at least the 1920s and recent study shows that they are parasites of other fungi as well as opportunistic, avirulent and plant symbionts (Harman *et al.*, 2004 and Harman, 2006).

The aim of present investigation to isolate pathogenic and non-pathogenic fungi from the rhizosphere and root and shoot samples of tomato to observe the occurrence of soil fungi.

## METHODOLOGY

The present study was planned to isolate and to identify the diseased samples of tomato from infected fields.

### Survey and Collection of Samples

Survey was carried out in the fields of *Solanum lycopersicum* near Institute of Agricultural Sciences, University of the Punjab, Lahore. During the survey, the diseased samples of shoot were collected from infested field of tomato. Soil and root samples were taken from rhizospheres of diseased plants with the help of spatula to a depth of about 15 cm. The spatula was applied perpendicular to the vertical surface of the profile (Azaz, 2003). All samples were collected in plastic bag.

### Laboratory Bioassays

***Preparation of solid media***

Malt extract medium (MEA) was prepared in water followed by autoclaving at 121°C at 15 lb/inch$^2$ for 75 minutes. Amoxal (500 mg capsule in 1000 ml of medium) was added to avoid bacterial contamination. The media was poured in the pre-sterilized Petri plates in aseptic condition.

***Isolation of Fungus from soil samples***

Soil dilution was prepared for the isolation of fungi from the soil and roots of infected plant. To prepare soil dilution five screw cap test tubes with 9 ml distilled water were autoclaved and arranged 1-5 into the Laminar Flow Hood for further processing. One-gram soil sample weighed and added to the first test tube shake thoroughly by Vortex mixer to allow mixing of soil sample and microorganisms, 10 fold serial dilutions were prepared (Sharpley, 1960). Serial dilutions were prepared from $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$ and $10^{-5}$. To obtaining fungal colonies, 3rd, 4th and 5th dilution was used. One milliliter (1 mL) of solution from third dilution were taken and spread on the media plates with the help of spreader. After each spreading, spreader was sterilized and this procedure was repeated for test tube 4$^{th}$ and 5$^{th}$. Plates were placed in an incubator at 22±3°C for 3 days after spreading (Arotupin, 2004).

***Isolation of Fungus from root and shoot samples***

Root and shoot samples of infected plant were firstly washed thoroughly under running tap water for isolation of fungal. About 0.3 cm root and shoot samples were surface sterilized in 3% sodium hypochlorite 1-3 minutes followed by rinsed thrice with sterilized distilled water (Jose *et al.*, 2012). Root and shoot were dried by placing them between the layers of sterile blotter papers and plated directly in 9 cm MEA Petri plates. The plates were incubated at 22±3°C and examined after 7 days (Amusa, 2001).

***Identification and purification of fungi***

Mixture of various soil borne fungi were grown on Malt extract agar (MEA) medium plates and a single spore of each fungi transferred to fresh MEA medium plate with the help of sterilized needle for further isolation and purification (Fang *et al.*, 1983). The cultures were identified at genus level on the basis of macroscopic (colonial morphology, color, texture, shape, diameter and appearance of colony) and microscopic characteristics (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia, and presence of sterile mycelium.

***Flask Bioassays***

Effect of fungicide i.e., Copper oxychloride at different concentrations (10%, 20%, 30%, 40% and 50%) on the growth of isolated fungi were studied in liquid broth. For that Malt extract medium was prepared in water followed by autoclaving at 121°C at 15 lb/inch$^2$ for 75 minutes. Amoxal (500 mg capsule in 1000 ml of medium) was added to avoid bacterial contamination. Each of five doses i.e. 10%, 20%, 30%, 40% and 50% of fungicide were added under aseptic conditions and media was again autoclaved. The fungicide containing medium in each flask were inoculated aseptically with 5 mm (diameter) inoculum-disc of the isolated fungi separately and incubated at 25±2°C for 7 days. The medium without any fungicide served as control.

The mycelial biomass was collected on pre-weighed filter papers and fresh weight was determined. For dry biomass determination, filtered biomass was oven dried overnight at 60°C.

## RESULTS

Five genera of fungi with several isolates were identified from soil root and shoot samples of tomato include *Alternaria, Fusarium, Mucor, Penicillium* and *Macrophomina phaesolina*. *Alternaria alternata*, *Fusarium oxysporum, Macrophomina phaseolina, Penicillium,* and *Mucor* were isolated from soil sample of tomato plant. *Fusarium oxysporum* was reported to be pathogenic on tomato and cause *Fusarium* wilt. *Macrophomina phaseolina* and *Fusarium oxysporum* were reported from root and shoot sample.

The result revealed that maximum growth inhibition of each fungus was observed at higher doses (40 to 50%) of fungicide. The fresh weight of *Penicillium* spp. Mucor, *A. Altrernata, M. Phaseolina* and *F. Oxysporum* was significantly reduced by 10 to 40% at lower doses of fungicide i.e., 10 to 30%. While, dry weight of each fungus was significantly inhibited by 30 to 70%. Whereas, at 40 and 50% of fungicide significantly exhibited maximum inhibition of each fungal growth by 50 to 99% both in fresh and dry biomass (Figures 3-7).

## CONCLUSION

The engrossed of different fungi in the infected area of tomato field is the alarming situation for the yield of tomato plant. Although in the present study revealed that the fungicides can be used to control the soil bore fungi, however there is a need of complete study to evaluate the impact of fungicide on the growth and yield of the crop.

## ACKNOWLEDGEMENTS

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## REFERENCES

Lichtenzveig, J., J. Anderson, G. Thomas, O. Richard and K. Singh. 2006. Inoculation and growth with soilborne pathogenic fungi. Medicago truncatula handbook. 1-10.

Raaijmakers, J.M., T.C. Paulitz, C. Steinberg, C. Alabouvette and Y.M. Loccoz. 2009. The Rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant soil. 321:341-361.

Azaz A.D. 2003. Isolation and identification of soil borne fungi in fields irrigated by GAP in harran plain using two isolation methods. Turkish. Journal of Botany. 27:83-92.

Ahmad, J.S, and R. Baker.1987. Rhizosphere competence of *Trichoderma harzianum*. Phytopathology. 77:182-189.

Harman, G.E., C.R. Howell, A. Viterbo, L. Chet and M. Lorito. 2004. Trichoderma Species-opportunistic, avirulent Plant symbionts. Nature reviews, microbiology. 2: 43-56.

Harman, G.E. 2006. Overview of mechanisms and uses of Trichoderma spp. Phytopathology. 96:190-194.

Jose, G., M. Vicente, V. Ferraro, S. Burruano and L.V.L. Llorca. 2012. Fungal assemblages associated with roots of halophytic and non-halophytic plant species vary differentially along a salinity gradient. Environmental Microbiology.64 (3):668-79.

Amusa, N.A. 2001. Fungi associated with yam chips in storage and the effects on the chip nutrient composition. Moor Journal of Agricultural Research. 2:35-39.

Gaddeyya, G., P.S. Niharika, P. Bharathi and P.K.R. Kumar. 2012. Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. Advances in Applied Sciences Research. 3:2020-2026.

**Table 1:** Different fungi isolated from diseased sample of tomato.

| Fungi isolated from soil | Fungi isolated from roots | Fungi Isolated from shoot |
|---|---|---|
| *Penicillium* spp. | *Macrophomina phaseolina* | *Macrophomina phaseolina* |
| *Alternaria alternata* | *Fusarium oxysporum* | *Fusarium oxysporum* |
| Mucor | - | - |
| Macrophomina phaseolina | - | - |
| *Fusarium oxysporum* | - | - |

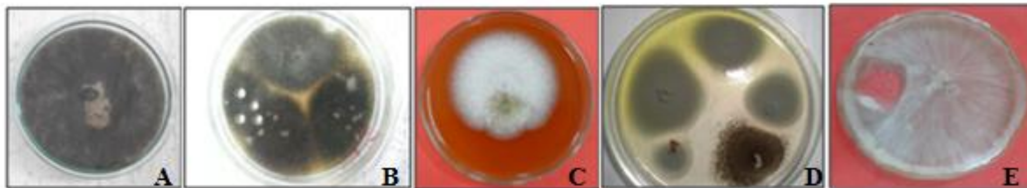


**Figure 1:** A: Alternaria alternata; B: Macrophomina phaseolina; C: Mucor; D: Penicillium spp. E: Fusarium oxysporum

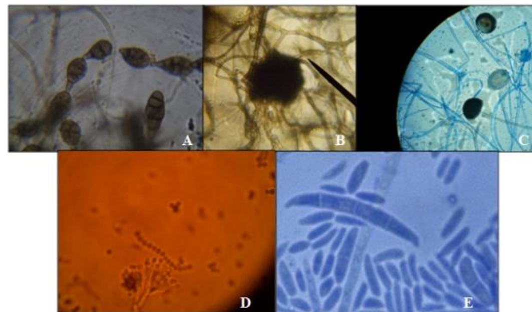


**Figure 2:** Micrscopic study of fungi. A: spores of *Alternaria alternate*, B: Perithecia of *Macrophomina phaseolina*, C: Mucor, D: *Penicillium spp.*, E: spores of *Fusarium oxysporum*.

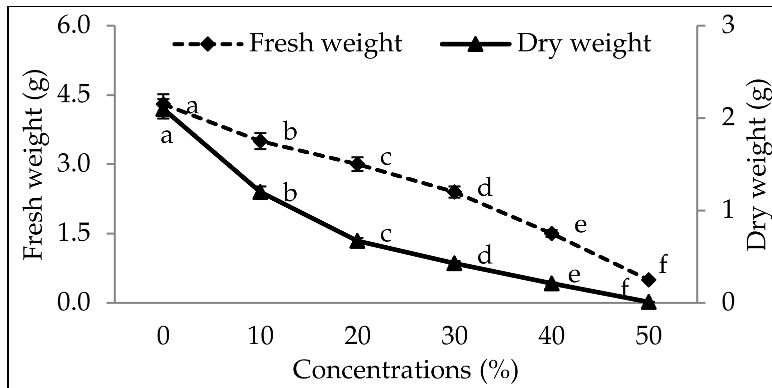


**Figure 3:** Impact of copper oxychloride on the growth of *Penicillium* spp. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey's Test.

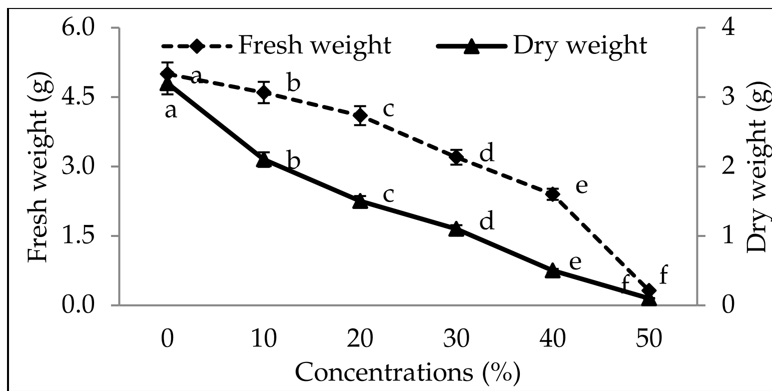


**Figure 4:** Impact of copper oxychloride on the growth of Mucor. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey's Test.

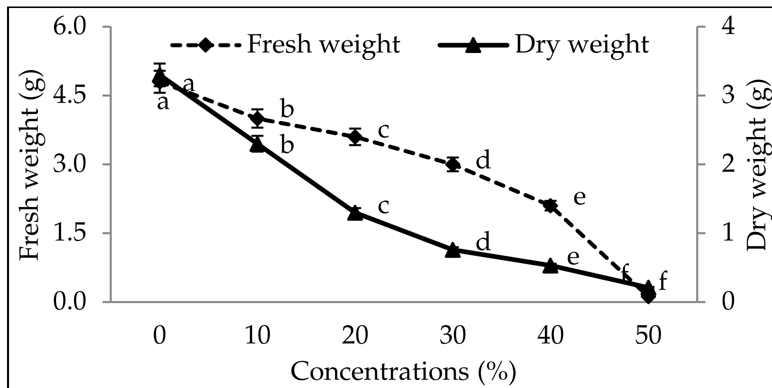


**Figure 5:** Impact of copper oxychloride on the growth of *A. alternata*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey's Test.

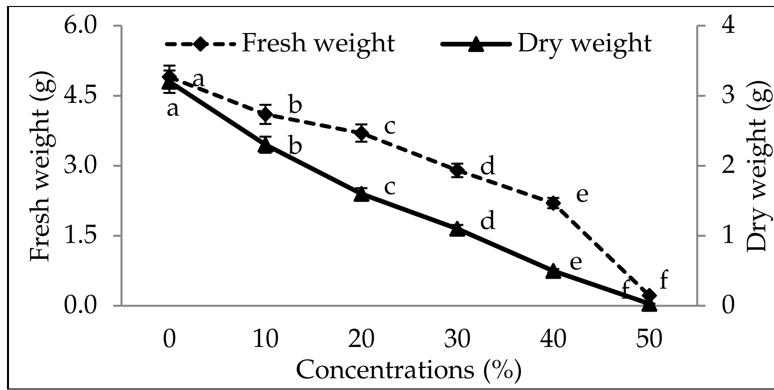


**Figure 6:** Impact of copper oxychloride on the growth of *M. phaseolina*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey's Test.

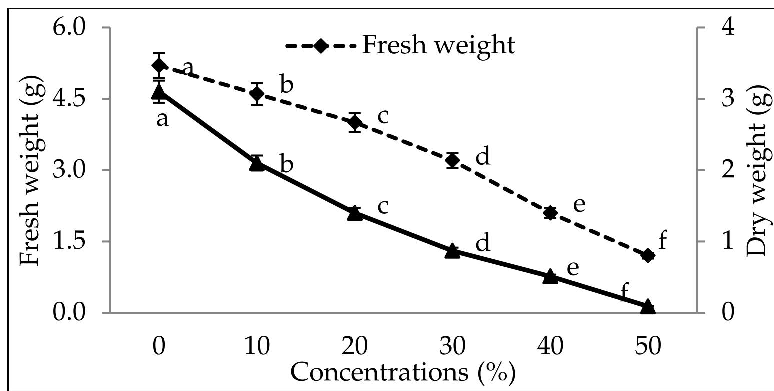


**Figure 7**: Impact of copper oxychloride on the growth of *F. oxysporum*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Tukey's Test.