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EFFECT OF NEEM LEAVES AS SOIL AMENDMENT ON SOUTHERN BLIGHT DISEASE, GROWTH AND PHYSIOLOGY OF CHILI

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

A B S T R A C T

A pot experiment was conducted to evaluate the effect of *Sclerotium rolfsii* inoculation and soil amendment with leaves of *Azadirachta indica* A. Juss. on southern blight disease, growth and physiology of chili (*Capsicum annuum* L.). The highest disease incidence (60%) and plant mortality (46%) were recorded in positive control where *S. rolfsii* was inoculated without soil amendment. Application of lower doses (1% and 2%) generally enhanced disease incidence. However, in 3% soil amendment, minimum disease incidence (27%) and plant mortality (27%) were recorded. *S. rolfsii* significantly reduced root and shoot growth as well as leaf chlorophyll and protein content. None of the soil amendment treatments significantly improved crop growth over positive control treatment. However, 3% soil amendment significantly enhanced chlorophyll content over positive control. In contrast, all the soil amendment treatments significantly declined leaf protein contents as compared to negative and positive control treatments. Application of different doses of *A. indica* significantly enhanced polyphenol oxidase (PPO) and peroxidase (PO) activities as compared to both the control treatments. This study concludes that soil amendment with 3% dry leaves of *A. indica* significantly decreased disease incidence and plant mortality in chili caused by *S. rolfsii,* possibly by enhancing production of defence related enzymes viz. PPO and PO.

Keywords: *Azadirachta indica,* disease management, plant physiology, *Sclerotium rolfsii,* southern blight.

INTRODUCTION

Chili is world famous 'wonder spice' that has been domesticated over 6000 years back, native to America, discovered by Christopher Columbus in 1493 (Olawuyi *et al*., 2014). In Pakistan, chili is a significant cash crop among vegetables occupying an area of 62.7 thousand hectares with the total production of 150.3 thousand tons having an average yield of 2.7 tons ha^{-1} (Sultana *et al*., 2014). Average production of 2.7 tons ha^{-1} is far below than the potential yield of other chili growing countries of the world. Therefore, it holds 1.5% share in country GDP (Anonymous, 2008-2009). For a couple of years, the production and export of chili in Pakistan are declining mainly due to poor quality of seed, mal-culturing practice and diseases (Khatoon *et al*., 2012; Sahar *et al*., 2013). *Sclerotium rolfsii* is a notorious fungal phytopathogen that generally prevails in a warm, humid climate and now has become a major constraint in chili production globally by causing southern blight disease (Kalmesh and Gurjar 2001; Dagnoko *et al*., 2013; Madhuri and Gayathri, 2014). Due to prevalence of *S. rolfsii* over diverse environmental extremes, the disease caused by pathogen is also becoming a threat to chili growing area of Pakistan (Jabeen *et al*., 2014). Generally, sclerotia and mycelium in the soil or plant is the main source of infection to other plants. Sclerotia are hard shelled survival structure that can produce that can survive for 5-7 years due to the presence of melanin in it until the onset of conducive conditions for apothecia formation. Rain splash, irrigation and tools, etc. are ways of disease spread through sclerotia (Okereke and Wokocha, 2007). Under optimum environmental conditions (25-35 °C and 25-35% relative humidity), the fungus produces a bulk of white, cottony and wool like hyphae from sclerotia. When fungus is inside host tissue, it produces more mycelium and forms more sclerotia and finally injures the underlying tissue through the production of its metabolites (Wyllie, 1962). Leaves shows wilting and drying. Mature infected plants of

* Corresponding Author:
Email: aamnaa29@yahoo.com

standing crop can collapse and suddenly die downward if host fails to respond or responds very slowly. However, on close examination deep cracks can be observed near collar region of infected plants. Roots become shredded and unhealthy, with freshly infected area are being covered with white mycelium (Kalmesh and Gurjar, 2001).

Due to hazardous effects of fungicides, management of *S. rolfsii* through exploring the role of allelopathic plants of family Meliaceae is attractive ecofriendly option. In this regard, *Azadirachta indica* (neem), traditional tree that is well known for remarkable antifungal properties (Farooq *et al*., 2010; Nweke, 2015) is an excellent plant to study. Neem being member of Meliaceae is rich in terpenoids. Many structurally related compounds like limonoids salannin, nimbin, 3-desacetylsalannin and -desacetylnimbin have been isolated and recognized to have antifungal potential (Johnson *et al*., 1996; Jarvis *et al*., 1999; Salazar *et al*., 2015). Therefore, the present study was conducted to assess the effect of leaf dry biomass of neem as a soil amendment to manage the southern blight disease in chili.

MATERIALS AND METHODS

An experiment was carried out in plastic pots, each containing 4 kg sandy loam soil. Before filling in the pots, soil was fumigated for one week using formaldehyde to kill any pathogen present there. *S. rolfsii* inoculum was prepared on boiled and autoclaved pearl millet seed. This inoculum was mixed in the soil of respective pots. Dry leaf biomass of *A. indica* was mixed in the pot soil at 1, 2, 3 and 4% (w/w) in combination with *S. rolfsii* inoculum. Negative control was without *S. rolfsii* and leaf biomass amendment while positive control contained *S. rolfsii* inoculum without leaf amendment. There were six treatments in total viz. i)- negative control, ii)- positive control, iii)- 1% *A. indica* leaf biomass (ALB) + *S. rolfsii* (SR), iv)- 2% ALB + SR, v)- 3% ALB + SR, and vi)- 4% ALB + SR. Pots were watered and left for one week under natural environmental conditions. There were 3 replicates of each treatment.

One month old seedlings of chili were transplanted in the pots at 5 plants per pot. Pots were watered and arranged in a completely randomized design under natural environmental conditions. Plants were harvested after 105 days of transplantation and data regarding disease incidence, plant mortality, shoot growth (including leaves, stems and fruits) and root growth were recorded. All the data were analyzed by one way ANOVA followed by LSD method to delineate treatment means at P≤0.05 using computer software Statistics 8.1.

RESULTS AND DISCUSSION

Effect of soil amendment on disease incidence and plant mortality: There were no disease symptoms observed in negative control. In positive control, there were 60% disease incidence and 46% plant mortality, respectively, which were increased to 86% each in treatment where the soil was amended with 1% leaves of *A. indica.* Disease incidence and plant mortality were 66% each in 2% soil amendment that were reduced to minimum (27% each) in 3% soil amendment treatment. Although the disease incidence in the highest dose of soil amendment (4%) was low (30%), however, plant mortality was high (53%) in this treatment possibly because of the allelopathic nature of *A. indica* (Figure 1).

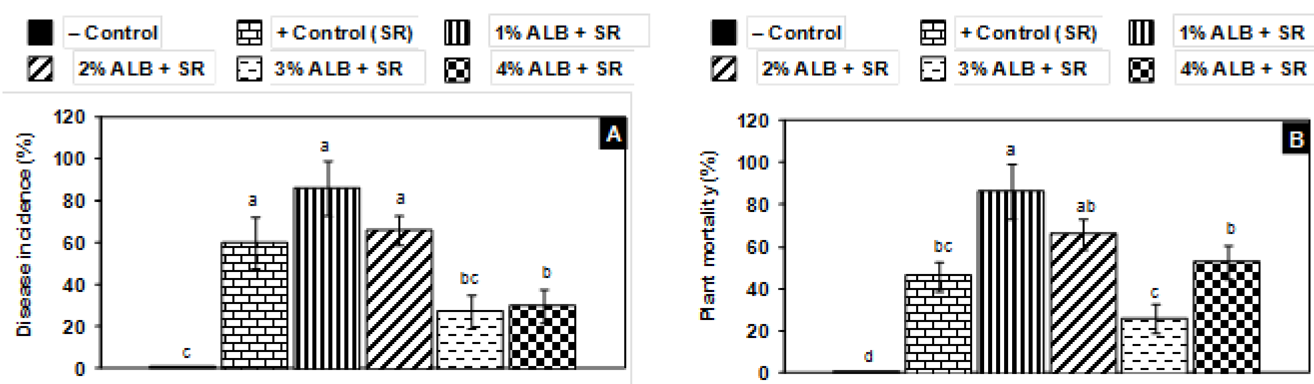

Figure 1 (A & B). Effect of soil amendment by *Azadirachta indica* leaf biomass (ALB) on disease incidence and plant mortality in chilli due to *Sclerotium rolfsii* (SR). Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD method.

Effect of soil amendment on plant growth: The highest values for length (24 cm) as well as fresh (4.53 g) and dry (0.92 g) biomass of the shoot were recorded in negative control. *S. rolfsii* inoculation (positive control) significantly (P ≤ 0.05) reduced fresh and dry biomass of shoot by 60% and 63% over negative control, respectively. Soil amendment with 3% *A. indica* leaves markedly improved shoot growth under *S. rolfsii* biotic stress resulting in 37% and 35% increase in fresh and dry biomass over positive control, respectively (Figure 2).

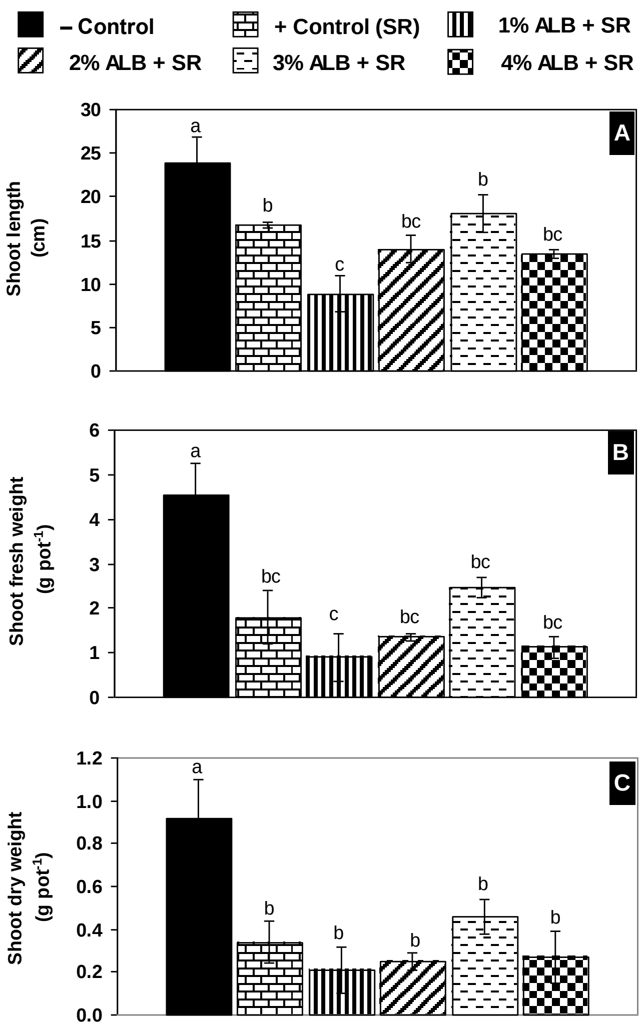

Figure 2 (A-C). Effect of *Sclerotium rolfsii* (SR) and soil amendment by *Azadirachta indica* leaf biomass (ALB) on shoot growth of chilli. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD method.

S. rolfsii significantly reduced various root growth parameters viz. length, and fresh and dry biomass by 57%, 62% and 64%, respectively, over negative control. In general, the effect of various doses of *A. indica* leaf biomass as soil amendment was insignificant. However, a 3% leaf amendment markedly increased length, and fresh and dry biomass of root by 24%, 28% and 29% over positive control, respectively (Figure 3).

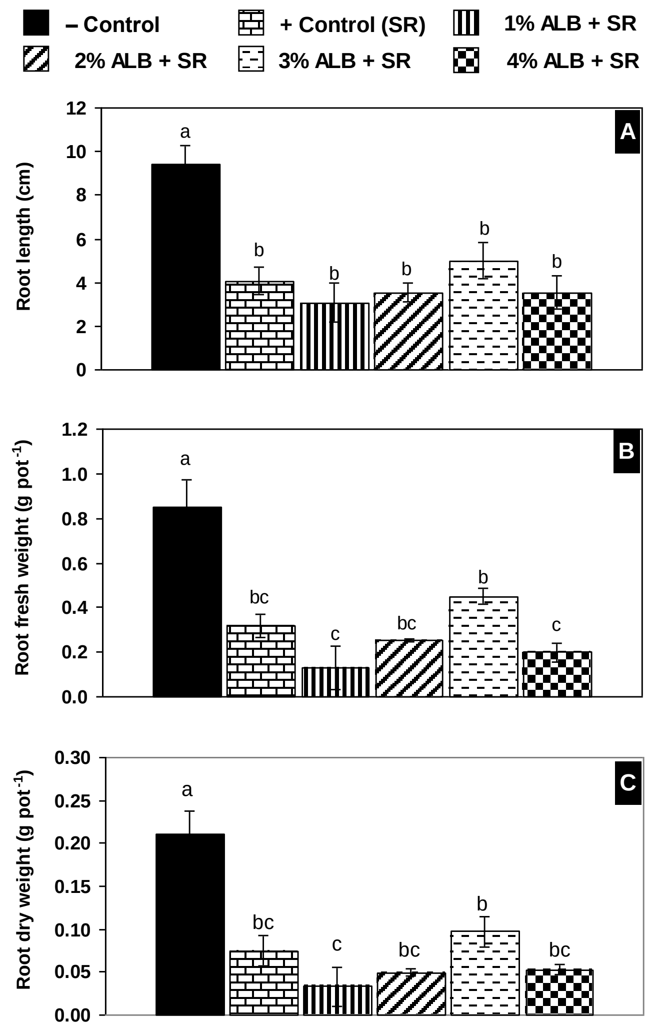

Figure 3 (A-C). Effect of *Sclerotium rolfsii* (SR) and soil amendment by *Azadirachta indica* leaf biomass (ALB) on root growth of chili. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD method.

Toxicity of *S. rolfsii* could probably occur by the toxins and acids of pathogenic fungus that could affect uptake of important minerals in plants. Which later on may change normal functioning of plant possibly by increasing respiration rate, membrane degradation (Wyllie, 1962; Orcutt and Nilsen 2000), abnormal functioning of stomata and abrupt transpiration with excessive loss of water (Aducci *et al.*, 1997; Kalmesh and Gurjar, 2001) resulted in plant wilting (Madhuri and Gayathri, 2014). It could be assumed that disease management and increase in plant biomass by 4% leaf dry biomass might be due to increase in resistance in plant, possibly by changing biotic and abiotic soil characteristics along with nutrient availability in favor of chili plant. Besides, specified concentrations of particular allelochemicals naturally may have growth promoting potential in plant by synergistical association with microbes.

Effect of soil amendment on host plant physiology: The highest chlorophyll content (3.88 mg g^{-1}) were recorded in negative control which were significantly reduced by 55% due to *S. rolfsii* inoculation. Application of 3% leaf biomass as soil amendment significantly enhanced chlorophyll content by 78% over positive control (Figure 4 A). Photoinhibition and photodestruction of chlorophyll pigments due to the inhibition of some specific enzymes necessary for the production of chlorophyll pigment might occur under biotic stress of *S. rolfsii* (Dugdale *et al*., 2000). So far, the increase in the total content of chlorophyll due to 3% soil amendment might be due to positive effects on source-sink balance and rubisco activity (Kasai , 2008).

The highest quantity of protein content (2.64 mg g^{-1}) were noted in negative control. *S. rolfsii* inoculation significantly suppressed this parameter over negative control. All the doses of *A. indica* leaf amendment failed to improve protein content under *S. rolfsii* biotic stress. In general, protein content was further declined due to application of *A. indica* leaf biomass in combination with *S. rolfsii* as compared to *S. rolfsii* alone inoculation. Three percent leaf biomass amendment was proved less inhibitory than rest of the leaf biomass amendment treatments (Figure 4 B). The significant decrease in the protein content as a result of pathogen infection could be related with generation of reactive oxygen species (ROS) during hypersensitive response (HR) (Houssien *et al*., 2010). The initiation of ROS production is one of the first events following the recognition of a pathogen by the plant (Baker and Orlandi, 1995). Variations in response of total protein content due to effect of soil amendmenats could be related with the variation of the demand for substrates, necessary to the production of plant defense mechanisms induced by *S. rolfsii* treatment (Guzzo *et al*.,, 2004).

S. rolfsii inoculation had an insignificant effect on polyphenol oxidase (PPO) activity. However, application of different doses of dry leaf biomass of *A. indica* significantly enhanced this parameter over positive control. The effect was more pronounced in lower doses of soil amendment viz. 1% and 2% than in higher doses viz. 3% and 4%. There was 95–323% increase in polyphenol oxidase activity due to different doses of soil amendment over control (Figure 4 C).

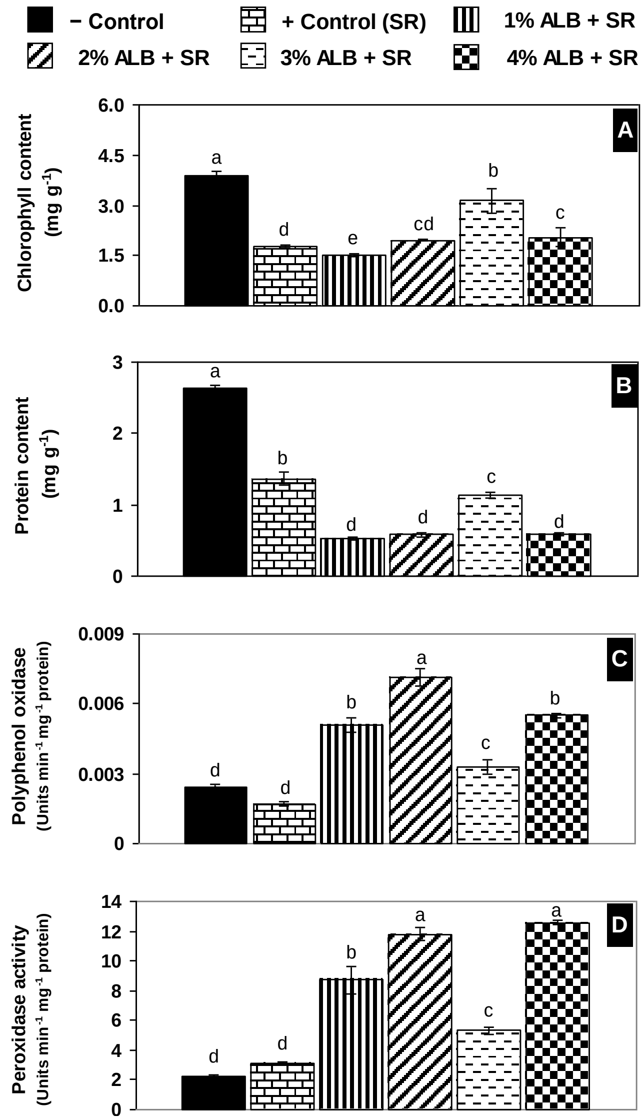

Figure 4 (A-D). Effect of *Sclerotium rolfsii* (SR) and soil amendment by *Azadirachta indica* leaf biomass (ALB)on various physiological parameters of chilli. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD method.

PPO is one of the polyphenolic compounds redox catalyzing enzyme and the induction of its activity under stress indirectly could indicate the involvement of antioxidative polyphenols in stressed plant (Gholizadeh and Kohnehrouz, 2010). The role of phenolic compounds in defense mechanism against stress by plant pathogens is well established (Agamy *et al*., 2013). Phenolic compounds provide disease resistance either by hypersensitive cell death or lignifications of cell walls or increased content of phenol itself toxic to pathogen (Nicholson and Hammerschimdt, 1992).

The effect of *S. rolfsii* and different doses of *A. indica* leaf biomass as soil amendment on peroxidase (PO) activity of chili plant was similar to the effect of these treatments on polyphenol oxidase activity (Figure 4 D). Stimulation in PO activity after providing infected soil with leaf biomass might show oxidation of phenols, suberization and lignifications of host plant cells during the defense reaction against pathogenic agents (Ashry and Mohamed, 2011).

From the present study, it is concluded that 3% dry leaves of *A. indica* as a soil amendment can significantly decrease the incidence of southern blight disease and plant mortality in *C. annuum,* possibly by overproduction of defence related enzymes namely PPO and PO that strengthened the defence mechanism to combat the biotic stress.

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