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PHYTOCHEMICAL MANAGEMENT OF COLLAR ROT OF CHILI WITH LEAF BIOMASS OF *EUCALYPTUS CAMALDULENSIS*

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A B S T R A C T

Sclerotium rolfsii Sacc. is a devastating soil-borne fungal pathogen that causes collar rot disease in chili (*Capsicum annuum* L.) and causes pronounced yield losses. In the present study, *S. rolfsii* inoculated soil was amended with 1, 2, 3 and 4% (w/w) dry leaves of *Eucalyptus camaldulensis* and their effect was studied on disease incidence, mortality, growth and physiology of the host plant. In positive control, there was 73% disease incidence that was further enhanced to 93% in 1% soil amendment treatment. However, further increase in dose of soil amendment (2% and 3%) decreased disease incidence to 66% and 53%, respectively. A similar effect of soil amendment was observed on plant mortality. A 3% dose of *E. camaldulensis* leaf biomass alleviated biotic stress of *S. rolfsii* and increased leaf dry biomass of chili by 67% as compared to positive control. Chlorophyll content and polyphenol oxidase activity were significantly lower in *E. camaldulensis* amended treatments over positive control. Protein content was gradually increased by increasing leaf amendment dose while reverse was recorded in case of peroxidase activity. The present study concludes that soil amendment with 3% leaf dry biomass of *E. camaldulensis* can alleviate biotic stress of *S. rolfsii* on growth of chili to some extent.

Keywords: Disease incidence, *Eucalyptus camaldulensis,* Peroxidase activity**,** protein content, *Sclerotium rolfsii,* soil amendment.

INTRODUCTION

Sclerotiumrolfsii is a soil-borne fungus belongs to basidiomycete, generally persists in warm humid climate and causes disease over 500 plants including chili (Madhuri and Gayathri, 2014). Infection in host plant can occur through complex infection structures (infection cushions), natural openings and wounds (Dodman and Flentje, 1970; Parmeter, 1970). After infection, the fungus produces oxalic acid and tissue degrading enzymes which causes poisoning of plant and break down of cell wall. Base of stem is covered with fluffy white mycelium and results in collar rot. Mature mycelia produce sclerotia, which germinate to produce either individual hyphal strands on its surface or mycelial aggregates bursting through its surface and cause new infection (Ferreira and Boley, 1992). Infection results in wilting of and drying of whole plant.

Up till now, *S. rolfsii* is considered to be a difficult pathogen to be managed by available methods due to extensive host range, prolific growth and resistant sclerotia (El-Said, 2012; Sennoi *et al.,* 2013). Amongst different management options, removal of infected plant is generally suggested but this practice is discouraged due to difficultly in its application in field. Soil heating is ineffective because of survival of some of the sclerotia and losses to crop as well. Soil solarization is expensive and also difficult to apply in field (Mullen, 2001). Crop rotation with crops susceptible to the pathogen could results in intensified spread of disease in following years (Rodriguez-Kabana *et al*., 1974; Lockweed, 1998). Fungicide application is not an appropriate alternative due to persistent nature of the pathogen, high cost and scarcity of the chemicals (Yaqub and Shahzad, 2009; Adesegun *et al*., 2012; Amin *et al*., 2014). Drawback of various management strategies against *S. rolfsii* has altered the research direction of scientists toward use of natural alternatives for management this pathogen such as extracts and biomass of allelopathic plants (Iqbal and Javaid, 2012; Khan and Javaid, 2013; Javaid and Iqbal, 2014).

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Eucalyptus camaldulensis, family Myrtaceae is an allelopathic tree cultivated throughout tropics, and commonly occupying open waste spaces and grasslands, road sides, river banks and wetlands (Abubakar, 2010). It is famous for its medicinal uses as well as antifungal potential due to presence of phenols, tannins, saponins and cardenolide etc. (Abubakar, 2010; Barra *et al*., 2010; Siramon *et al*., 2013). Considerable literature is available on *in vitro* antifungal potential of leaf essential oil of many *Eucalyptus* spp. including *E. grandis, E. camaldulensis*, and *E. citriodora* against several pre- and post-harvest fungi like *Aspergillus clavatus*, *Aspergillus niger, Penicillium citrinum Chaetomium globosum* and *Cladosporium cladosporioides* etc. (Su *et al*., 2006). Likewise, Bashir and Tahira (2012) showed aqueous, methanolic and *n*-hexane leaf extracts of *E. camaldulensis* hold significant antimycotic potential against *Fusarium solani*. Recently, Shafique *et al*. (2015) revealed 85% management of Fusarium wilt of chili caused by *F. oxysporum* through methanolic leaf extract of *E. citriodora*. However, literature is scanty on *in vivo* disease management potential of leaf biomass of *E. camaldulensis*. It has been revealed that phytochemicals in leaf triggers plant defense mechanism by activating function of antioxidant enzymes (peroxidase, catalase and phenyl ammonia lyase etc.) during growth and development of plant often and protect them against microbial attack (Haralampiodis *et al*., 2001). Production of reactive oxygen species (ROS) are obvious events linked with changes in activity of antioxidant enzymes. Peroxidase is multipurpose enzymes provides protection against pathogens by phenols oxidation, host cell wall suberization and lignifications. Polyphenol functions by oxidation of phenols (Ashry and Mohamed, 2012). Antifungal action of plant phytochemicals comprised of inhibition of various activities of fungal pathogens like fungal extracellular enzymes, oxidative phosphorylation, spore germination and mycelial growth (Chérif*et al*., 2007). According to Ayepola and Adeniy (2008), tannin in leaf could bind proteins thereby inhibiting cell protein synthesis in microbial cell. Current investigation was performed to determine influence of *E. camaldulensis* leaf dry biomass as soil amendment against collar rot disease by assessing growth and physiology of chili.

MATERIALS AND METHODS

Preparation of inoculum: Pearl millet seeds (300 g) were soaked in water for two hours and boiled mildly. Excessive water was drained and boiled seeds were autoclaved at 121 °C and 304 kPas pressure for 30 minutes in plastic bags. After cooling, bags were inoculated with actively growing culture of *S. rolfsii* and incubated at 27 °C for 10 days.

Preparation of pots: Plastic pots were filled with sandy loam soil at 4 kg soil per pot. Before filling in the pots, soil was fumigated with formalin for one week. *S. rolfsii*inoculum (20 g pot^{-1}) was thoroughly mixed in pot soil and watered and left for one week. Negative control was without fungal inoculum. However, same amount of boiled and autoclaved pearl millet seeds were mixed in the pot soil. In fungal inoculated pots, dried and powdered leaves of *E. camaldulensis* were mixed at 1, 2, 3 and 4% (w/w). Positive control treatment was without dry leaf amendment. Pots were irrigated and left for one week for release of allelochemicals from leaves and their interaction with the fungal pathogen.

Experimental design and treatments: One month old seedlings of chili were transplanted in pots at 6 seedlings per pot. Pots were watered. Experiment was carried out in a completely randomized design with three replications. There were following 6 treatments:

T_1 Negative control
T_2 Positive control (*S. rolfsii*)
T_3 *S. rolfsii* + 1% *E. camaldulensis*
T_4 *S. rolfsii* + 2% *E. camaldulensis*
T_5 *S. rolfsii* + 3% *E. camaldulensis*
T_6 *S. rolfsii* + 4% *E. camaldulensis*

Harvesting and data analysis: Plants were harvested 105 days after transplantation. Disease incidence, plant mortality as well as roots and shoots length and fresh weight were recorded. Plants materials were dried at 70 °C and dry weights were recorded. All the data were analyzed by ANOVA. Treatment means were separated at 5% level of significance by applying computer software Statistics 8.1.

RESULTS AND DISCUSSION

Effect of soil amendment on disease incidence and plant mortality: There was 73% disease incidence in positive control that was further enhanced to 93% due to application of 1% dry leaf biomass of *E. camaldulensis.* Further increase in dose of soil amendment (2-4%) decreased disease incidence to 66% and 53%. In general, the difference in disease incidence among various *S. rolfsii* inoculated treatments with and without soil amendment was insignificant. The effect of *S. rolfsii* and soil amendment on plant mortality was similar to that of the effect on disease incidence (Figure 1).

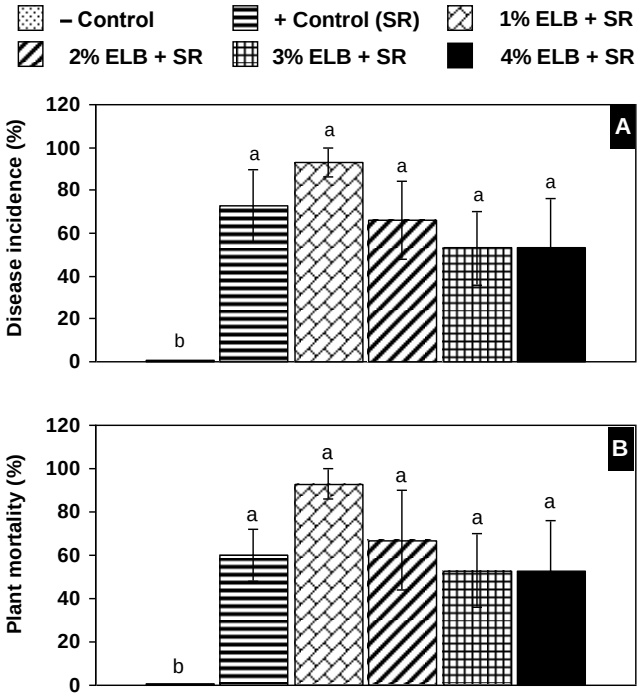

Figure 1. Effect of soil amendment by *Eucalyptus camaldulensis* leaf biomass (ELB) on disease incidence and plant mortality in chili due to *Sclerotium rolfsii* (SR). Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

Pathogenicity of *S. rolfsii* to chili might be attributed to occurrence of oxalic, gallic, ferulic, cinnamic, chlorogenic and indole-acetic acid in sclerotial filtrate (Amber *et al*., 2012). Reduction in disease incidence with increased dose of *E. camaldulensis* might be due to increase in amount of antioxidant compounds e.g. phloroglucinol, flavonoids and tannins (Amakura*et al*., 2002), that likely to inhibit growth of *S. rolfsii*.

Effect of soil amendment on plant growth: The effect of *S. rolfsii* and different doses (1-4%) of *E. camaldulensis* leaf biomass application as soil amendment had insignificant effect on shoot fresh and dry biomass. However, 3% dose of *E. camaldulensis* leaf amendment alleviated biotic stress of *S. rolfsii* and increased leaf dry biomass of chili by 67% over positive control (Figure 2). All the *S. rolfsii* inoculated treatments showed lower root length and dry biomass than negative control. The effect was more severe in *E. camaldulensis* leaf amended treatments. However, the adverse effect of *S. rolfsii* on root fresh and dry biomass was less pronounced in 3% *E. camaldulensis* leaf amendment treatment (Figure 3). Presently, highest applied dose (4%) showed less improvement in plant growth and biomass than that of 3%. It might be due to concentration depended action of allelochemicals, while these allelochemicals could work as parts plant defense mechanism against biotic stresses. At higher concentration, these may block important physiological and metabolic processes of plant (Farooq*et al*., 2013).

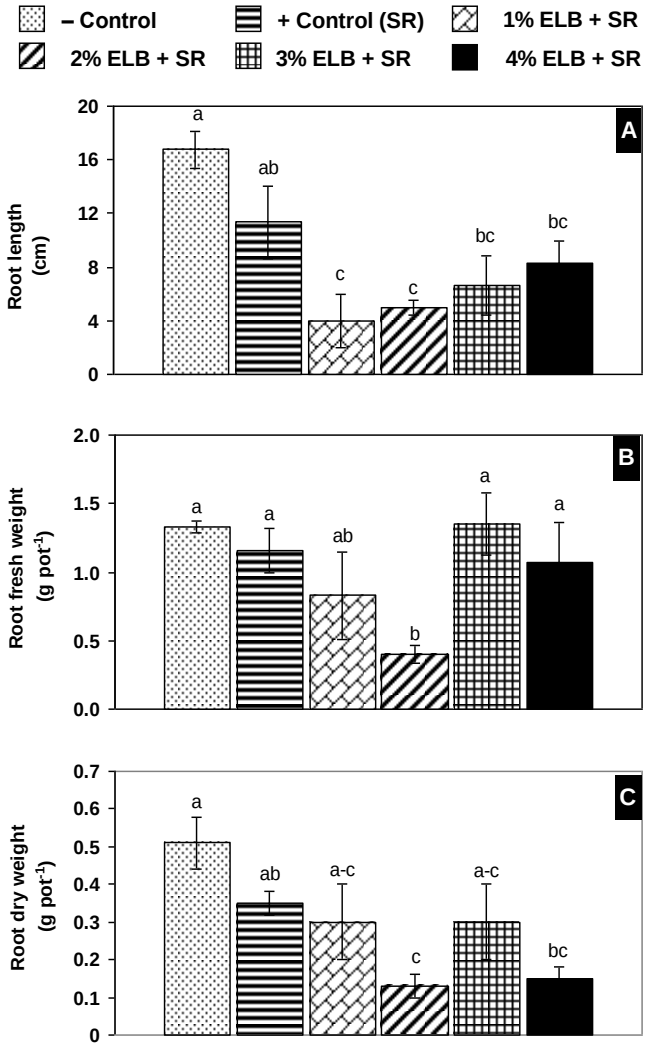

Figure 2. Effect of *Sclerotiumrolfsii* (SR) and soil amendment by *Eucalyptus camaldulensis* leaf biomass (ELB) on shoot growth of chili. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

So far, 1% and 2% also found less effective than 3% could be due to lower concentration of allelochemicals to combat with *S. rolfsii* (Lankau , 2012). Root is in direct contact with soil, therefore, drastic effect of root growth and biomass could be obvious probably generated by synergistic interaction of allelochemicals and *S. rolfsii.*

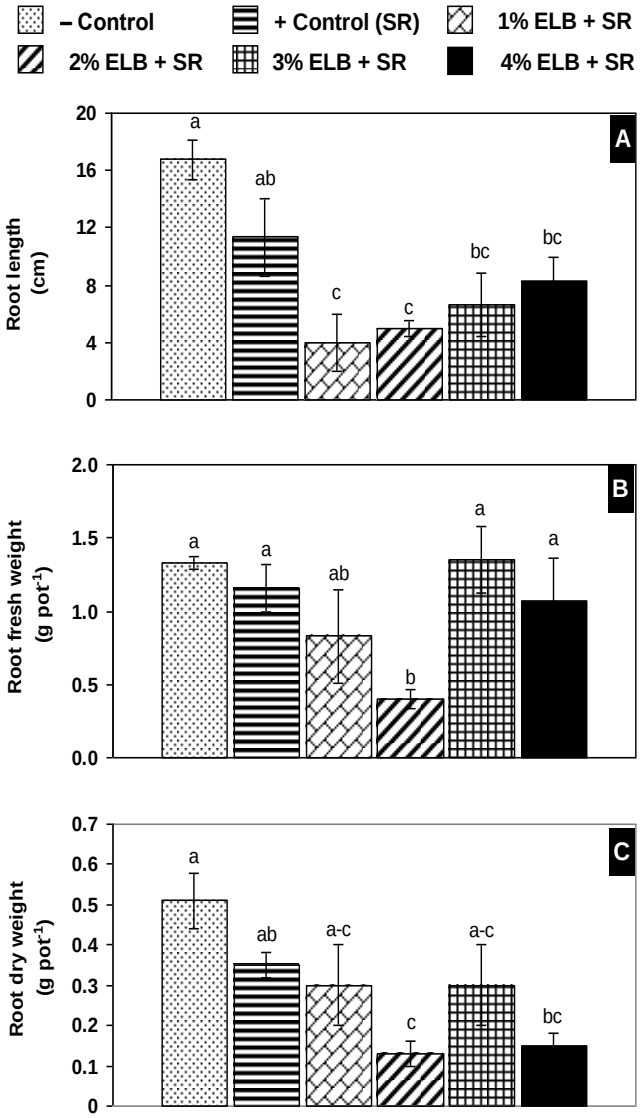

Figure 3. Effect of *Sclerotium rolfsii* (SR) and soil amendment by *Eucalyptus camaldulensis* leaf biomass (ELB) on root growth of chili. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by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

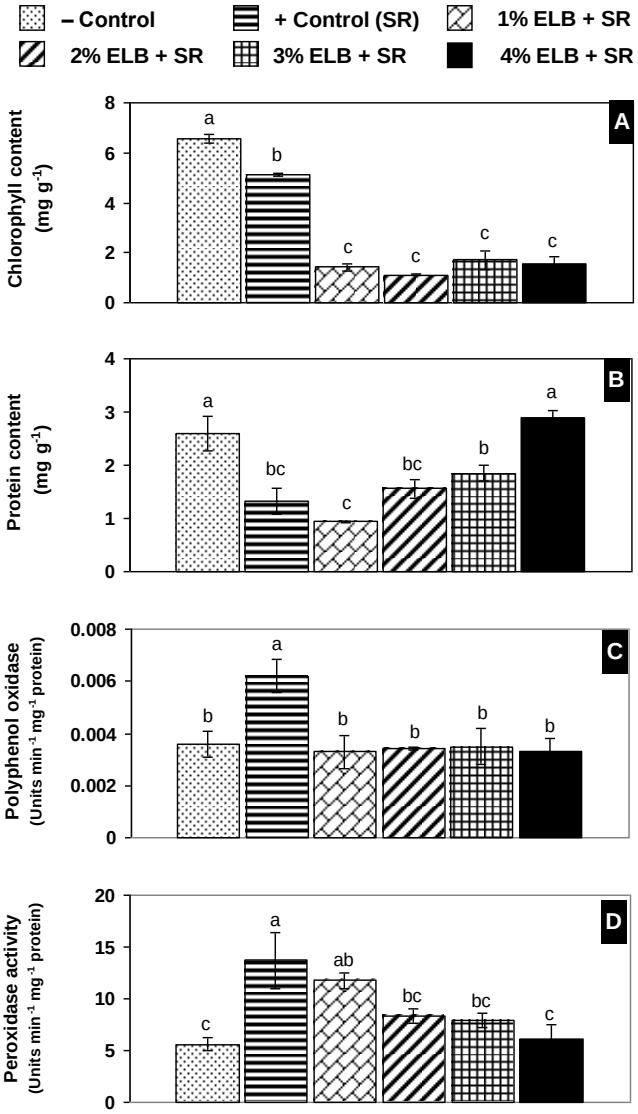

Figure 4. Effect of *Sclerotium rolfsii* (SR) and soil amendment by *Eucalyptus camaldulensis* leaf biomass (ELB) on various physiological parameters of chili. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

Effect of soil amendment on host plant physiology: The highest chlorophyll contents (6.56 mg g^{-1}) were recorded in negative control which were significantly reduced by 22% in positive control. Application of leaf biomass amendment significantly reduced chlorophyll contents than negative as well as positive control treatments (Figure 4 A). Reduction in chlorophyll content followed by programmed cell death might be consequences of fungal toxins (Howlett, 2006) So far, variation in total content of chlorophyll due to different biofungicides might be due to changes in stomatal conductance, source-sink balance and effect on rubisco activity (Kasai, 2008).

S. rolfsii inoculation significantly reduced protein contents by 49% over negative control. Application of *E. camaldulensis*leaves amendments enhanced protein contents. There was a gradual increase in protein contents with an increase in quantity of *E. camaldulensis* leaves biomass. The highest protein contents were recorded in 4% leaf amendment treatments that were significantly greater by 118% over positive control treatments (Figure 4 B). Van Loon (1997) documented

presence of stress protein in plants after stress, however, the content of protein may decline due to increase in total amino acid pool (Parida*et al*., 2004). Increase in protein content due to soil amendment might be due to synthesis of nitrogenous compound that possibly increase in total free amino acid and proline content (Da La Rosa-Ibarca and Maiti, 1995) and could reveal different levels of resistance acquired by chili plant against *S. rolsii*.

Polyphenol oxidase (PPO) activity was significantly increased by 72% due to soil inoculation with *S. rolfsii.* However, application of different doses of leaf biomass of *E. camaldulensis* leaves lowered the quantity of this parameter at par with negative control (Figure 4 C). The lowest peroxidase (PO) activity (5.6 units min^{-1} mg^{-1} protein) was recorded in negative control. Inoculation of *S. rolfsii* significantly enhanced this parameter to its maximum in positive control (13.70 units min^{-1} mg^{-1} protein). Application of *E. camaldulensis*leaves as soil amendment gradually decreased this parameter as compared to positive control with an increase in dose of the leaf biomass (Figure 4 D). Generation of defense related enzymes including PPO and PO has been demented by Avdiushko *et al*. (1993) in cucumber leaf and Bhagat & Chakraborty (2010) in tea leaf due to infection of *S. rolfsii*. Reduction in activity of PPO and PO due to soil amendment might indicate low requirement of plant for the induction of antioxidants.

CONCLUSION

The present study concludes that collar rot disease of chili can be managed by applying 3% dry leaf biomass of *E. camaldulensis*as soil amendment. Further studies are needed to evaluate the effect of soil amendment with this plant material under field conditions.

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